Three New Xanthones from the Fungus *Penicillium* sp. NH-7-1

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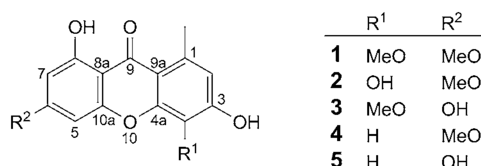
Three new xanthones, drimiopsins G – I (**1** – **3**, resp.), and two known congeners, griseoxanthone C (**4**) and norlichexanthone (**5**), were isolated from a fungal isolate, *Penicillium* sp. NH-7-1. The structures of all compounds were determined on the basis of extensive spectroscopic analyses, as well as by comparison with literature reports, and the structure of compound **1** was further confirmed by single-crystal X-ray diffraction.

Introduction. – Xanthones are widespread in nature, commonly occurring in a number of higher plant families and fungi [1][2]. Some fungal species are well-known as sources of xanthone derivatives, *e.g.*, *Penicillium raistrickii* [3], *Actinoplanes* sp. [4], *Phomopsis* sp. [5], *Ascodesmis sphaerospora* [6], and *Humicola* sp. [7]. Xanthones are known to have a variety of interesting biological profiles, *e.g.*, antibacterial, antifungal, antioxidant, antiviral, anti-inflammatory, and anti-carcinogenic activities [8]. With the aim of discovering new natural products from wetland-derived fungi, we examined a rarely explored area – Tianjin ancient lagoon wetland from which we had isolated 46 fungal strains. We performed chemical analyses of the secondary metabolites produced by isolated fungi based on high performance liquid chromatography/mass spectrometry (HPLC/MS) and HPLC/UV profiles. One of the hits resulting from our efforts was the strain NH-7-1 which was identified as *Penicillium* sp. by its 18S rRNA gene sequence (99% similarity to that of the strain *Penicillium* sp. Cs/2/3) and was shown to produce xanthone derivatives. On this basis, the current secondary-metabolite investigation was undertaken, which afforded three new xanthone derivatives **1** – **3**, in addition to two known congeners (*Fig. 1*). The structures were elucidated by means of MS, and 1D- and 2D-NMR experiments, and by comparisons with reports in the literature.

Results and Discussion. – *Isolation and Structure Elucidation*. The mycelium and culture broth of *Penicillium* sp. NH-7-1 were extracted with 80% acetone and AcOEt,



	R1	R2
1	MeO	MeO
2	OH	MeO
3	MeO	OH
4	H	MeO
5	H	OH

Fig. 1. *Structures of compounds* **1** – **5**

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respectively. Since the HPLC profiles of the two extracts were nearly identical, they were combined and further purified by a combination of column chromatography on silica gel and *Sephadex LH-20* to afford three new xanthones drimiopsins G – I (**1** – **3**, resp.), and two known related derivatives, griseoxanthone C (**4**) [9] and norlichexanthone (**5**) [10].

Compound **1** was obtained as colorless crystals. The HR-ESI-MS showed the *quasi*-molecular-ion peak at *m*/*z* 303.0632 ($[M+H]^+$, $C_{16}H_{15}O_6^+$; calc. for 303.0790), in accordance with the molecular formula $C_{16}H_{14}O_6$, which indicated ten degrees of unsaturation. Strong absorption bands accounting for OH (3423 cm^{-1}), CO (1653 cm^{-1}), and aromatic groups (1615, 1572, 1453 cm^{-1}) were observed in its IR spectrum. The ^1H- and ^{13}C-NMR spectra of **1** (*Table*) displayed signals for all 16 C-atoms and 14 H-atoms, including those of a xanthone skeleton (δ(C) 136.7, 116.9, 156.9, 133.4, 152.2, 92.5, 166.1, 97.5, 163.2, 103.4, 182.2, 111.3, 156.9), with three aromatic H-atoms (δ(H) 6.71 (*s*), 6.58 (*d*, $J=2.0$), 6.30 (*d*, $J=2.0$)), two MeO groups (δ(H) 3.85 (*s*), 3.87 (*s*)), and one phenolic OH group (δ(H) 13.42). The HMBCs (*Fig. 2*) of Me(1) (δ(H) 2.67) with C(1) (δ(C) 136.7), C(2) (δ(C) 116.7), and C(9a) (δ(C) 111.3), of H–C(2) (δ(H) 6.71) with C(4) (δ(C) 133.4), and C(9a) suggested that the Me group should be located at C(1). The location of the two MeO groups at C(4) and C(6) were supported by HMBCs of MeO(4) (δ(H) 3.85) with C(4), MeO(6) (δ(H) 3.87) with C(6) (δ(C) 166.1). One OH group located at C(8) was confirmed by HMBCs of the OH H-atom signal (δ(H) 13.42) with C(7) (δ(C) 97.5) and C(8) (δ(C) 163.2), indicating a phenolic H-atom that is H-bonded to the neighboring CO group; and another phenolic OH group, of which the H-atom signal which was undetected should be located at C(3) (δ(C) 156.9). The typical H-atom signals (δ(H) 6.71 (*s*), 6.58 (*d*, $J=2.0$), and 6.30 (*d*, $J=2.0$)) also supported the 1,3,4-trisubstitution for ring *A*, and 6,8-disubstitution for

