New Lactone and Xanthone Derivatives Produced by a Mangrove Endophytic Fungus *Phoma* sp. SK3RW1M from the South China Sea

by **Jia-Hui Pan**[a]), **Juan-Juan Deng**[a]), **Yi-Guang Chen**[a]), **Jun-Ping Gao**[a]), **Yong-Cheng Lin***[a]), **Zhi-Gang She**[a]), and **Yu-Cheng Gu**[b])

[a]) School of Chemistry and Chemical Engineering, Sun Yat-sen University, 135 Xingang West Road, Guangzhou 510275, P. R. China (phone/fax: +86-20-84039623; e-mail: ceslyc@mail.sysu.edu.cn)

[b]) *Syngenta*, Jealott's Hill International Research Centre, Bracknell, Berkshire, UK RG42 6EY

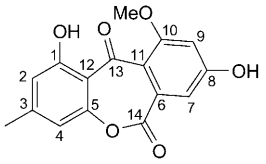
A new lactone, 1,8-dihydroxy-10-methoxy-3-methyldibenzo[*b*,*e*]oxepine-6,11-dione (**1**), and two new xanthones, 1-hydroxy-8-(hydroxymethyl)-6-methoxy-3-methyl-9*H*-xanthen-9-one (**2**) and 1-hydroxy-8-(hydroxymethyl)-3-methoxy-6-methyl-9*H*-xanthen-9-one (**3**), were isolated from a mangrove endophytic fungus *Phoma* sp. SK3RW1M collected from the South China Sea. This is the first report on xanthone derivatives isolated as secondary metabolites from *Phoma* species. Their structures were elucidated by spectroscopic methods, mainly 1D- and 2D-NMR techniques, and the structure of compound **2** was confirmed by X-ray crystallography. Cytotoxicity assays showed that compounds **1**–**3** were inactive against KB and KBv200 cells.

Introduction. – The genus *Phoma* contains many terrestrial as well as marine-derived fungi. These microorganisms secrete a number of metabolites to their surroundings including phytotoxin, and antitumor, antimicrobial, and anti-HIV compounds. It has been established as a rich source of structurally diverse and biologically active natural compounds [1][2]. Xanthone derivatives are endowed with diverse pharmacological profiles, including antihypertensive, antioxidative, antithrombotic, and anticancer activities [3]. Studies on structure–activity relationships of xanthone derivatives were reviewed comprehensively [4]. As part of our on-going program to search for new bioactive natural compounds in the South China Sea [5], an endophytic fungus, *Phoma* sp. SK3RW1M, has been isolated from the roots of *Avicennia marina* (FORSK.) VIERH., collected in Shankou mangrove, Guangxi, P. R. China, and two new xanthones, together with a new lactone, were isolated and identified. To the best of our knowledge, there is no previous report on xanthone derivatives as secondary metabolites from *Phoma* species. Cytotoxicity assays showed that compounds **1**–**3** were inactive against KB and KBv200 cells ($IC_{50} > 50$ μg ml^{-1}).

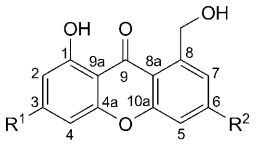
Results and Discussion. – Repeated column chromatography of the AcOEt extract from the fermentation broth of the fungal strain *Phoma*. sp. SK3RW1M yielded compounds **1**–**3**[1]).

Compound **1** was isolated as a pale yellow amorphous powder. Its molecular formula was determined as $C_{16}H_{12}O_6$ on the basis of its HR-EI-MS (*m*/*z* 300.0630 (M^+;

[1]) Arbitrary numbering; for systematic numbering and names, see *Exper. Part.*



1[1])



