Chemistry of Natural Compounds, Vol. 46, No. 3, 2010

A NEW XANTHONE DERIVATIVE FROM MANGROVE ENDOPHYTIC FUNGUS No. ZSU-H16

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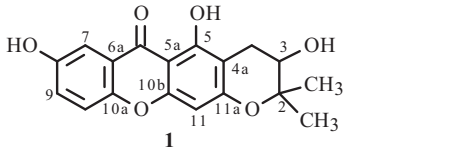
UDC 547.815

*A new xanthone derivative, 3,5,8-trihydroxy-2,2-dimethyl-3,4,4-trihydro-2*H,*6*H*-pyrano[3,2-*b*]-xanthen-6-one (**1**), together with three known compounds, 5,8-dihydroxy-2,2-dimethyl-2*H,*6*H*-pyrano[3,2-*b*]xanthen-6-one (**2**), cyclo-(*N-O-*methyl-L-Trp-L-Ile-D-Pip-L-2-amino-8-oxo-decanoyl) (**3**), and cyclo-(Phe-Tyr) (**4**), was isolated from the mangrove endophytic fungus No. ZSU-H16 obtained from the South China Sea coast. Their structures were elucidated by spectroscopic methods, mainly 1D and 2D NMR spectroscopic techniques. Compound **1** exhibited cytotoxicity against KB and KB_V 200 cells with IC_{50} values greater than 50 μg/mL, respectively.*

Keywords: mangrove endophytic fungus, xanthone derivative, metabolites.

In our continuing studies on fungal metabolites, we have isolated many significant new bioactive compounds from marine mangrove endophytic fungi [1–8]. Xanthone derivatives are widespread in nature, commonly occurring in a number of higher plant families and fungi [9, 10]. Now we report a new xanthone derivative, 3,5,8-trihydroxy-2,2-dimethyl-3,4,4-trihydro-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one (**1**), together with three known compounds, 5,8-dihydroxy-2,2-dimethyl-2*H*,6*H*-pyrano[3,2-*b*] xanthen-6-one (**2**), cyclo-(*N-O*-methyl-L-Trp-L-Ile-D-Pip-L-2-amino-8-oxo-decanoyl) (**3**), and cyclo-(Phe-Tyr) (**4**), from the mangrove endophytic fungus No. ZSU-H16 from the South China Sea coast.

Compound **1** was obtained as a yellow amorphous solid, mp 272–274°C. Its molecular formula was determined as $C_{18}H_{16}O_6$, with eleven degrees of unsaturation, on the basis of HR-EI-MS (*m/z* 328.0935 $[M]^+$, calcd for $C_{18}H_{16}O_6$, 328.0941) and NMR spectral data (Table 1).



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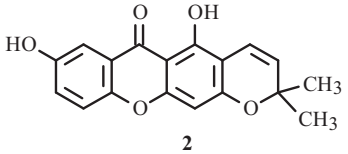

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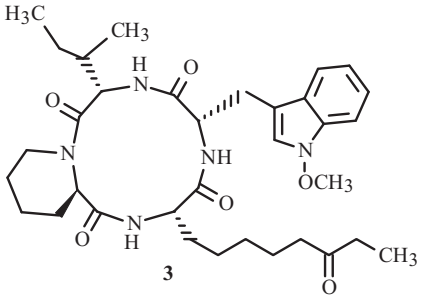

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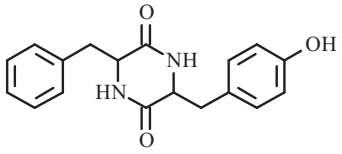

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 0009-3130/10/4603-0348 ©2010 Springer Science+Business Media, Inc.