Table. *^1H- and ^{13}C-NMR Data of* **1** – **3** (at 600 and 150 MHz, resp.; in (D_6)DMSO)

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1		136.7 (*s*)		131.6 (*s*)		136.6 (*s*)
2	6.71 (*s*)	116.9 (*d*)	6.68 (*s*)	115.9 (*d*)	6.68 (*s*)	116.7 (*d*)
3		156.9 (*s*)		151.6 (*s*)		156.4 (*s*)
4		133.4 (*s*)		131.1 (*s*)		133.3 (*s*)
4a		152.2 (*s*)		151.6 (*s*)		152.0 (*s*)
5	6.58 (*d*, $J=2.0$)	92.5 (*d*)	6.51 (*d*, $J=2.0$)	92.4 (*d*)	6.31 (*d*, $J=2.0$)	93.9 (*d*)
6		166.1 (*s*)		166.0 (*s*)		165.6 (*s*)
7	6.30 (*d*, $J=2.0$)	97.5 (*d*)	6.29 (*d*, $J=2.0$)	97.1 (*d*)	6.12 (*d*, $J=2.0$)	98.5 (*d*)
8		163.2 (*s*)		163.3 (*s*)		163.5 (*s*)
8a		103.4 (*s*)		103.3 (*s*)		102.3(*s*)
9		182.2 (*s*)		182.6 (*s*)		181.9 (*s*)
9a		111.3 (*s*)		111.5 (*s*)		111.4 (*s*)
10a		156.9 (*s*)		156.9 (*s*)		156.9 (*s*)
Me–C(1)	2.67 (*s*)	23.2 (*q*)	2.65 (*s*)	22.8 (*q*)	2.67 (*s*)	23.2 (*q*)
*Me*O–C(4)	3.85 (*s*)	61.2 (*q*)			3.83 (*s*)	61.1 (*q*)
*Me*O–C(6)	3.87 (*s*)	56.5 (*q*)	3.87 (*s*)	56.4 (*q*)		
*H*O–C(8)	13.42 (*s*)		13.47 (*s*)		13.42 (*s*)	

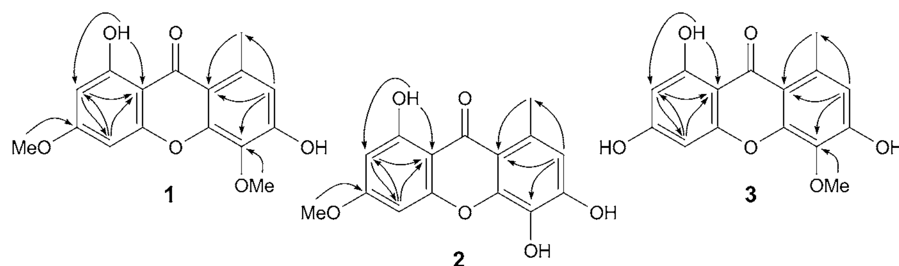

Fig. 2. *Key HMBCs of* **1**–**3**

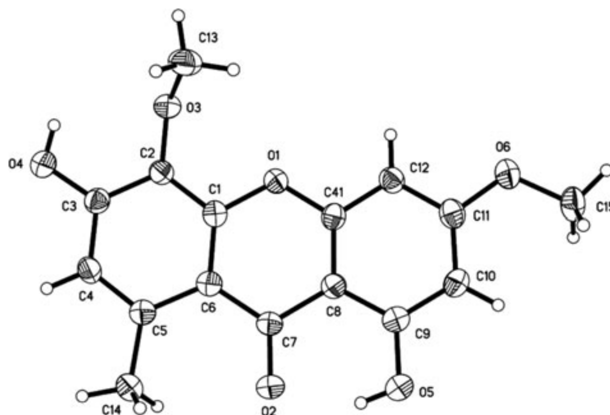

Fig. 3. *ORTEP Diagram of the crystal structure of* **1**[1])