2[1]) R^1 = Me, R^2 = MeO
3[1]) R^1 = MeO, R^2 = Me

calc. 300.0634)) and NMR data. ^{1}H- and ^{13}C-NMR spectra (*Table 1*) revealed the presence of one aromatic Me group (δ(C) 22.4, δ(H) 2.41), one aromatic MeO group (δ(C) 57.1, δ(H) 3.96), and a OH group strongly chelated to a CO group (δ(H) 12.32). Signals of the two sets of *meta*-coupled aromatic H-atoms at δ(H) 7.18 (*d*, $J = 2.4$) and 6.98 (*d*, $J = 2.4$), and δ(H) 6.87 (br. *d*, $J = 1.2$) and 6.66 (br. *d*, $J = 1.2$) implied the presence of two 1,3-disubstitued aromatic rings. The IR ($\tilde{\nu}_{max}$ 1651 and 1738 cm^{-1}) and ^{13}C-NMR (δ(C) 179.8 and 169.4) spectroscopic data indicated that compound **1** has a conjugated ketone and a conjugated lactone moiety in the structure. The HMBC detected between H–C(7) (δ(H) 6.98) and the lactone C-atom C(14) (δ(C) 169.4) suggested that the lactone group was at the *ortho*-position to H–C(7). HMBC cross-peaks between HO–C(1), Me(3), and H–C(2) placed the Me group at C(3). The position of the MeO group was assigned to C(10) due to the strong HMBC from MeO–C(10) to C(10). HMBCs were also observed between H–C(9), and C(7), C(8),

Table 1. *^{1}H- and ^{13}C-NMR Data of* **1**[a]). Recorded in (D_6)DMSO, at 400 and 100 MHz, respectively, δ in ppm, J in Hz.

	δ(H)	δ(C)	HMBC (H → C)[b])
C(1)	–	161.0	–
H–C(2)	6.67 (br. *d*, $J = 1.2$)	111.8	1, 4, 3-Me
C(3)	–	149.3	–
H–C(4)	6.87 (br. *d*, $J = 1.2$)	107.7	2, 3-Me, 5 (w), 12
C(5)	–	155.7	–
C(6)	–	137.1	–
H–C(7)	6.98 (*d*, $J = 2.4$)	112.3	9, 10 (w), 11, 14
C(8)	–	158.2	–
H–C(9)	7.18 (*d*, $J = 2.4$)	101.7	7, 8, 10
C(10)	–	165.4	–
C(11)	–	110.2	–
C(12)	–	106.4	–
C(13)	–	179.8	–
C(14)	–	169.4	–
HO–C(1)	12.32 (*s*)	–	1, 2, 12
Me–C(3)	2.41 (*s*)	22.4	2, 3
HO–C(8)	–	–	–
*Me*O–C(10)	3.96 (*s*)	57.1	10

[a]) Arbitrary atom numbering as indicated in the *Formulae*. For systematic names, see *Exper. Part.* [b]) w = Weak.

and C(10), and between H–C(7), and H–C(9), C(10) (weak), and C(11). This indicated that the remaining OH group was at C(8). Considering all the above results, the structure of compound **1** was elucidated as 1,8-dihydroxy-10-methoxy-3-methyldibenzo[*b*,*e*]oxepine-6,11-dione. The assignments of ^{1}H- and ^{13}C-NMR data were supported by the data of similar structures in the literature [6–8].

