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THREE XANTHONES FROM A MARINE-DERIVED MANGROVE ENDOPHYTIC FUNGUS

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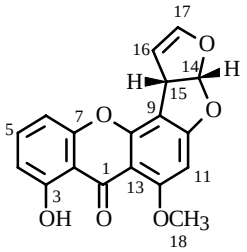
*Three xanthones were produced from the marine-derived endophytic fungus isolate 1850 from a leaf of Kandelia candel from an estuarine mangrove in Hong Kong. Their structures were elucidated as sterigmatocystin (**1**), dihydrosterigmatocystin (**2**), and secosterigmatocystin (**3**) by analysis of spectroscopic data.*

Key words: xanthone, marine, fungus, mangrove.

Marine fungus, especially mangrove endophytic fungus, has proved to be an abundant resource for novel natural compounds [1–13]. During our screening for novel structures from marine-derived mangrove endophytic fungus from the South China Sea, a fungal strain (isolate 1850) was found to produce three xanthones (**1–3**). We report herein their isolation, structural elucidation, and biological activities.

Cultures of endophytic fungus isolate 1850 were filtered through cheesecloth; the mycelia were dried by air and extracted with methanol, and the concentrate was repeatedly chromatographed on silica gel using gradient elution from petroleum to ethyl acetate to afford compounds **1** and **2** as pale-yellow needles. The filtrate was concentrated below 50°C and extracted with ethyl acetate. The ethyl acetate extract was also repeatedly chromatographed on silica gel using gradient elution from petroleum to ethyl acetate to afford compound **3** as pale-yellow needles.

Compound **1** has the molecular formula $C_{18}H_{12}O_6$ established by FABMS from the peak at *m/z* 325 $[M+H]^+$ and elemental analysis, and suggests the presence of 13 unsaturated degrees. The spectral properties of compound **1** indicate that it might be a derivative of 1-hydroxyxanthone. The UV absorption agrees with those recorded for many hydroxylated and/or methoxylated xanthones [14]. The IR spectra show a strong band at 1649 cm^{-1}, which is assigned to the stretching vibration of a xanthone hydrogen-bonded carbonyl group, and the hydrogen bond is further confirmed by the downfield signal of hydroxyl at δ_H 13.28. Thus the ten unsaturated degrees was explained by the structure of 1-hydroxyxanthone. The COSY spectrum revealed a contiguous sequence from H-4 to H-6. In the HMBC spectrum, the correlation between C-3 and the proton of -OH, and the correlation between C-12 and H-18 respectively, were observed.



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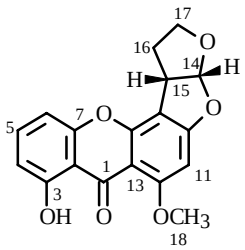

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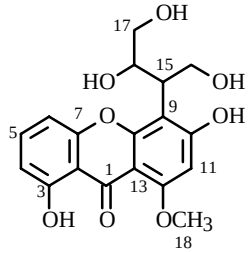

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TABLE 1. NMR Data of Compound **1** (DMSO-d_6, ppm, J/Hz, TMS)

Atom	δ_C (DEPT)	δ_H	HMBC	COSY
1	180.4 (C)			
2	108.2 (C)		H-4,5,6,OH	
3	161.3 (C)		H-4,5,OH	
4	110.7 (CH)	6.74 m	H-5,6,OH	H-5
5	136.2 (CH)	7.62 (dd, J = 8.5, 1.0)		H-4,6
6	106.4 (CH)	6.99 (dd, J = 8.5, 1.0)	H-4,5	H-5
7	154.5 (C)		H-5,6	
8	153.3 (C)			
9	106.5 (C)		H-11,14,15	
10	164.4 (C)		H-11,14	
11	91.0 (CH)	6.71 s		
12	162.9 (C)		H-11,18	
13	105.0 (C)		H-11	
14	113.3 (CH)	6.97 (d, J = 7.0)	H-15,16,17	H-15
15	47.2 (CH)	4.86 (tt, J = 7.0, 2.0)	H-14,16,17	H-14,16
16	102.6 (CH)	5.53 (dd, J = 3.0, 2.0)	H-14,15,17	H-15,17
17	145.5 (CH)	6.74 m	H-14,15,16	H-16
18	56.8 (CH_3)	3.90 s		
OH		13.28 s		

The chemical shifts indicate the presence of only one olefinic bond (representing one unsaturated degree), thus leaving another two unsaturated degrees. This can only be explained as the presence of two rings. The COSY spectrum revealed another contiguous sequence from H-14 to H-17. In the HMBC spectrum, the correlations between C-9 and H-11, 14, 15, and the correlations between C-10 and H-11, 14, respectively, established the structure of the bisfuranic ring.