TABLE 1. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), and HMBC Data for **1** (DMSO-d_6, δ, ppm, J/Hz)

C atom	δ_C (DEPT)	δ_H	HMBC
2	83.1 (C)		H-3, 4, 2-CH_3, 3-OH
3	71.2 (CH)	4.13 (m)	H-4, 2-CH_3, 3-OH
4	25.6 (CH_2)	3.18 (1H, dd, J = 14.2, 10.2)	H-3, 3-OH
		2.94 (1H, dd, J = 14.2, 6.5)	H-3, 3-OH
4a	105.2 (C)		H-3, 4, 11, 5-OH
5	161.1 (C)		H-4, 5-OH
5a	104.7 (C)		H-11, 5-OH
6	181.9 (C)		H-7
6a	126.0 (C)		H-7, 10
7	109.2 (CH)	7.43 (d, J = 2.8)	H-9, 8-OH
8	155.1 (C)		H-7, 9, 10, 8-OH
9	121.3 (CH)	7.31 (dd, J = 9.0, 2.8)	H-7, 10, 8-OH
10	115.1 (CH)	7.52 (d, J = 9.0)	H-9
10a	150.2 (C)		H-7, 9, 10
10b	158.1 (C)		H-11
11	96.3 (CH)	6.50 (s)	
11a	158.2 (C)		H-4
2-CH_3	21.8 (CH_3)	1.47 (6H, s)	H-3
3-OH		4.35 (m)	
5-OH		12.96 (s)	
8-OH		10.09 (s)	

The ^{13}C NMR and DEPT spectra showed 18 carbon signals including two methyls, one sp^3 methylene, one sp^3 methine, one quaternary sp^3 carbon, four sp^2 methines, and nine quaternary sp^2 carbons (one of which is carbonyl). The eleven degrees of unsaturation consisted of six double bonds, one carbonyl group, and four cyclic systems. The UV absorption bands at 236, 258, 294, and 378 nm and IR (KBr) absorption bands at 3225, 1658, 1605, 1574, and 1488 cm^{-1} suggested the presence of the xanthone skeleton. The signal at δ 12.96 in the ^{1}H NMR spectrum of **1** (DMSO-d_6) indicated that a hydroxyl was chelated to a carbonyl group. The ^{1}H NMR spectrum also exhibited one aromatic proton at δ 6.50 (s) and three coupled aromatic protons at 7.31 (dd, J = 9.0, 2.8 Hz), 7.52 (d, J = 9.0 Hz), and 7.43 (d, J = 2.8 Hz), indicating that one aromatic ring was monosubstituted and the other was trisubstituted.

The two phenolic hydroxyl groups (δ_H 12.96; 10.09) were located, respectively, at C-5 (δ_C 161.1) and C-8 (δ_C 155.1), as determined by the HMBC correlations of the former proton with C-4a, C-5, and C-5a, and of the latter proton with C-7, C-8, and C-9. The HMBC correlations from H-4 to C-2, C-3, C-4a, C-5, and C-11a showed that the methylene (C-4) was located between the carbon (C-3) and the aromatic ring (C-4a). Two singlet methyls (δ_H 1.47, δ_C 21.8) were attached to C-2, as shown by the HMBC correlations with C-2 and C-3. The hydroxyl group (δ_H 4.13) was located at C-3 (δ_C 71.2) as shown by the HMBC correlations from OH-3 to C-2, C-3, and C-4. Finally, the structure of compound **1** was determined to be 3,5,8-trihydroxy-2,2-dimethyl-3,4,4-trihydro-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one.

Furthermore, three known compounds were identified as 5,8-dihydroxy-2,2-dimethyl-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one (**2**), cyclo-(*N*-*O*-methyl-L-Trp-L-Ile-D-Pip-L-2-amino-8-oxo-decanoyl) (**3**), and cyclo-(Phe-Tyr) (**4**) by comparison of their spectroscopic data with the literature [11–13], respectively.

Primary bioassays showed that **1** exhibited weak cytotoxicity against KB and KB_V 200 cells (IC_{50} > 50 μg/mL).