Compound **2** possesses the molecular formula $C_{16}H_{14}O_5$ derived from HR-EI-MS (*m*/*z* 286.0836 (M^+; calc. 286.0841)) indicating ten degrees of unsaturation. The ^{13}C-NMR and DEPT spectra of **2** revealed 16 C-atom resonances attributable to nine quaternary C-atoms (δ(C) 182.9, 164.9, 161.6, 160.0, 155.6, 148.5, 144.8, 112.7, 107.1), four CH groups (δ(C) 114.8, 111.6, 106.9, 100.0), one CH_2 group (δ(C) 65.3), and two Me groups (δ(C) 55.9, 22.5). It was also shown that seven of the ten elements of unsaturation within **2** were twelve aromatic C-atoms and a CO group (δ(C) 161.6), which was supported by an IR absorption at $\tilde{\nu}_{max}$ 1659 cm^{-1}. The remaining three degrees of unsaturation indicated that the molecule was tricyclic. UV_{max} Absorptions at 235, 250, 303, 351 nm also suggested the presence of a xanthone nucleus [9]. In the ^{1}H-NMR spectrum, two sets of *meta*-coupled aromatic H-atom signals at δ(H) 6.89 (*d*, $J = 2.4$) and 6.80 (*d*, $J = 2.4$), and 6.69 (*d*, $J = 0.8$) and δ(H) 6.62 (*d*, $J = 0.8$) indicated that compound **2** possessed two 1,3-disubstitued aromatic rings. ^{1}H- and ^{13}C-NMR spectra showed the characteristic signals of one aromatic Me group (δ(C) 22.5, δ(H) 2.42), one aromatic CH_2OH group (δ(C) 65.3, δ(H) 4.91, 2 H, *s*), one aromatic MeO group (δ(C) 55.9, δ(H) 3.93), and a H-bonded phenolic OH group (δ(H) 12.63). Further confirmation of the substituent pattern on the rings was obtained from HMBC data shown in *Table 2*. In the HMBC spectrum, correlations from the chelated HO–C(1) and the aromatic Me–C(3) to H–C(2) and C(9a) indicated that Me group was at C(3). This was confirmed by the COSY peaks between Me–C(3), and H–C(2) and H–C(4), and the HMBCs between Me–C(3), and C(2), C(3), and C(4). The HMBC spectrum also showed ^3J cross-peaks from the equivalent O–CH_2 H-atoms to (7) and C(8a), establishing attachment of the HO–CH_2 group to C(8). Thus, the remaining MeO group was assigned to C(6), *i.e.*, the *meta*-position of C(8), which was further confirmed by the strong correlation between MeO–C(6) and C(6). On the basis of these data, the compound was elucidated as 1-hydroxy-8-(hydroxymethyl)-6-methoxy-3-methyl-9*H*-xanthen-9-one and confirmed by X-ray diffraction analysis (see *Table 3* and *Fig.*).

Compound **3** was isolated as a yellow-brown amorphous powder. HR-EI-MS Data (*m*/*z* 286.0835 (M^+; calc. 286.0841)) along with the NMR results revealed that compound **3** has a molecular formula of $C_{16}H_{14}O_5$, which is the same as **2**. The UV, IR, and NMR data (*Table 2*) were similar to those of **2**, suggesting this compound has a xanthone framework with the same substitution pattern of **2**. In its ^{1}H-NMR spectrum, the peak with a chemical shift at δ(H) 12.86 was due to the phenolic H-atom (HO–C(1)), bonded with the CO O-atom. The HMBCs from CH_2O H-atoms (HO–CH_2–C(8)) to H–C(7), C(8), and C(8a) placed the CH_2OH group at C(8). The ^{13}C-NMR spectrum showed that the chemical shift of C(3) shifted from δ(C) 148.5 in **2** to 166.8 in **3**, whereas that of C(6) shifted from δ(C) 164.9 in **2** to 146.7 in **3**, indicating the interchange between the Me and MeO group. This was supported by the upfield shifting of H–C(2) and H–C(4), and the downfield shifting of H–C(5) and H–C(7), and the ^3J cross-peaks in HMBC spectrum from MeO–C(3) to C(3), and from Me(6)

Table 2. *^{1}H- and ^{13}C-NMR Data of* **2** *and* **3**[a]). Recorded in $CDCl_3$, at 400 and 100 MHz, respectively, δ in ppm, J in Hz.

	2			**3**		
	δ(H)	δ(C)	HMBC	δ(H)	δ(C)	HMBC
C(1)	–	161.6	–	–	163.6	–
H–C(2)	6.62 (*d*, *J* = 0.8)	111.6	1, 4, 3-Me	6.36 (*d*, *J* = 2.4)	97.3	1, 4, 9a
C(3)	–	148.5	–	–	166.8	–
H–C(4)	6.69 (*d*, *J* = 0.8)	106.9	2, 4a, 9a, 3-Me	6.42 (*d*, *J* = 2.4)	92.4	2, 4a, 9a
C(4a)	–	155.6	–	–	157.9	–
C(5)	6.80 (*d*, *J* = 2.4)	100.0	6, 7, 8a, 10a	7.22 (br. *d*)	117.8	6, 7, 8a, 10a
C(6)	–	164.9	–	–	146.7	–
H–C(7)	6.89 (*d*, *J* = 2.4)	114.8	5, 6, 8a, 8-CH_2OH	7.12 (br. *d*)	127.2	8a, 8-CH_2OH, 6-Me
C(8)	–	144.8	–	–	142.5	–
C(8a)	–	112.7	–	–	116.5	–
C(9)	–	182.9	–	–	182.8	–
C(9a)	–	107.1	–	–	104.4	–
C(10a)	–	160.0	–	–	157.2	–
HO–C(1)	12.63 (*s*)	–	1, 2, 9a	12.86 (*s*)	–	1, 2, 9a
Me–C(3) or *Me*O–C(3)	2.42 (*s*)	22.5	2, 3, 4, 9a	3.90 (*s*)	55.8	3
*Me*O–C(6) or *Me*–C(6)	3.93 (*s*)	55.9	6	2.48 (*s*)	21.8	5, 6, 7
HO–*CH_2*–C(8)	4.91 (*s*)	65.3	7, 8a	4.94 (*s*)	65.2	7, 8, 8a