The large proton coupled constant (J = 7.0 Hz) between H-14 and H-15 indicates the *cis*- conformation in the bisfuranic ring. Compound **1** and sterigmatocystin [15] are levorotatory, suggesting that they have the same absolute configurations at C-14 and C-15, that is, 14*R*, 15*S*, and further proving that the structure of compound **1** is in agreement with that of sterigmatocystin [15].

The FABMS of compound **2** showed a molecular ion peak at *m/z* 327 $[M + H]^+$, wich was 2 more than that of compound **1**. This number is equal to adding two hydrogen atoms to compound **1**. The ^1H and ^{13}C NMR spectra of compound **2** are more similar to those of compound **1**. The differences between them were that there were less signals of the olefinic bond between C-16 and C-17 but more signals of the two saturated CH_2 in compound **2** than in compound **1**. It is very clear that compound **2** is a derivative of compound **1**, in which the olefinic bond was hydrogenated. Thus, compound **2** was elucidated as dihydrosterigmatocystin [15, 16]. Compound **2** seems to be derived from the enzymatic hydrogenation of sterigmatocystin.

The third xanthone, compound **3**, was obtained from the fermentation liquid. The FABMS showed a molecular ion peak at *m/z* 363 $[M+H]^+$ that was 36 more than that of compound **2**. This number is equal to adding two water molecules to compound **2**. The spectral properties and the co-occurrence of compound **3** with compounds **1** and **2** indicate their structural relationship. Its ^1H and ^{13}C NMR spectra support the presence of the xanthone part of the molecule. The fact that there are four hydroxyl signals of one phenol at δ_H 11.65 (br.s) and three alcohols at δ_H 3.35 (br.s, 2OH) and 4.60 (br.s, OH) in compound **3** more than in compounds **1** and **2**, suggested that compound **3** is the hydrolysis product of compounds **1** or **2**. Therefore, compound **3** was elucidated as 3,8-dihydroxy-4-(2,3-dihydroxy-1-hydroxymethylpropyl)-1-methoxyxanthone [17], and designated as secosterigmatocystin. Compound **3** seems to be derived from the enzymatic degradation of sterigmatocystin or dihydrosterigmatocystin.

In the primary bioassay, compound **1** showed weak cytotoxic activity against tumor cell lines Bel-7402 and NCIH-460 with IC_{50} values of 96.53 μg/mL and 72.52 μg/mL, respectively. All three xanthones did not exhibit significant inhibitory activity against human DNA topoisomerase type (hTOP) with IC_{50} values greater than 100 μg/mL.

TABLE 2. NMR Data of Compounds **2** and **3** (DMSO-d_6, ppm, J/Hz, TMS)

Atom	Compound **2**		Compound **3**	
	δ_C (DEPT)	δ_H	δ_C (DEPT)	δ_H
1	180 (C)		180.8 (C)	
2	108.1 (C)		109.8 (C)	
3	161.3 (C)		163.5 (C)	
4	110.5 (CH)	6.73 (dd, J = 8.5, 1.0)	109.8 (CH)	6.68 (d, J = 8.5)
5	136.0 (CH)	7.61 (t, J = 8.5)	135.7 (CH)	7.58 (t, J = 8.5)
6	106.1 (CH)	6.94 (dd, J = 8.5, 1.0)	106.2 (CH)	6.91 (d, J = 8.5)
7	154.4 (C)		157.3 (C)	
8	153.8 (C)		154.5 (C)	
9	105.4 (C)		107.8 (C)	
10	165.8 (C)		161.1 (C)	
11	90.2 (CH)	6.60 s	96.8 (CH)	6.41 s
12	162.9 (C)		160.0 (C)	
13	104.8 (C)		103.9 (C)	
14	113.4 (CH)	6.55 (d, J = 5.5)	60.3 (CH_2)	3.86 m
15	43.3 (CH)	4.25 m	39.8 (CH)	4.21 m
16	30.7 (CH_2)	2.45 m; 2.24 m	70.8 (CH)	3.61 m
17	67.2 (CH_2)	4.10 m; 3.54 m	64.2 (CH_2)	3.29 m
18	56.5 (CH_3)	3.89 s	55.8 (CH_3)	3.85 s
OH		13.38 s		13.39 s
OH				11.65 br.s
OH				4.60 br.s
2OH				3.35 br.s

