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XANTHONE DERIVATIVES FROM THE FERMENTATION PRODUCTS OF AN ENDOPHYTIC FUNGUS OF *PHOMOPSIS AMYGDALI*

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Abstract – Two new xanthones, 1,5-dihydroxy-3-(2-oxopropyl)-6-methoxy-carbonylxanthone (**1**) and 1-hydroxy-3-(2-oxopropyl)-8-methoxycarbonyl-xanthone (**2**), together with four known xanthones (**3**-**6**) were isolated from the fermentation products of a *Phomopsis* fungus. Their structures were elucidated by spectroscopic methods, including extensive 1D- and 2D-NMR techniques. Compound **1** showed high cytotoxicity against A549 and PC3 cell with IC_{50} values of 3.5 and 2.8 μM. Compound **2** showed high cytotoxicity against PC3 cell with IC_{50} value of 2.4 μM. The other compounds also showed moderate cytotoxicity for some tested cell lines with IC_{50} values between 5.2-8.6 μM.

The *Phomopsis* species known as an important phytopathogenic genus contains more than 900 species named from a wide range of hosts.[1] These microorganisms produce a number of secondary metabolites with various biological activities, including antimicrobial,[2,3] antifungal,[4,5] antimalarial,[6,7] antitumor,[7-9] and the like. The xanthone derivatives are important metabolites isolated from the *Phomopsis* genus, and they appeals to medicinal chemists because of their pronounced pharmacological effects.[10,11]

With the aim of multipurpose utilization endophytic fungus of isolated from the rhizome of *Paris polyphylla* var. *yunnanensis* and identify bioactive natural products, the phytochemical investigation on fermentation products of the endophytic fungus *Phomopsis amygdali*. was carried out. As a result, two new xanthones (**1**-**2**), together with four known xanthones (**4**-**6**), were isolated from this extract. The

structures of new compounds were elucidated on the basis of a comprehensive analysis of the ^{1}H NMR, ^{13}C NMR and 2D NMR spectra. In addition, cytotoxicities of compounds **1**-**5** were evaluated. The details of the isolation, structure elucidation and cytotoxicity of the compounds are reported in this article.