[a]) Arbitrary atom numbering as indicated in the *Formulae*. For systematic names, see *Exper. Part.*

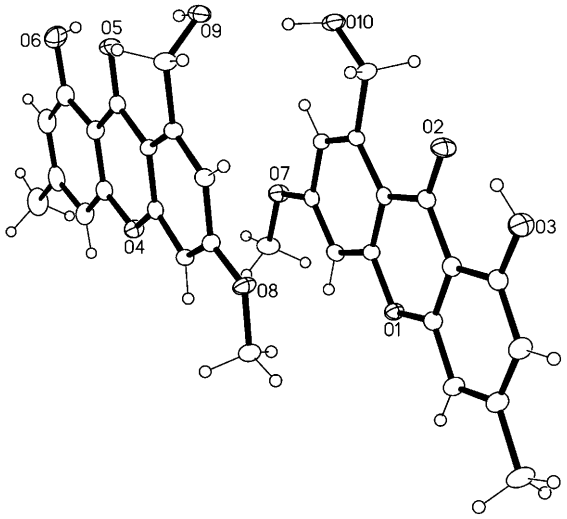


Figure. *X-Ray crystal structure of compound* **2**

Table 3. *X-Ray Crystal and Refinement Data of Compound* **2**[a])

Solvent for crystallization	$CHCl_3$
Empirical formula	$C_{16}H_{14}O_5$
M_r [Da]	286.27
Crystal size	$0.46 \times 0.32 \times 0.22$ mm
Unit-cell dimensions:	
a [Å]	8.2950(16)
b [Å]	11.080(2)
c [Å]	14.567(3)
α [°]	75.472(3)
β [°]	87.308(3)
γ [°]	84.711(3)
Volume [Å^3]	1290.0(4)
λ (MoK_α) [Å]	0.71073
Crystal system	monoclinic
Space group	$P1$
Z	4
Temp. [K]	173(2)
Calc. density [Mg/m^3]	1.474
Absorption coefficient [mm^{-1}]	0.110
θ-Range [°] for data collection	1.44 – 26.00
F (000)	600
Refinement method	Full-matrix least-squares on F^2
Data, restraints, parameters	4981, 0, 395
Reflections collected	4981
Goodness-of-fit on F^2	1.129
Final R_1 indices ($I > 2\sigma(I)$)	0.0810 $wR_2 = 0.2661$
R Indices (all data)	$R_1 = 0.1144$, $wR_2 = 0.2855$
Largest diff. peak and hole	0.816, 0.353 e Å^{-3}
Limiting indices	$-10 \le h \le 10$, $-13 \le k \le 13$, $-17 \le l \le 17$

[a]) CCDC-740933 contains the supplementary crystallographic data for this article. These data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/data_request/cif.

to C(5), C(6), and C(7). Thus, the structure of compound **3** was identified as 1-hydroxy-8-(hydroxymethyl)-3-methoxy-6-methyl-9*H*-xanthen-9-one.

Cytotoxic activities of compounds **1**–**3** were evaluated against KB and KBv200 cells, as there was no report on the antitumor activity of compounds that shared the similar C-atom skeleton with **1**. Preliminary results indicated that they were all inactive.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Co.*, P. R. China). M.p.: *Fisher–Johns* hot-stage apparatus; uncorrected. UV Spectra: *Shimadzu UV-2501PC* spectrophotometer, in anh. MeOH; λ_{max} (log ε) in nm. IR Spectra: *Bruker EQUINOX 55*

spectrophotometer, as KBr pellets; in cm^{-1}. NMR Spectra: *Bruker AVANCE 400* NMR spectrometer, in (D_6)DMSO or $CDCl_3$; δ in ppm, J in Hz. EI-MS: *DSQ* EI-mass spectrometer; in m/z. HR-EI-MS: *MAT95XP* high-resolution mass spectrometer; in m/z.