EXPERIMENTAL

General Methods. NMR data were recorded on Varian Inova-500 NB and Varian Mercury-Plus 300 NB spectrometers, with TMS as internal standard. EI-MS spectrum was acquired on a VG-ZABHS mass spectrometer, and HR-EI-MS spectrum was obtained on a VG Autospec-500 mass spectrometer. IR spectra were obtained on a Nicolet 5DX-FTIR spectrophotometer, and UV spectra were measured on a Shimadzu UV-240 spectrophotometer. The melting point was determined on an X-4 micro-melting point apparatus and was uncorrected. Column chromatography was carried out on silica gel (200–300 mesh; Qingdao Haiyang chemicals).

Fungus Material and Culture Conditions. The fungus ZSU-H16 is an endophytic fungus isolated from the leaves of the mangrove tree *avicennia* from the South China Sea coast. It is apospory and its general species have not been identified. Starter cultures were maintained on cornmeal seawater agar. Plugs of agar supporting mycelia growth were cut and transferred aseptically to a 250 mL Erlenmeyer flask containing 100 mL of liquid medium (glucose 10 g/L, peptone 2 g/L, yeast extract 1 g/L, NaCl 2.5 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) and incubated at room temperature for 30 days.

Extraction and Separation of Metabolites. The cultures (120 L) were filtered through cheesecloth. The filtrate was concentrated to 3.5 L below 60°C and extracted five times by shaking with an equal volume of ethyl acetate. Removing ethyl acetate under reduced pressure by a rotary evaporator yielded the extracts (a sticky brown liquid, 60 g). The extracts was separated by column chromatography on silica gel and eluted with petroleum ether–ethyl acetate (9:1, 7:3, 5:5, and 3:7) to give fractions A, B, C, and D. Then, fraction B was repeatedly purified by column chromatography on silica gel using petroleum ether–ethyl acetate (9:1, 8:2, 7:3, and 6:4), giving fractions B_1, B_2, B_3, and B_4. Fraction B_3 was subjected to column chromatography on silica gel eluting with petroleum ether–ethyl acetate (8:2) to give compound **1** as a yellow amorphous solid (6 mg). Fraction B_4 was purified by column chromatography on silica gel with petroleum ether–ethyl acetate (7:3) to afford compound **2** as yellow needles (8 mg). Fraction C was purified by column chromatography on silica gel with petroleum ether–ethyl acetate (6:4), giving compound **3** as colorless needles (20 mg). Fraction D was purified by column chromatography on silica gel with petroleum ether–ethyl acetate (5:5) to give compound **4** as a colorless solid (16 mg).

3,5,8-Trihydroxy-2,2-dimethyl-3,4,4-trihydro-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one (1). Yellow amorphous solid, mp 272–274°C; $[\alpha]_D^{20}$ –95.6° (*c* 0.20, CH_3OH). UV spectrum (CH_3OH, λ_{max}, nm) (log ε): 236 (4.75), 258 (4.56), 294 (4.42), 378 (4.20). IR spectrum (KBr, ν, cm^{-1}): 3225 (OH), 2935, 2876, 1658 (C=O), 1605, 1574, 1488 (Ph), 1266, 1220, 1165. Mass spectrum (EI^+, *m*/*z*, I_{rel}, %): 328 (12) $[M]^+$, 310 [M – H_2O] (15), 300 [M – CO] (20), 286 (15), 240 (38), 226 (100), 204 (25),126 (10). Mass spectrum (HR-EI^+, *m*/*z*, I_{rel}, %): 328.0935 (26.9) $[M]^+$, (calcd for $C_{18}H_{16}O_6$, 328.0941). ^1H, ^{13}C NMR, see Table 1.

5,8-Dihydroxy-2,2-dimethyl-2*H*,6*H*-pyrano[3,2-*b*] xanthen-6-one (2). Yellow needles, mp 266–268°C. ^1H NMR (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 13.25 (1H, s), 10.06 (1H, s), 7.46 (1H, d, J = 9.1), 7.41 (1H, d, J = 2.7), 7.28 (1H, dd, J = 9.0, 2.8), 6.59 (1H, d, J = 9.9), 6.39 (1H, s), 5.76 (1H, d, J = 10.0), 1.44 (6H, s). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 180.4 (C), 160.6 (C), 157.3 (C), 157.1 (C), 154.3 (C), 149.5 (C), 128.7 (CH), 125.3 (C), 120.5 (CH), 119.6 (CH), 114.8 (CH), 108.3 (CH), 104.1 (C), 103.2 (C), 94.8 (CH), 78.6 (C), 28.4 (2 × CH_3).

