A NEW ISOCHROMAN FROM THE MARINE ENDOPHYTIC FUNGUS 1893#

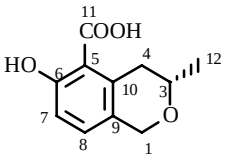
Guangying Chen,[1] Yongcheng Lin,[2] L. L. P.Vrijmoed,[3] and Wang-Fun Fong[3]

UDC 547.314

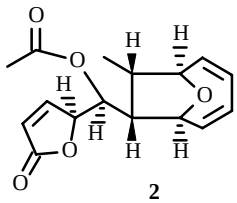
A new isochroman, 6-hydroxy-3-methylisochroman-5-carboxylic acid, and six known compounds were isolated from the culture of marine-derived mangrove fungus 1893#. Their structures were elucidated by analysis of spectroscopic data.

Key words: isochroman, marine, fungus, mangrove.

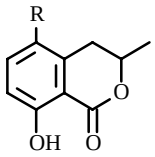
Marine microorganisms have proved to be rich sources of bioactive secondary metabolites, and numerous compounds with potent biological activities and unique chemical structures have been isolated [1]. In the course of our search for novel bioactive compounds from marine mangrove fungi [2–6], the extract of the endophytic fungus No. 1893 was found to exhibit cytotoxicity toward NCI4460 and Bel-7402, and high activities against Heliothis armigera (Huehner) and Sinergasilus spp. This endophytic fungus strain was collected from the dropper of Kandelia candel from an estuarine mangrove on the South China Sea coast. Two new lactones 1893A and B have been isolated from its fermentation broth [7], which have attracted one group to synthesize them since their report [8]. In the ongoing research on metabolites from the fungus we adopted industrial fermentation to replace experimental fermentation. A new compound, 6-hydroxy-3-methylisochroman-5-carboxylic acid (**1**), together with six known compounds, mycoepoxydiene (**2**) [9], 5-carboxylmellein (**3**) [10], 5-methylmellein (**4**) [11], pyrrole-2-carboxylic acid (**5**) [12], 3-methylhydantoin (**6**) [13], and cyclo(L-Ala-L-Tyr) (**7**) [14] were isolated from the fermentation broth of this fungus. Compound **2** was the analog of 1893A and B. Their structures were elucidated by spectroscopic methods.



1

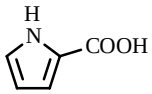


2

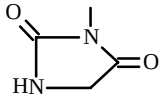


3, 4

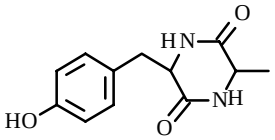
3: R = COOH; **4:** R = CH_3



5



6



7

Compound **1** was obtained as brown oils, and determined to have the molecular formula $C_{11}H_{12}O_4$ by HREI-MS data (*m/z* 209.0840, calc. 209.0813). In the ^{13}C NMR spectrum, six olefinic carbon signals (δ 138.6, 137.7, 136.7, 127.6, 115.9, 108.7), one methine-bearing oxygen signals (δ 75.4), two methylene signals (δ 62.6, 31.2), and one methyl group (δ 21.0) were observed. The six unsaturation equivalents required by the molecular formula indicated that the compound has two rings.

1) Department of Chemistry, Hainan Normal University, Haikou 571158, China; 2) Department of Applied Chemistry, Zhongshan University, 510275, Guangzhou, P.R. China, Fax: 0086-20-84039623, e-mail: yclin48@hotmail.com; 3) Department of Biology and Chemistry, City University of Hong Kong, Hong Kong. Published in Khimiya Prirodnykh Soedinenii, No. 2, pp. 114-116, March-April, 2006. Original article submitted July 4, 2005.

