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NEW XANTHONES FROM *COMASTOMA PULMONARIUM* AND THEIR ANTI-TOBACCO MOSAIC VIRUS ACTIVITY

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Abstract – Two new xanthones, pulmonarxanthone A (**1**) and pulmonarxanthone B (**2**), together with four known ones (**3**-**6**), were isolated from the whole plants of *Comastoma pulmonarium*. The structures of compounds **1–6** was elucidated by spectroscopic methods including extensive 1D- and 2D-NMR techniques. Compounds **1–6** were also evaluated for their anti-tobacco mosaic virus (Anti-TMV) activity. The results showed that all the isolated compounds exhibited weak anti-TMV activity with inhibition rates in the range of 14.4–22.3%.

Comastoma pulmonarium (Turcz.) Toyokuni, belong to Gentianaceae family, is a plant of 5–30 cm in height, alpine annual inhabiting meadow slopes, alpine meadows and river banks at altitudes ranging from 2170 to 4800 m.[1] In China, it has been used as traditional Chinese medicine for treatment of hepatitis, encephalalgia, and pharyngalgia by Tibetan people.[2] Previous phytochemical studies on *C. pulmonarium* have revealed the presence of flavonoids,[2] xanthones,[3] and triterpenes.[3] The xanthone derivatives are important metabolites isolated from the higher plants and the fermentation products of microorganisms, and they appeal to medicinal chemists because of their pronounced pharmacological effects.[4-7] Motivated by a search for more new bioactive metabolites from this plant, we now investigated the chemical constituents of the whole plants of *C. pulmonarium* in shangri-la Prefecture, Yunnan Province. This lead to the isolation of six xanthones (**1**–**6**), including two new compounds (**1** and **2**). In

this paper, we report the isolation, structure elucidation, and anti-TMV activity of the isolated compounds.

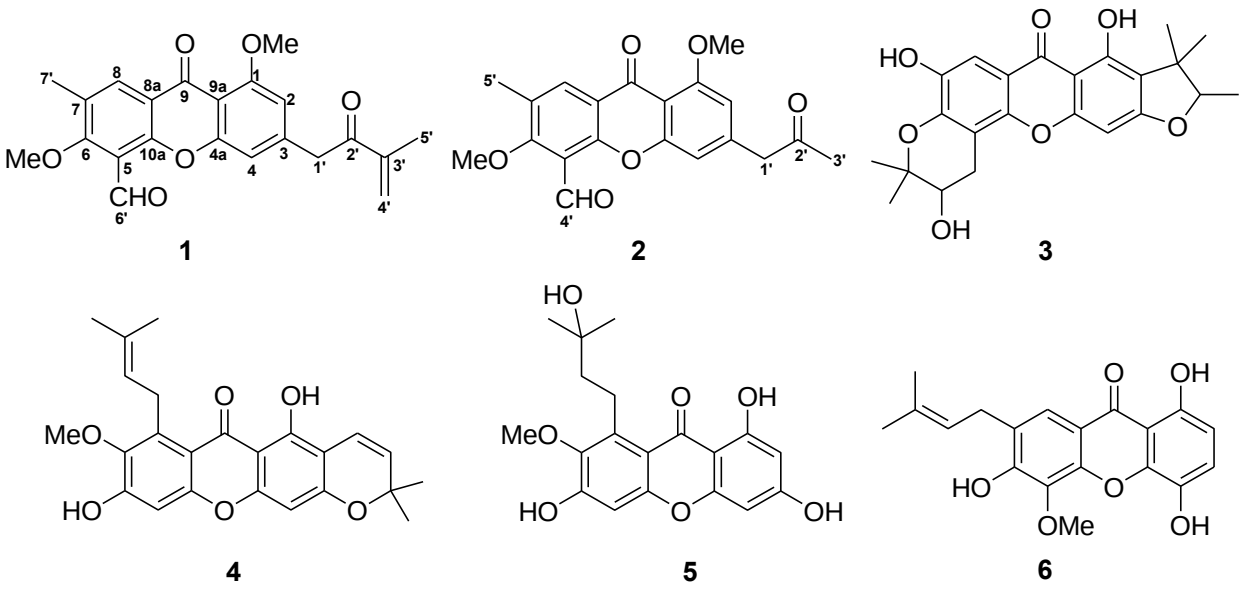

Figure 1. Chemical structures of compounds **1**-**6** from *C. pulmonarium*.

