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A NEW XANTHONE DERIVATIVE FROM THE CO-CULTURE BROTH OF TWO MARINE FUNGI (STRAIN No. E33 AND K38)

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*A new xanthone derivative, 8-hydroxy-3-methyl-9-oxo-9*H*-xanthene-1-carboxylic acid methyl ether (**1**), was isolated from the co-culture broth of two mangrove fungi (strain No. K38 and E33) isolated from the South China Sea coast. The structure of **1** was determined by comprehensive spectroscopic methods, especially 2D NMR techniques. Primary bioassays showed that compound **1** has inhibitory activity against five microorganisms, including* Gloeasporium musae *and* Peronophthora cichoralearum *etc.*

Keywords: marine mangrove fungus, xanthone, antifungal activity.

Marine-derived fungi have become more and more important as a rich source of structurally new natural products with a wide range of biological activities [1, 2]. As part of our studies on the metabolites of mangrove fungi from the South China Sea coast, we examined the chemical constituents of two mangrove fungi (strain No. K38 and E33) [3–5]. In this report, we describe the isolation and characterization of 8-hydroxy-3-methyl-9-oxo-9*H*-xanthene-1-carboxylic acid methyl ether (**1**), a new xanthone derivative that showed activity against several fungi. Among these fungi, *Gloeasporium musae* and *Peronophthora cichoralearum* were more sensitive to **1**. The antibiotic was obtained from the mixed fermentation broth of the two mangrove fungi (strain No. K38 and E33). It was not detected when either strain was cultured individually. Mixed fermentation has been used in the food industry and to enhance enzyme production [6, 7]. M. Cueto and his colleagues have begun to explore the use of the method for the discovery of antibiotics that show efficacy against drug-resistant pathogens [8]. The isolation of 8-hydroxy-3-methyl-9-oxo-9*H*-xanthene-1-carboxylic acid methyl ether (**1**) demonstrates that mixed fermentation can lead to new antibiotics and suggests that this method may have utility for drug research.

A 200 L fermentation broth was concentrated and extracted with ethyl acetate. The extract was repeatedly chromatographed on silica gel to obtain compound **1** from the 15% ethyl acetate/petroleum fraction as yellow needles. Compound **1** has the molecular formula $C_{16}H_{12}O_5$ determined by FAB-MS (*m/z* 285 $[M + H]^+$) and elemental analysis, which led to the conclusion that compound **1** had 11 degrees of unsaturation. The ^{13}C NMR spectrum of **1** (Table 1) revealed 16 carbon signals. Furthermore, the DEPT spectrum confirmed that this compound had two methyl, five methine, and nine quaternary carbon atoms. It was also shown that 8 of the 11 elements of unsaturation in **1** were due to the 12 aromatic carbons and two carbonyl groups (δ 180.8, 169.7). The other three degrees of unsaturation indicated that the molecule was a tricyclic compound. In the ^1H NMR spectrum, a hydrogen-bonded hydroxyl group signal appeared at δ 12.31 (1H, s). The signals at δ 7.59 (1H, dd, J = 7.8, 8.4 Hz), 6.91 (1H, dd, J = 8.4, 1.2 Hz), and 6.80 (1H, dd, J = 7.8, 1.2 Hz) showed the presence of a 1,2,3-trisubstituted benzene ring. The compound also had two meta-coupled aromatic protons at δ 7.35 (1H, d, J = 1.2 Hz) and 7.15 (1H, d, J = 1.2 Hz), a methyl group at δ 2.52 (3H, s) and a methoxyl group at δ 4.03 (3H, s). This NMR data suggested that compound **1** was a xanthone derivative with one hydroxyl, one carboxylic acid methyl ester, and one methyl group. HMQC spectral data enabled all the proton signals to be assigned to their directly attached carbons. On the basis of its downfield chemical shift, the phenolic proton signal at δ 12.31 (8-OH) should be hydrogen-bonded with the carbonyl oxygen. In the HMBC spectrum, the correlations from H-12 to C-11 showed the presence of a $COOCH_3$ group.

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TABLE 1. ^{1}H and ^{13}C NMR Data for **1** ($CDCl_3$, TMS, δ, J/Hz)

C atom	δ_H	δ_C (DEPT)	^{1}H–^{1}H COSY	HMBC
1		133.3 (C)		
2	7.15 (d, J = 1.2)	124.2 (CH)	H-4	C-4, 9a, 10, 11
3		147.0 (C)		
4	7.35 (d, J = 1.2)	119.2 (CH)	H-2	C-2, 4a, 9a, 10
5	6.91 (dd, J = 1.2, 8.4)	106.9 (CH)	H-6, 7	C-4b, 7, 8a
6	7.59 (dd, J = 7.8, 8.4)	136.9 (CH)	H-5, 7	C-4b, 8
7	6.80 (dd, J = 1.2, 7.8)	110.8 (CH)	H-5, 6	C-5, 8a
8		161.7 (C)		
9		180.8 (C)		
9a		115.3 (C)		
4a		156.2 (C)		
4b		155.8 (C)		
8a		108.9 (C)		
10	2.52 (3H, s)	22.0 (CH_3)		C-2, 3, 4
11		169.7 (C)		
12	4.03 (3H, s)	53.2 (CH_3)		C-11
8-OH	12.31 (1H, br.s)			C-7, 8, 8a