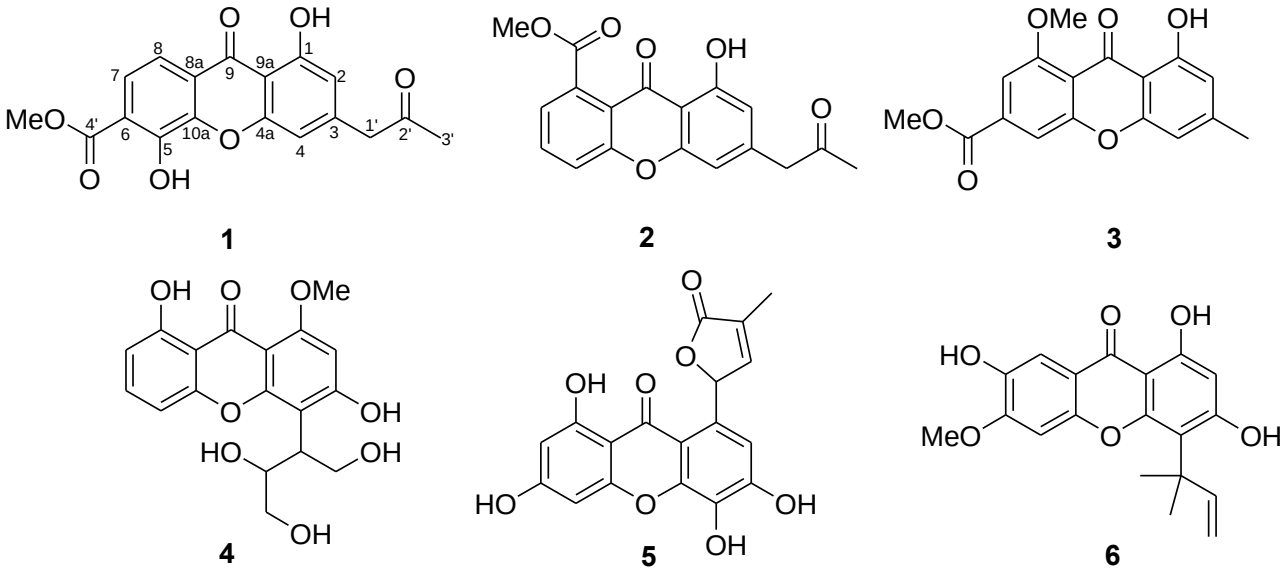

Figure 1. The structures of compounds **1** - **6**

A 70% aq. acetone extract prepared from fermentation products of the endophytic fungus *Phomopsis amygdali* was subjected repeatedly to column chromatography on Silic gel, Sephadex LH-20, RP-18 and Preparative HPLC to afford compounds **1**-**6**, including two new xanthones, named 1,5-dihydroxy-3-(2-oxopropyl)-6-methoxycarbonylxanthone (**1**) and 1-hydroxy-3-(2-oxopropyl)-8-methoxycarbonyl-xanthone (**2**), together with four known xanthones, yicathin B (**3**),[12] secosterigmatocystin (**4**),[13] garcinexanthone F (**5**),[18] and hypericumxanthone A (**6**).[15] The structures of the compounds **1**-**6** were as shown in Figure 1, and the ^{1}H and ^{13}C NMR data of **1** and **2** were listed in Table 1.

Compound **1** was obtained as pale yellow gum. The HRESIMS showed the quasi-molecular ion peak at *m/z* 365.0632 $[M+Na]^{+}$ (calc. for 365.0637), in accordance with the molecular formula $C_{18}H_{14}NaO_7$, which indicated 12 degrees of unsaturation. Its UV spectrum showed the maximum absorption at 322, 250, and 210 nm. Strong absorption bands accounting for hydroxy (3413 cm^{-1}), carbonyl (1726, 1718, 1650 cm^{-1}), and aromatic groups (1597, 1538, 1463 cm^{-1}) could also be observed in its IR spectrum. The ^{1}H and ^{13}C NMR spectra of **1** (Table 1) displayed signals for all 18 carbons and 14 protons, including a xanthone skeleton (δ_C 162.4, 108.8, 143.2, 105.1, 154.5, 119.2, 126.0, 120.7, 181.9, 156.5, 124.9, 118.5, and 151.9) with

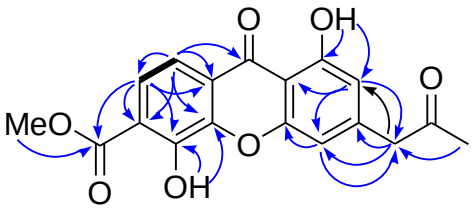

Figure 2. Key HMBC ( ) correlations of **1**

four aromatic protons [δ_H 6.88 d (2.2), 7.02 d (2.2), 7.61 d (8.2), 7.41 d (8.2)], one 2-oxopropyl unit (δ_C 48.3, 206.2, 30.2; δ_H 2.27, 1.51 s),[16] one methoxycarbonyl group (δ_C 168.9, 52.7; δ_H 4.15 s),[17] and two phenolic hydroxy groups (δ_H 12.12 s, 12.47 s). The HMBC correlations (Figure 2) of H-1′ (δ_H 2.27) with C-2 (δ_C 108.8), C-3 (δ_C 143.2), and C-4 (δ_C 105.1), of H-2 (δ_H 6.88) and H-4 (δ_H 7.02) with C-1′ (δ_C 48.3) suggested the 2-oxopropyl unit should be located at C-3. Tow phenolic hydroxy groups located at C-1, and C-5 were supported by the HMBC correlations of one phenolic hydroxy proton signal (δ_H 12.12) with C-1 (δ_C 162.4), C-2 (δ_C 108.8), and C-9a (δ_C 118.5); and of another phenolic hydroxy proton signal (δ_H 12.47) with C-5 (δ_C 154.5), C-6 (δ_C 119.2), and C-10a (δ_C 151.9), respectively. The methoxycarbonyl group at C-6 was supported by HMBC correlations of H-7 (δ_H 7.61) with the ester carbonyl carbon (δ_C 168.9), of H-8 (δ_H 7.41) with C-9 (δ_C 181.9), and no correlation was observed between H-8 and the ester carbonyl. The typical protons signals [δ_H 6.88 d (2.2), 7.02 d (2.2), 7.61 d (8.2), 7.41 d (8.2)] also supported the 5,6-disubstituted for ring B, and 1,3-disubstituted for ring C of the xanthone skeleton. Therefore, compound **1** was assigned as 1,5-dihydroxy-3-(2-oxopropyl)-6-methoxycarbonylxanthone.