0009-3130/06/4202-0138

TABLE 1. NMR Data of **1** ($CDCl_3$, δ, ppm, J/Hz)

C atom	δ_C (DEPT)	δ_H	COSY	HMBC
1	62.6 (CH_2)	4.62 (d, J = 12.5)		H-8
3	75.4 (CH)	4.73 (ddd, J = 3, 6, 9.5)	H-4b, 12	H-4a, 4b, 12
4	31.2 (CH_2)	a 3.20 (dd, J = 3, 17)	H-4b	H-12
		b 2.88 (dd, J = 12, 17)	H-3,4a	
5	108.7 (C)			H-4, 6-OH
6	138.6 (C)			6-OH, H-8
7	115.9 (CH)	6.90 (d, J = 8.5)	H-8	6-OH, H-8
8	136.7 (CH)	7.46 (d, J = 8.5)	H-7	H-7
9	127.6 (C)			H-1,4
10	137.7 (C)			H-3,4
11	162.1 (C)			
12	21.0 (CH_3)	1.57 (dd, J = 6, 10)	H-3	H-3

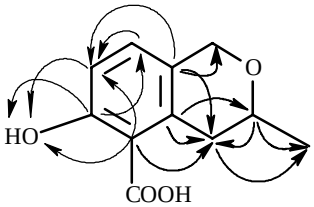


Fig. 1. Correlation of the HMBC of compound **1**.

The IR spectrum of **1** showed the presence of one hydroxy group with band at 3280 cm^{-1} and one carboxyl group with band at 1685 cm^{-1} (at δ 162.1 in the ^{13}C NMR spectrum). The chelation of the phenolic hydroxy group with the carboxyl group was confirmed by the lowfield signal in the ^1H NMR spectra at δ 11.1. In the ^1H NMR spectrum, there were two vicinal aromatic protons at δ 7.46 (d, 8.5 Hz) and 6.90 (d, 8.5 Hz). In the COSY spectrum, the correlation between H-3 and H-12 located CH_3-12 at C-3. In the HMBC spectrum, the correlations between C-1 and H-8 and the correlations between C-5 and H-4, H-7 and between C-9 and H-1, H-4, H-7 respectively, established the overall structure of **1** (Table 1 and Fig. 1).

The coupling constant of 12.5 Hz between H-3 and H-4b indicated that H-3 is located at the *a*-bond position, so the methyl group is at the *e*-bond position. In mono-substituted isochromans, the substituent group in C-1 or C-3 lying at the *e*-position was more stable than at the *a*-position [15].

EXPERIMENTAL

General Procedures. IR spectra were obtained using an EQUINOX55-A590/3F FT-IR spectrometer; UV spectra were obtained on a Shimadzu UV-2501PC spectrophotometer; NMR spectra were recorded on a Varian Unity INOVA 500NB NMR spectrometer (500 MHz for ^1H and 125 MHz for ^{13}C) using TMS as internal standard; EIMS spectra were performed on a VG-ZAB-HS mass spectrometer and HREIMS spectrum was performed on a VG Autospec-500 mass spectrometer.

Fungus Material and Purification. A strain of the fungus (no. 1893) was isolated from the dropper of Kandelia candel from an estuarine mangrove on the South China Sea coast. 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 a 300 L fermenter containing 170 L of GYT medium, incubated at 30°C for 80 h. The 170 L cultures were filtered through cheesecloth. The filtrate was concentrated to 3.5 L below 50°C and extracted five times by shaking with equal volumes of ethyl acetate. The combined extracts were chromatographed repeatedly on silica gel using gradient elution from petroleum to ethyl acetate to obtain mycoepoxydiene (**2**, 50 mg) [9], 3-methylhydantoin (**6**, 15 mg), [13], cyclo(L-Ala-L-Tyr) (**7**, 35 mg) [14], 5-carboxylmellein (**3**, 15 mg) [10], 5-methylmellein

(**4**, 18 mg) [11], and pyrrole-2-carboxylic acid (**5**, 20 mg) [12] respectively. Semipreparative HPLC (mobile phase: methanol–water 1:1) of the combined fractions 15 & 20 resulted in the compound 6-hydroxy-3-methylisochroman-5-carboxylic acid (**1**, t_R = 11.3 min, 10 mg). The structure of **1** was determined by a combination of IR, HR-EIMS, UV, and extensive 1D and 2D NMR analyses.