X-Ray Crystallography. The X-ray diffraction data for compound **2** were obtained with an *Xcalibur Nova 1000 CCD* diffractometer (MoK_α radiation, graphite monochromator; *cf. Table 3* and *Fig.*).

Fungal Strain. Phoma sp. SK3RW1 M was isolated from the roots of *Avicennia marina* (FORSK.) VIERH., collected in Shankou mangrove, Guangxi, P. R. China. Axenic cultures of this strain was deposited with the Department of Applied Chemistry, Sun Yat-sen University, Guangzhou, P. R. China, and maintained on cornmeal seawater agar with the access code SK3RW1M.

Fermentation, Extraction, and Isolation. Plugs of agar, supporting mycelial growth, were cut and transferred aseptically to a 250-ml *Erlenmeyer* flask containing 100 ml of liquid GYP medium (glucose (10 g l^{-1}), peptone (2 g l^{-1}), yeast extract (1 g l^{-1}), and NaCl (3 g l^{-1})). The flask was incubated at 28° on a rotary shaker for 5–7 d, and then the mycelium was aseptically transferred to 500-ml *Erlenmeyer* flasks containing 250 ml of liquid medium. The flasks were incubated at 28° in standing culture for 30 d. The cultures (150 l) were filtered through cheesecloth. The filtrate was concentrated to 5 l below 50° under reduced pressure, and the residue was extracted five times by shaking with equal volumes of AcOEt. The combined soln. was evaporated to dryness under vacuum, and the extract was chromatographed on SiO_2 using a gradient elution from petroleum ether (PE) to AcOEt. Compound **1** (12 mg), **2** (33 mg), and **3** (8 mg), were obtained from the 20% AcOEt/PE fraction.

*1,8-Dihydroxy-10-methoxy-3-methyldibenzo[*b,e*]oxepine-6,11-dione* (**1**). Pale-yellow, amorphous powder. M.p. 268–269°. UV (MeOH): 235, 251, 303, 350. IR (KBr): 3226, 1738, 1651, 1608, 1456, 1424, 1392, 1271, 1218. NMR: see *Table 1*. EI-MS: 300 (M^+), 282, 256, 227. HREI-MS: 300.0630 (M^+, $C_{16}H_{12}O_6^+$; calc. 300.0634).

*1-Hydroxy-8-(hydroxymethyl)-6-methoxy-3-methyl-9*H*-xanthen-9-one* (**2**). Yellow-brown, amorphous powder. M.p. 214–215°. UV (MeOH): 201, 205, 235, 250, 266, 303, 351. IR (KBr): 3382, 1659, 1604, 1562, 1274, 1215. NMR: see *Table 2*. EI-MS: 286 (M^+), 268, 257, 240. HREI-MS: 286.0835 (M^+, $C_{16}H_{14}O_5^+$; calc. 286.0841).

*1-Hydroxy-8-(hydroxymethyl)-3-methoxy-6-methyl-9*H*-xanthen-9-one* (**3**). Yellow-brown, amorphous powder. M.p. 219–220°. UV (MeOH): 205, 235, 250, 266, 303, 350. IR (KBr): 3404, 1660, 1609, 1565, 1278, 1213. NMR: see *Table 2*. EI-MS: 286 (M^+), 268, 257, 240. HREI-MS: 286.0836 (M^+, $C_{16}H_{14}O_5^+$; calc. 286.0841).

Cytotoxicity Assay. KB and KBv200 cells were harvested and seeded in 96-well plates at 3.0×10^3 cells/well in a final volume of 190 µl. After 24 h of incubation, 10 µl of cytotoxic agent or compound vehicles were added to each well. After 68 h, 10 µl of MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) soln. was added to each well. After 4 h, DMSO (100 µl) was added. The concentrations required to inhibit cell growth by 50% (IC_{50}) were calculated from the cytotoxicity curves using *Bliss*'s software.

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