EXPERIMENTAL

M.p.: uncorrected. IR: Bruker Vector 22 FT-IR spectrophotometer, KBr pellets, in cm^{-1}. ^{1}H-, ^{13}C-, DEPT, ^{1}H, ^{1}H-COSY, HMQC, HMBC NMR: Varian Inova 500 NB spectrometer, δ in ppm, J/Hz, TMS. UV: Shimadzu UV-2501 PC spectrophotometer. FABMS: VG-ZAB-HS mass spectrometer. Elemental analysis: Elementar Vario EL CHNS-O elemental analyzer.

Fungus Material and Purification. A fungal strain (isolate 1850) was isolated from a leaf of *Kandelia candel* from an estuarine mangrove in Hong Kong which species was unidentified. Starter cultures were maintained on cornmeal seawater agar. Plots of agar supporting mycelial growth were cut and transferred aseptically to a 250 mL Erlenmeyer flask containing 200 mL of liquid medium (glucose 10 g/L, peptone 2 g/L, yeast extract 1 g/L, NaCl 3.5 g/L, pH 7.0). The flask was incubated at 30°C on a rotatory shaker for 5–7 days. The mycelium was aseptically transferred to 500 mL Erlenmeyer flasks containing 200 mL liquid medium. The flasks were incubated at 30°C for 35–40 days.

The 120 L cultures were filtered through cheesecloth. The mycelia were dried by air and extracted five times with 5 L methanol, and the concentrate was repeatedly chromatographed on silica gel using gradient elution from petroleum to ethyl acetate to obtain a mixture of compounds **A** and **B** from the petroleum-ethyl acetate (7:3) fraction. The mixture was purified by preparative TLC to give compounds **1** (60 mg) and **2** (8 mg). The culture filtrate was concentrated below 50°C and extracted five times by shaking with an equal volume of ethyl acetate. The ethyl acetate extract was also repeatedly chromatographed on silica gel using gradient elution from petroleum to ethyl acetate to obtain compound **3** (13 mg) from the ethyl acetate fraction.

Sterigmatocystin (1): pale-yellow needles, $C_{18}H_{12}O_6$, FABMS (*m/z*) 325 $[M+H]^+$, mp 249–251°C. $[\alpha]_D^{25}$ –319° (*c* 0.15, acetone). IR spectrum (KBr, ν, cm^{-1}): 3428, 3103, 2923, 2852, 1649, 1629, 1590, 1484, 1460. UV (acetone, λ_{max}, nm): 329, 316. ^{1}H, ^{13}C NMR see Table 1.

Dihydrosterigmatocystin (2): pale-yellow needles, $C_{18}H_{14}O_6$, FABMS (*m/z*): 327 $[M+H]^+$, mp >300°C. $[\alpha]_D^{25}$ –146.5° (*c* 0.198, $CHCl_3$). IR spectrum (KBr, ν, cm^{-1}): 3431, 3333, 3131, 1641, 1614, 1453, 1401, 1087. UV ($CHCl_3$, λ_{max}, nm): 324, 249. ^1H, ^{13}C NMR see Table 2.

Secosterigmatocystin (3): pale-yellow needles, $C_{18}H_{18}O_8$, FABMS (*m/z*): 363 $[M+H]^+$, mp 236–240°C. $[\alpha]_D^{25}$ +6.26° (*c* 0.335, CH_3CH_2OH). IR (KBr, ν, cm^{-1}): 3418 (br), 2924, 1646, 1608, 1510, 1480, 1412, 1301, 1271, 1238, 1207, 1142, 1098, 1063, 1004, 921, 875, 817. UV (CH_3CH_2OH, λ_{max}, nm): 331, 247, 233, 207. ^1H, ^{13}C NMR see Table 2.

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