The whole plants of *C. pulmonarium* were extracted with 70% aqueous acetone. The extract was subjected repeatedly to column chromatography on silica gel, RP-18, and semi-preparative RP-HPLC separation to afford compounds **1**–**6**. Their structures were shown in Figure 1. The ^{1}H and ^{13}C NMR data of compounds **1** and **2** were listed in Table 1. By comparing the spectral data previously reported, the known compounds were identified as cudratrixanthone I (**3**),[8] 9-hydroxycalabaxanthone (**4**),[9] xanthochymone A (**5**),[10] and 1,4,6-trihydroxy-5-methoxy-7-prenylxanthone (**6**).[11]

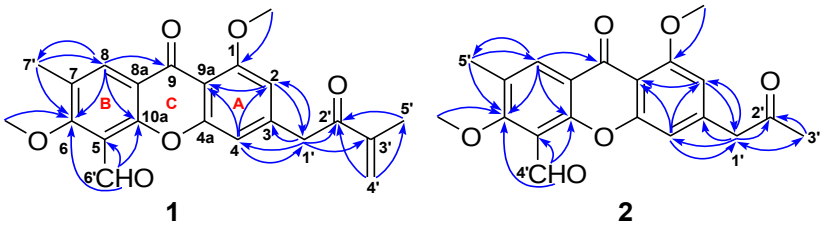

Figure 2. Selected HMBC (H ↷ C) correlations of compounds **1** and **2**.

Compound **1** was isolated as a pale yellow gum. The HRESIMS showed the quasi-molecular ion peak at *m/z* 403.1150 $[M + Na]^+$ (calc. for 403.1158, $C_{22}H_{20}O_6Na$), in accordance with the molecular formula $C_{22}H_{20}O_6$, which indicated 13 degrees of unsaturation. Its UV spectrum showed the absorption maxima at 318, 253, and 210 nm. Strong absorption bands accounting for carbonyl (1685 and 1650 cm^{-1}) and aromatic groups (1600, 1537, and 1462 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 22 carbons and 20 protons, including a xanthone skeleton (δ_C 161.9 s, 108.3 d, 141.3 s, 106.2 d, 114.3 s, 165.7 s, 119.4 s, 136.2 d, 177.9 s, 156.9 s, 118.5 s, 112.2 s, and 152.3 s) with three aromatic protons [δ_H 6.85 d (1.8), 6.64 d (1.8), and 7.70 s],[12] a 2-oxo-3-methylbut-3-enyl group (δ_C 38.1 t, 201.1 s, 144.2 s, 123.9 t, and 18.2 q; δ_H 4.63 s, 5.86, 6.12 brs,

and 2.02 s),[8] an aldehyde group (δ_C 191.7; δ_H 9.92),[13] a methyl group (δ_C 16.6 q; δ_H 2.39 s), and two methoxy groups (δ_C 56.1 q and 61.0 q; δ_H 3.80 s and 3.83 s). The HMBC correlations of H-1′ (δ_H 4.63) with C-2 (δ_C 108.3), C-3 (δ_C 141.3), and C-4 (δ_C 106.2), and of H-2 (δ_H 6.85) and H-4 (δ_H 6.64) with C-1′ (δ_C 38.1), suggested the 2-oxo-3-methylbut-3-enyl group should be located at C-3 (Figure 2). The aldehyde group located at C-5 was supported by the HMBC correlations of aldehyde proton signal (δ_H 9.92) with C-5 (δ_C 114.3), C-6 (δ_C 165.7), and C-10a (δ_C 152.3). The methyl group at C-7 was supported by HMBC correlations of methyl proton (δ_H 2.39) with C-6 (δ_C 165.7), C-7 (δ_C 119.4), and C-8 (δ_C 136.2), and of H-8 (δ_H 7.70) with C-7′ (δ_C 16.6). Finally, the methoxy groups located at C-1 and C-6 were supported by the HMBC correlations of methoxy proton signals (δ_H 3.80 and 3.83) with C-1 (δ_C 161.9) and C-6 (δ_C 165.7), respectively. Therefore, compound **1** was assigned as shown and given the trivial name of pulmonarxanthone A.