Compound **2** was assigned the molecular formula $C_{18}H_{14}NaO_6$ by its HRESIMS at *m/z* 349.0680 $[M+Na]^+$. The ^1H and ^{13}C NMR spectra of **2** are similar to those of compound **1**. The difference between compounds **1** and **2** was due to the disappearance of a phenolic hydroxy group signal in **2**, and the ^1H NMR data variation for ring B. The typical protons signals [δ_H 7.44 d (8.2), 7.66 t (8.2), 7.74 d (8.2)] suggested that the substituent group on ring B should be at C-5 or C-8. A methoxycarbonyl group located at C-8 were supported by HMBC correlations of H-7 (δ_H 7.74) with the ester carbonyl carbon (δ_C 169.9), and no correlation was observed between proton signal and the carbonyl (δ_C 180.4). Accordingly, the structure of **2** was determined as shown.

Table 1. ^1H and ^{13}C NMR data of compounds **1** and **2** (δ in ppm, in C_5D_5N, 500 and 125 MHz)

No.	Compound **1**		Compound **3**	
	δ_C (m)	δ_H (m, *J*, Hz)	δ_C (m)	δ_H (m, *J*, Hz)
1	162.4 s		162.8 s	
2	108.8 d	6.88 d (2.2)	109.7 d	6.90 d (2.2)
3	143.2 s		143.9 s	
4	105.1 d	7.02 d (2.2)	105.6 d	7.03 d (2.2)
5	154.5 s		119.2 d	7.44 d (8.2)
6	119.2 s		130.3 d	7.66 t (8.2)
7	126.0 d	7.61 d (8.2)	122.1 d	7.74 d (8.2)
8	120.7 d	7.41 d (8.2)	134.1 s	
9	181.9 s		180.4 s	
4a	156.5 s		156.8 s	
8a	124.9 s		117.2 s	
9a	118.5 s		107.9 s	
10a	151.9 s		155.0 s	
1′	48.3 t	2.27 s	48.9 t	2.30 s
2′	206.2 s		206.2 s	
3′	30.2 q	1.51 s	30.5 q	1.51 s
4′	168.9 s		169.9 s	
4′-OMe	52.7 q	4.15 s	53.0 q	4.07 s
Ar-OH-1		12.12 s		12.61 s
Ar-OH-5		12.47 s		

Xanthones are known to exhibit cytotoxic effects.[6,10,18] Since the cytotoxicity of

compounds **1**-**5** had not been reported, the cytotoxicity of **1**-**5** were tested using a previously reported procedure.[19] All treatments were performed in triplicate. In the MTT assay, the IC_{50} was defined as the concentration of the test compound resulting in a 50% reduction of absorbance compared with untreated cells. The cytotoxic abilities against NB4, A549, SHSY5Y, PC3, and MCF7 tumor cell lines by MTT-assay (with taxol as the positive control) were shown in Table **2**. Compound **1** showed high cytotoxicity against A549 and PC3 cell with IC_{50} values of 3.5 and 2.8 μM. Compound **2** showed high cytotoxicity against PC3 cell with IC_{50} value of 2.4 μM. The other compounds also showed moderate cytotoxicity for some tested cell lines with IC_{50} values between 5.2-8.6 μM.