Cyclo-(*N*-*O*-Methyl-L-Trp-L-Ile-D-Pip-L-2-amino-8-oxo-decanoyl) (3). Colorless needles, mp 195–197°C. ^1H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.71 (1H, d, J = 7.5), 7.54 (1H, d, J = 7.5), 7.38 (1H, dt, J = 1.0, 7.5), 7.36 (1H, m), 7.22 (1H, m), 7.19 (1H, dt, J = 0.6, 7.5), 6.97 (1H, d, J = 6.0), 6.66 (1H, d, J = 10.2), 5.22 (1H, br.d, J = 4.8), 4.85 (1H, t, J = 10.2), 4.39 (1H, br.q, J = 8.1), 4.19 (3H, s), 4.02 (1H, s), 4.00 (1H, m), 3.64 (1H, dd, J = 15, 10), 3.44 (1H, dd, J = 15, 7.5), 3.14 (1H, m), 2.39 (2H, m), 2.35 (2H, m), 2.12 (2H, m), 2.00 (3H, m), 1.77 (2H, m), 1.68 (2H, m), 1.60 (2H, m), 1.49 (2H, m), 1.23 (2H, m), 1.21 (2H, m), 1.19 (3H, t, J = 7.5), 1.06 (3H, t, J = 7.5), 0.98 (3H, d, J = 6.6). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 211.4 (C), 175.2 (C), 174.1 (C), 174.0 (C), 171.3 (C), 132.1 (C), 123.4 (C), 122.4 (CH), 121.8 (CH), 119.6 (CH), 118.6 (CH), 108.2 (CH), 106.8 (C), 65.6 (CH_3), 61.0 (CH), 54.1 (CH), 53.8 (CH), 50.7 (CH), 43.9 (CH_2), 42.0 (CH_2), 35.8 (CH_2), 34.7 (CH), 29.2 (CH_2), 28.7 (CH_2), 25.4 (2 × CH_2), 25.3 (CH_2), 24.8 (CH_2), 24.2 (CH_2), 23.5 (CH_2), 19.4 (CH_2), 15.7 (CH_3), 10.8 (CH_3), 7.9 (CH_3).

Cyclo-(Phe-Tyr) (4). Colorless solid, mp 291–293°C. ^1H NMR (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 9.04 (1H, s), 7.65 (2H, br.s), 7.26 (2H, t, J = 7.5), 7.20 (1H, t, J = 7.5), 7.05 (2H, d, J = 7.0), 6.85 (2H, d, J = 8.5), 6.66 (2H, d, J = 8.5), 3.95 (1H, m), 3.89 (1H, m), 2.63 (1H, dd, J = 14.0, 7.0), 2.24 (1H, dd, J = 14.0, 7.0). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 165.9 (C), 165.8 (C), 155.7 (C), 136.5 (C), 130.62 (2 × CH), 129.6 (2 × CH), 128.1 (2 × CH), 126.4 (C), 126.4 (CH), 114.8 (2 × CH), 55.6 (CH), 55.4 (CH), 39.2 (CH_2), 38.86 (CH_2).

Bioasays. The cytotoxic assays were performed using the MTT assay method [14]. Compound **1** exhibited cytotoxicity against KB and KB_V 200 cells with IC_{50} values greater than 50 μg/mL, respectively.

ACKNOWLEDGMENT

This research work was supported by a grant from the Natural Science Foundation of Guangxi Province (0832081), the Science Foundation of Guangxi University for Nationalities (200702YJ18), the National Natural Science Foundation of China (20072058), and the 863 Foundation of China (2006AA09Z422).

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