ring *B* of the xanthone skeleton. Ultimately, the structure of **1** was further confirmed by single-crystal X-ray diffraction (*Fig. 3*). Thus, compound **1** was identified as 3,8-dihydroxy-4,6-dimethoxy-1-methylxanthone.

Compound **2** was deduced to have the molecular formula $C_{15}H_{12}O_6$ with ten degrees of unsaturation, having one CH_2 unit less than **1**, on the basis of HR-ESI-MS data (*m*/*z* 289.0710 $[M+H]^+$; calc. 289.0634). The NMR data were very similar to those of **1**. However, signals for the MeO group resonating at δ(H) 3.85 and δ(C) 61.2 in **1** disappeared in the NMR spectra of **2**, which was also confirmed by 2D-NMR correlations. Therefore, compound **2** was elucidated as 3,4,8-trihydroxy-6-methoxy-1-methylxanthone.

Compound **3** was obtained as white powder, and the molecular formula, $C_{15}H_{12}O_6$, was deduced from HR-ESI-MS (*m*/*z* 289.0706 $[M+H]^+$). According to the molecular formula and NMR data, **3** was determined to possess almost the same pattern as **2**, differing only in the signals due to the presence of a MeO group at C(4) and a OH group at C(6), which were also confirmed by 2D-NMR correlations. Accordingly, compound **3** was identified as 3,6,8-trihydroxy-4-methoxy-1-methylxanthone.

[0]) Different atom numberings are used in the text and the table.

Compounds **4** and **5** were identified by comparing their ^{1}H- and ^{13}C-NMR data with those reported in the literature.

Financial supports from the *National Natural Science Foundation of China* (Nos. 21302214 and 31400026), the *973 Foundation of China* (No. 2012CB721104), the *863 Foundation of China* (No. 2012AA02A704), the *Science & Technology Plan Project of Tianjin City of China* (No. 11ZCKFSY07900) are gratefully acknowledged.

Experimental Part

General. Column chromatography (CC): commercial silica gel (SiO_2; *Qingdao Haiyang Chemical Group Co.*; 200–300 mesh) and *Sephadex LH-20* (*Amersham Biosciences*). UV Spectra: *Shimadzu UV-1800* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Thermo Scientific Nicolet 6700* spectrophotometer; in cm^{-1}. Semi-prep. HPLC: *Shimadzu 6-AD* apparatus equipped with a UV detector and a *Shim-pack PREP-ODS (H) kit* (20×250 mm, 5 µm) column; flow rate, 8 ml/min. ^{1}H- and ^{13}C-NMR spectra: *Bruker AVANCE III 600* spectrometer; at 600 and 150 MHz, resp.; δ in ppm, J in Hz. HR-ESI-MS: *Bruker microQ-TOF II* mass spectrometer; in m/z.

Fungal Material. The fungal strain *Penicillium* sp. NH-7-1 was isolated from the soil of Qilihai Ancient Coastal and Wetland National Nature Reserve, Tianjin City, P. R. China, in October 2011. The fungal strain is deposited in our laboratory at $-80°$. The producing strain was prepared on Potato Dextrose agar slants and stored at 4°.

Fermentation, Extraction, and Isolation. *Penicillium* sp. NH-7-1 was grown under static conditions at r.t. for 45 d in 50 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), yeast extract (3 g/l), KH_2PO_4 (0.5 g/l), $MgSO_4 \cdot 7\,H_2O$ (0.3 g/l), and NaCl (5 g/l). The fermented broth (*ca.* 15 l) was filtered through cheesecloth to separate it into filtrate and mycelia. The filtrate was concentrated under reduced pressure to *ca.* 1/4 of the original volume and then extracted three times with AcOEt to give an AcOEt soln., while the mycelia was extracted three times with 80% acetone. The acetone soln. was concentrated under reduced pressure to afford an aq. soln., which was extracted three times with AcOEt to give another AcOEt soln. Both AcOEt solns. were combined and concentrated under reduced pressure to give a crude extract (12 g).