6-Hydroxy-3-methylisochroman-5-carboxylic Acid (1): brown oil, $C_{11}H_{12}O_4$.

UV spectrum (C_2H_5OH, λ_{max}, nm): 316, 245, 220.

IR spectrum ($CHCl_3$, ν, cm^{-1}): 3280 (OH), 2980, 2930, 1655 (C=O), 1620, 1495 (Ph), 1367, 1205, 873.

Mass spectrum (HR-EIMS, *m/z*): 209.0840 (calc. 209.0813).

^1H, ^{13}C NMR see Table 1.

Mycoepoxydiene (2): colorless needles, mp 184~186°C; $[\alpha]_D^{20}$ +27.0 (*c* 0.054, C_2H_5OH).

IR spectrum (KBr, ν, cm^{-1}): 2965, 1741 (C=O), 1719 (C=O), 1631 (C=C), 1370, 1265, 1235, 1073, 1027.

Mass spectrum (FAB^+, *m/z*, I_{rel}, %): 291 (18) $[M+1]^+$, 231 (14) $[M-CH_3COOH-H]^+$, 154 (100), 136 (90), 107 (35); EA (%): C 66.12, H 6.23, O 27.65. $C_{16}H_{18}O_5$ (calc. C 66.20, H 6.20, O 27.60).

^1H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.02 (1H, dd, J = 6, 10), 6.21 (1H, d, J = 10), 6.09 (1H, dd, J = 4.5, 10), 6.03 (1H, dd, J = 6, 11), 5.92 (1H, dd, J = 5.5, 10), 5.89 (1H, dd, J = 5.5, 11), 5.07 (1H, dd, J = 2.5, 6), 4.48 (1H, dd, J = 2.5, 11), 4.31 (1H, d, J = 4.5), 4.27 (1H, dd, J = 6, 6), 3.05 (1H, dd, J = 6, 11), 3.01 (1H, m), 2.03 (3H, s), 1.13 (3H, d, J = 7).

^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 170.0, 162.1, 140.2, 137.5, 136.9, 126.3, 125.1, 124.4, 86.4, 77.6, 75.9, 63.1, 52.6, 50.1, 20.6, 14.1.

5-Carboxylmellein (3): colorless needles, mp 242–243°C.

^1H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 12.92 (1H, brs), 11.67 (1H, s), 8.07 (1H, d, J = 9), 6.96 (1H, d, J = 9), 4.76 (1H, m), 3.80 (1H, dd, J = 3, 17.5), 3.02 (1H, dd, J = 12, 17.5), 1.45 (3H, d, J = 5.5).

^{13}C NMR (500 MHz, DMSO-d_6, δ, ppm): 169.8, 167.2, 165.0, 143.1, 138.7, 119.7, 115.7, 108.7, 75.3, 32.6, 20.6.

5-Methylmellein (4): colorless needles, mp 131–133°C.

^1H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 10.99 (1H, s), 7.28 (1H, d, J = 9), 6.81 (1H, d, J = 9), 4.69 (1H, m), 2.93 (1H, dd, J = 3.5, 17), 2.72 (1H, dd, J = 12, 17), 2.19 (3H, s), 1.54 (3H, d, J = 6.5).

Pyrrole-2-carboxylic Acid (5): white grainy solid, mp 202–204°C (decomposed).

^1H NMR (500 MHz, CD_3COCD_3, δ, ppm, J/Hz): 10.80 (1H, br), 7.04 (1H, d, J = 1.5), 6.85 (1H, d, J = 2.5), 6.20 (1H, d, J = 1.5, 2.5).

^{13}C NMR (500 MHz, CD_3COCD_3, δ, ppm): 162.21, 124.2, 123.6, 115.9, 110.4.