Table 2. Cytotoxic activity of compounds **1** - **5**

Compounds	NB4	A549	SHSY5Y	PC3	MCF7
1	7.2	3.5	8.5	2.8	7.9
2	6.2	5.2	>10	2.4	8.7
3	8.2	>10	7.7	>10	8.5
4	>10	>10	8.2	>10	>10
5	7.5	>10	>10	8.6	>10
taxol	0.03	0.02	0.2	0.2	0.1

NB4, human leukemia cell; A549, carcinomic human alveolar basal epithelial cell; SHSY5Y, human neuroblastoma cell; PC3, human prostate cancer cell; MCF7, human breast adenocarcinoma cell.

EXPERIMENTAL

General. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. IR spectra were obtained in KBr disc on a Bio-Rad Wininfmred spectrophotometer. ESI-MS were measured on a VG Auto Spec-3000 MS spectrometer. ^{1}H, ^{13}C and 2D NMR spectra were recorded on Bruker DRX-500 instrument with TMS as internal standard. Column chromatography was performed on silica gel (200-300 mesh), or on silica gel H (10~40 μm, Qingdao Marine Chemical Inc., China). Second separate was used an Agilent 1100 HPLC equipped with ZORBAX-C_{18} (21.2 mm × 250 mm, 7.0 μm) column and DAD detector.

Fungal Material. The culture of *Phomopsis* sp. was isolated from the rhizome of *Paris polyphylla* var. *yunnanensis* collected from Shizhong, Yunnan, People's Republic of China, in 2009. The strain was identified by one of authors (Gang Du) based on the analysis of the ITS sequence (Genbank Accession number KF308686). It was cultivated at room temperature for 7 days on potato dextrose agar at 28 °C. Agar plugs were inoculated into 250 mL Erlenmeyer flasks each containing 100 mL potato dextrose broth and cultured at 28 °C on a rotary shaker at 180 rpm for five days. Large scale fermentation was carried out in 200 Fernbach flasks (500 mL) each containing 100 g of rice and 120 mL of distilled H_2O. Each flask was inoculated with 5.0 mL of cultured broth and incubated at 25 °C for 45 days.

Extraction and Isolation. The fermentation products were extracted four times with 70% acetone (4 × 10 L) at room temperature and filtered. The crude extract (103 g) was applied to silica gel (200–300 mesh)

column chromatography, eluting with a $CHCl_3$-acetone gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions A–F. The further separation of fraction A (9:1, 18.5 g) by silica gel column chromatography, eluted with petroleum ether-EtOAc (9:1, 8:2, 7:3, 6:4, 1:1), yielded mixtures A1–A5. Fraction A2 (8:2, 5.4 g) was subjected to preparative HPLC (65% MeOH, flow rate 12 mL/min) to give **2** (12.6 mg), **3** (16.7 mg), and **6** (8.22 mg). The further separation of fraction A3 (7:3, 4.6 g) by silica gel column chromatography, and preparative HPLC (60% MeOH, flow rate 12 mL/min) to give **1** (9.76 mg), **4** (13.8 mg), and **5** (18.4 mg).

Cytotoxicity Assay. The cytotoxicity tests for the isolates were performed by against NB4, A549, SHSY5Y, PC3, and MCF7 tumor cell lines by MTT-assay (with taxol as the positive control).[19]

1,5-Dihydroxy-3-(2-oxopropyl)-6-methoxycarbonylxanthone (**1**): Obtained as a yellow gum; UV (MeOH) λ_{max} (log ε) 210 (4.17), 256 (3.62), 315 (3.79) nm; IR (KBr) ν_{max} 3410, 3067, 2918, 2874, 1726, 1718, 1650, 1597, 1538, 1463, 1385, 1134, 1062, 867, 765 cm^{-1}; ESIMS *m/z* (positive ion mode) 365 $[M+Na]^+$; HRESIMS (positive ion mode) *m/z* 365.0632 $[M+Na]^+$ (calcd $C_{18}H_{14}NaO_7$ for 365.0637).

1-Hydroxy-3-(2-oxopropyl)-8-methoxycarbonylxanthone (**2**): Obtained as a yellow gum; UV (MeOH) λ_{max} (log ε) 210 (4.22), 250 (3.82), 322 (3.87) nm; IR (KBr) ν_{max} 3413, 3057, 2908, 2870, 1728, 1715, 1646, 1600, 1542, 1457, 1380, 1124, 1057, 871, 760 cm^{-1}; ESIMS *m/z* (positive ion mode) 353 $[M+Na]^+$; HRESIMS (positive ion mode) *m/z* 349.0680 $[M+Na]^+$ (calcd $C_{18}H_{14}NaO_6$ for 349.0688).