The AcOEt extract (12 g) was separated into nine fractions, *Frs. 1–9*, by CC (SiO_2; stepwise gradients of petroleum ether (PE)/CH_2Cl_2, 0 to 100% (v/v)) and CH_2Cl_2/and MeOH, 0 to 100% (v/v)). *Fr. 7* (1.5 g) was separated into seven fractions, *Frs. 7-1–7-7*, by CC (SiO_2; PE 0 to 100% (v/v)). *Fr. 7-7* was separated into five fractions by CC (*Sephadex LH-20*; MeOH/CH_2Cl_2 1:1 (v/v)). *Fr. 7-7-2* was further purified by semiprep. HPLC (75% MeOH) to give **2** (t_R 18.7 min; 10 mg) , **3** (t_R 16.2 min; 5 mg) and **5** (t_R 15.1 min; 8 mg). *Fr. 8* (1.2 g) was purified by CC (SiO_2; PE 0 to 100% (v/v)) to afford five fractions. *Fr. 8-2* was also purified by CC (*Sephadex LH-20*; MeOH/CH_2Cl_2 1:1 (v/v)) and 100% MeOH), before separation by semiprep. HPLC (80% MeOH) to give compound **1** (t_R 28.2 min; 11 mg) and **4** (t_R 32.1 min; 10 mg).

Drimiopsin G (*=3,8-Dihydroxy-4,6-dimethoxy-1-methyl-9*H*-xanthen-9-one*; **1**). Colorless crystals. UV (MeOH): 235 (4.25), 311 (3.90). IR (KBr): 3423, 2920, 2850, 1653, 1615, 1572, 1513, 1453, 1281, 1202, 1162. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS: 303.0632 ($[M+H]^+$, $C_{16}H_{15}O_6^+$; calc. 303.0790).

Drimiopsin H (*=3,4,8-Trihydroxy-6-methoxy-1-methyl-9*H*-xanthen-9-one*; **2**). Light-yellow powder. UV (MeOH): 233 (4.22), 310 (3.82). IR (KBr): 3507, 3392, 2926, 2853, 1656, 1616, 1585, 1515, 1466, 1314, 1283, 1212, 1160, 1056. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS: 289.0710 ($[M+H]^+$, $C_{15}H_{13}O_6^+$; calc. 289.0634).

Drimiopsin I (*=3,6,8-Trihydroxy-4-methoxy-1-methyl-9*H*-xanthen-9-one*; **3**). White powder. UV (MeOH): 234 (4.20), 312 (3.85). IR (KBr): 3412, 2925, 2850, 1652, 1617, 1577, 1513, 1457, 1320, 1273, 1167, 1053. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS: 289.0706 ($[M+H]^+$, $C_{15}H_{13}O_6^+$; calc. 289.0634).

X-Ray Crystallography of **1**. *Crystal Data*. $C_{16}H_{14}O_6$, M_r 302.27; crystal size: $0.45 \times 0.08 \times 0.06$ mm^3; monoclinic; space group, $P2(1)/c$; unit cell dimensions, $a = 3.9220(3)$ Å, $b = 20.3770(18)$ Å, $c = 17.1870(16)$ Å, $\alpha = 90.00$, $\beta = 97.044(2)$, $\gamma = 90.00$, $V = 1363.2(2)$ Å^3, $Z = 4$, $D_{calc.} = 1.473$ Mg/m^3, and $F(000) = 632$. The reflection data were collected on an *Bruker FRAMBO CCD* area detector diffractometer using CuK_α radiation at 293 (2) K, $\lambda = 1.54178$. A total of 4,795 reflections were collected in the range $3.38° \leq \theta \leq 66.06°$, of which 2,396 unique reflections with $I > 2\sigma(I)$ were collected for the analysis. The structure was solved by direct methods (SHELXS-97) and expanded using *Fourier* techniques (SHELXL-97). The final R and wR_2 factors were 0.0759 and 0.2002, resp. Crystallographic data for the structure reported in this article has been deposited with the *Cambridge Crystallographic Data Centre* (accession No. CCDC-1018480).

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Received September 16, 2014