3-Methylhydantoin (6): colorless needles, mp 196–198°C.

^1H NMR (500 MHz, DMSO-d_6, δ, ppm): 8.60 (1H, s), 3.92 (2H, s), 2.98 (3H, s).

^{13}C NMR (500 MHz, DMSO-d_6, δ, ppm): 173.1, 156.9, 52.3, 28.6.

Cyclo(L-Ala-L-Tyr) (7): white needles, mp 264~265°C (decomposed).

^1H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 7.01 (1H, dd, J = 3, 8.5), 6.73 (1H, dd, J = 3, 8.5), 4.22 (1H, t, J = 4.5), 3.77 (1H, q, J = 7), 3.16 (1H, dd, J = 4.5, 13), 2.86 (1H, dd, J = 4.5, 13), 0.61 (3H, d, J = 7).

ACKNOWLEDGMENT

We wish to acknowledge financial support from the National Natural Science Foundation of China (20072058), the 863 Foundation of China (2003AA624010), the Natural Science Foundation of Guangdong Province, China (021732), and the Star Lake Biotechnology Co., Inc., Zhaoqing, Guangdong, China.

REFERENCES

1. D. Faulkner, *J. Nat. Prod. Rep.*, **18**, 1 (2001).
2. Y. C. Lin, H. J. Li, G. C. Jiang, S. N. Zhou, L. L. P. Vrijmoed, and E. B. G. Jones, *Indian. J. Chem.*, Sect. B, **41**B, 1542 (2002).

3. Y. C. Lin, X. Y. Wu, Z. J. Deng, J. Wang, S. N. Zhou, L. L. P. Vrijmoed, and E. B. G. Jones, *Phytochemistry*, **59**, 469 (2002).
4. Y. C. Lin, X. Y. Wu, S. Feng, G. C. Jiang, J. H. Luo, S. N. Zhou, L. L. P. Vrijmoed, E. B. G. Jones, K. Krohn, K. Steingroever, and F. Zsila, *J. Org. Chem.*, **66**, 6252 (2001).
5. Y. C. Lin, J. Wang, X. Y. Wu, S. N. Zhou, L. L. P. Vrijmoed, and E. B. G. Jones, *Aust. J. Chem.*, **55**, 225 (2002).
6. Y. C. Lin, X. Y. Wu, S. Feng, G. C. Jiang, J. H. Luo, S. N. Zhou, L. L. P. Vrijmoed, and E. B. G. Jones, *Tetrahedron Lett.*, **42**, 449 (2001).
7. G. Y. Chen, Y. C. Lin, L. Wen, L. L. P. Vrijmoed, and G. E. B. Jones, *Tetrahedron*, **59**, 4907 (2003).
8. K. Takao, H. Yashi, S. Yamamoto, D. Sasaki, S. Kawasaki, G. Watanabe, and K. Tadano, *J. Org. Chem.*, **69**, 8789 (2004).
9. P. Cai, A. T. McPhail, and E. Krainer, *Tetrahedron Lett*., **40**, 1479 (1999).
10. J. R. Andson, R. L. Edwards, and A. J. S. Whalley, *J. Chem. Soc., Perkin Trans I*, 2185 (1983).
11. K. Krohn, R. Bahramsari, U. Florke, K. Ludewig, C. Klichespory, A. Michel, H. J. Aust, S. Draeger, B. Schulz, and S. Antus, *Phytochemistry*, **45**, 313 (1997).
12. J. W. Scott, A. Focella, and U. O. Hengartner, *Synth. Commun*., **10**, 529 (1980).
13. S. Cortes, Z. K. Liao, D. Watson, and H. Kohn, *J. Med. Chem.*, **28**, 601 (1985).
14. W. J. Qin, L. B. Ma, Y. S. Wen, and L. Yang, *Zhongcaoyao*, **26**, 3 (1995).
15. E. W. Olefirowicz and E. I. Eliel, *J. Comput. Chem.*, **10**, 407 (1989).