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REFERENCES

1. D. Udayanga, X. Liu, E. H. C. McKenzie, E. Chukeatirote, A. H. A. Bahkali, and K. D. Hyde, *Fungal Divers.*, 2011, **50**, 189.
2. J. N. Choi, J. Kim, K. Ponnusamy, C. Lim, J. G. Kim, M. J. Muthaiya, and C. H. Lee, *J. Antibiot.*, 2013, **66**, 231.
3. H. Hussain, M. K. Tchimene, I. K. Ahmed, M. Steinert, S. Draeger, B. Schulz, and K. Krohn, *Nat. Prod. Commun.*, 2011, **6**, 1905.
4. H. Hussain, I. Ahmed, B. Schulz, S. Draeger, and K. Krohn, *Fitoterapia*, 2012, **83**, 523.

5. H. Hussain, K. Krohn, I. Ahmed, S. Draeger, B. Schulz, S. Pietro, and G. Pescitelli, *Eur. J. Org. Chem.*, 2012, **9**, 1783.
6. M. Isaka, A. Jaturapat, K. Rukseree, K. Danwisetkanjana, M. Tanticharoen, and Y. Thebtaranonth, *J. Nat. Prod.*, 2001, **64**, 1015.
7. C. Hemtasin, S. Kanokmedhakul, K. Kanokmedhakul, C. Hahnvajanawong, K. Soytong, S. Prabpai, and P. Kongsaeree, *J. Nat. Prod.*, 2011, **74**, 609.
8. E. Adelin, C. Servy, S. Cortial, H. Levaique, M. T. Martin, P. Retailleau, G. G. Le, B. Bussaban, S. Lumyong, and J. Ouazzani, *Phytochemistry*, 2011, **72**, 2406.
9. C. Hemtasin, S. Kanokmedhakul, K. Kanokmedhakul, C. Hahnvajanawong, K. Soytong, S. Prabpai, and P. Kongsaeree, *J. Nat. Prod.*, 2011, **74**, 609.
10. K. S. Masters and S. Brase, *Chem. Rev.*, 2012, **112**, 3717.
11. S. Kaul, S. Gupta, M. Ahmed, and M. K. Dhar, *Phytochem. Rev.*, 2013, **11**, 487.
12. R. R. Sun, F. P. Miao, J. Zhang, G. Wang, X. L. Yin, and N. Y. Ji, *Magn. Reson. Chem.*, 2013, **51**, 65.
13. F. Zhu and Y. C. Lin, *Chem. Nat. Comp.*, 2007, **43**, 132.
14. Y. Chen, H. Fan, G. Z. Yang, Y. Jiang, F. F. Zhong, and H. W. He, *Helv. Chim. Acta*, 2011, **94**, 662.
15. W. B. Xin, Z. J. Mao, G. L. Jin, and L. P. Qin, *Phytother. Res.*, 2011, **25**, 536.
16. Q. F. Hu, B. Zhou, X. M. Gao, L. Y. Yang, L. D. Shu, Y. Q. Shen, G. P. Li, C. T. Che, and G. Y. Yang, *J. Nat. Prod.*, 2012, **75**, 1909.
17. Q. F. Hu, D. Y. Niu, X. L. Li, Y. H. Qin, Z. Y. Yang, G. L. Zhao, Z. X. Yang, X. M. Gao, and Z. Y. Chen, *Heterocycles*, 2013, **87**, 1127.
18. J. X. Yang, S. X. Qiu, Z. G. She, and Y. C. Lin, *Chem. Nat. Comp.*, 2013, **49**, 31.
19. X. M. Gao, R. R. Wang, D. Y. Niu, C. Y. Meng, L. M. Yang, Y. T. Zheng, G. Y. Yang, Q. F. Hu, H. D. Sun, and W. L. Xiao, *J. Nat. Prod.*, 2013, **76**, 1052.