TABLE 2. The Antimicrobial Activity Study of **1** against Five Fungal Strains

Fungi	CK, cm	Treatments, cm	Rate of inhibition, %
Gloeasporium musae	6	2.8	53
Blumeria graminearum	6.5	6.2	4.6
Fusarium oxysporum	4.2	3.8	9.5
Peronophthora cichoralearum	3.1	1.6	48
Colletotrichum glocosporioides	5.0	3.4	28

Rate of inhibition% = [(CK average diameter of radial growth-Treatments average diameter of radial growth)]/CK average diameter of radial growth × 100.

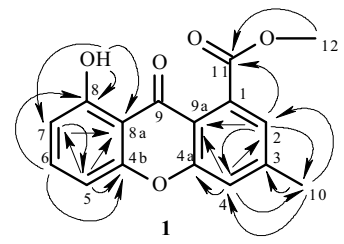

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Fig. 1. The correlations of the HMBC of **1**.

The positions of CH_3 (C-10) and $COOCH_3$ (C-11, C-12) groups were determined by the values of chemical shifts and HMBC correlations from H-10 to C-2,3,4; H-2 to C-4,9a,10,11; H-4 to C-2,4a,9a,10, and ^{1}H–^{1}H COSY correlations between H-2 and H-4. The HMBC correlations from OH to C-7, C-8, and C-8a indicated that OH is attached to C-8. On the basis of the above-mentioned results, the structure of compound **1** was determined to be 8-hydroxy-3-methyl-9-oxo-9*H*-xanthene-1-carboxylic acid methyl ether (Fig. 1).

In our present study, five kinds of fungi were examined. It was found that compound **1** has broad inhibitory activity against these microorganisms. Especially to two fungi, *Gloeasporium musae* and *Peronophthora cichoralearum*, compound **1** showed more sensitivity (Table 2).

EXPERIMENTAL

Melting points were detected on a Fisher-Johns hot-stage apparatus and are uncorrected. NMR data were recorded on a Varian Inova-500 NB spectrometer, using $CDCl_3$ as a solvent and TMS as an internal standard. Mass spectra were acquired on a VG-ZAB mass spectrometer. IR spectra were obtained on a Nicolet 5DX-FTIR spectrophotometer. Chromatography was carried out on a silica gel column (200–300 mesh; Qingdao Haiyang Chemicals).

Fungus Material and Culture Conditions. The fungi E33 and K38 were isolated from the South China Sea coast. They are both apospory and their general species have not been identified. Starter cultures were maintained on cornmeal seawater agar. Plugs of agar supporting mycelial growth were cut and transferred aseptically to a 250 mL Erlenmeyer flask containing 100 mL of liquid medium (glucose 10g/L, peptone 2 g/L, yeast extract 1 g/L, NaCl 30 g/L). The flask was incubated at 30°C on a rotary shaker for 5–7 days. The mycelium was aseptically transferred to 500 mL Erlenmeyer flasks containing culture liquid (200 mL). The flasks were then incubated at 30°C for 25 days.

Extraction and Separation of Metabolites. The cultures (200 L) were filtered through cheesecloth. The filtrate was concentrated to 5 L in vacuo below 50°C and extracted five times by shaking with an equal volume of ethyl acetate. The combined extracts were chromatographed repeatedly on a silica gel column using gradient elution from petroleum to ethyl acetate to obtain 8-hydroxy-3-methyl-9-oxo-9*H*-xanthene-1-carboxylic acid methyl ether (**1**) from the ethyl acetate–petroleum ether (15:85) fraction.

8-Hydroxy-3-methyl-9-oxo-9*H*-xanthene-1-carboxylic Acid Methyl Ether (**1**). $C_{16}H_{12}O_5$, yellow needles, FAB-MS *m/z*: 285, 283. IR (bands, neat, cm^{-1}): 3438, 2923, 2853, 1705, 1634, 1610, 1272, 1238, 822. ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 1.

Fungicidal Acivity of 1 *in vivo*. Antifungal activities of **1** were investigated by the disk assay method [9], using five representative fungi. These fungi were grown in potato dextrose agar (PDA) media at 28°C. The PDA plates were prepared with compound **1** (at a concentration of 100 μg/mL). Then, disks (6.0 mm in diameter) of epiphyte were placed on the plates. Similarly, disks of epiphyte were also placed on the innocuous plates (adding the same dosage sterile water) as blank comparison. All plates were incubated at 28°C for 1–3 days each, and the tests were repeated five times. Rate of inhibition % = [(CK average diameter of radial growth–Treatments average diameter of radial growth)]/CK average diameter of radial growth × 100. The results are shown in Table 2.

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