Pulmonarxanthone B (**2**) was also obtained as a yellow gum, and it was assigned the molecular formula of $C_{20}H_{18}O_6$ by HRESIMS at *m/z* 377.1008 $[M + Na]^+$. The ^1H and ^{13}C NMR spectra of **2** (Table 1) were very similar to those of **1**. The major structural difference was the presence of 2-oxopropyl group (δ_C 46.1 t, 206.6 s, 30.3 q; δ_H 4.41 s, 1.60 s) in **2** instead of a 2-oxo-3-methylbut-3-enyl group.[14] The HMBC correlations of H-1′ (δ_H 4.41) with C-2 (δ_C 108.1), C-3 (δ_C 138.4), and C-4 (δ_C 106.4), and of H-2 (δ_H 6.82) and H-4 (δ_H 6.64) with C-1′ (δ_C 46.1), suggested the 2-oxopropyl group located at C-3 (Figure 2). The other precise subsituents positions, one aldehyde group at C-5, one methyl group at C-7, and two methoxy groups located at C-1 and C-6, respectively, were also established by further analysis of HMBC correlations. The structure of **2** is therefore determined.

Table 1. ^1H and ^{13}C NMR Data of Compounds **1** and **2** (δ in ppm, in $CDCl_3$, 500 and 125 MHz).

No.	**1**		**2**	
	$d\delta_C$	δ_H (m. *J*, Hz)	$d\delta_C$	$d\delta_H$ (m. *J*, Hz)
1	161.9 s		161.3 s	
2	108.3 d	6.85 d (1.8)	108.1 d	6.82 d (1.8)
3	141.3 s		138.4 s	
4	106.2 d	6.64 d (1.8)	106.4 d	6.64 d (1.8)
5	114.3 s		114.0 s	
6	165.7 s		166.2 s	
7	119.4 s		119.8 s	
8	136.2 d	7.70 s	135.9 d	7.73 s
9	177.9 s		177.6 s	
4a	156.9 s		155.9 s	
8a	118.5 s		118.3 s	
9a	112.2 s		111.5 s	
10a	152.3 s		152.4 s	
1′	38.1 t	4.63 s	46.1 t	4.41 s
2′	201.1 s		206.6 s	
3′	144.2 s		30.3 q	1.60 s
4′	123.9 t	5.86, 6.12 brs	191.3 d	9.94 s
5′	18.2 q	2.02 s	17.0 q	2.32 s
6′	191.7 d	9.92 s		
7′	16.6 q	2.39 s		
1-OMe	56.1 q	3.80 s	56.2 q	3.80 s
6-OMe	61.0 q	3.83 s	61.2 q	3.86 s

Since some xanthones exhibit potential anti-TMV activity,[7,12,15] compounds **1**–**6** were tested for their anti-TMV activity. The inhibitory activities of compounds **1**–**6** against TMV replication were tested using the half-leaf method.[16] Ningnanmycin, a commercial product for plant disease in China, was used as a positive control. The antiviral inhibition rates of compounds **1**–**6** at the concentration of 20 μM were

listed in Table 2. The results showed that compounds **1**–**6** showed weak anti-TMV activity with inhibition rate in the range of 14.4–22.3%.

Table 2. TMV Infection Inhibition Activities of Compounds **1–6**

Compounds	Inhibition rates at 20 μM (%)	Compounds	Inhibition rates at 20 μM (%)
1	21.6 ± 2.7	**5**	14.8 ± 2.0
2	22.3 ± 2.8	**6**	14.4 ± 2.5
3	15.2 ± 2.2	ningnanmycin	33.5 ± 3.5
4	18.7 ± 2.4		

All results are expressed as mean ± SD; n = 3 for all groups.

EXPERIMENTAL

General Experimental Procedures. UV spectra were obtained on a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on a DRX-500 NMR spectrometer with TMS as internal. Unless otherwise specified, chemical shifts (δ) are expressed in ppm with reference to the solvent signals. HRESIMS was performed on a VG Autospec-3000 spectrometer. Semipreparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with Zorbax PrepHT GF (21.2 mm × 25 cm) or Venusil MP C_{18} (20 mm × 25 cm) columns. Column chromatography was performed using silica gel (200–300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany), and MCI gel (75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). The fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 5% H_2SO_4 in EtOH.

Plant Material. The whole plants of *Comastoma pulmonarium* (Turcz.) Toyokuni were collected in shangri-la Prefecture, Yunnan Province, People's Republic of China, in September 2012. The identification of plant material was verified by Prof. Ning Yuan (Yunnan University of Nationalities). A voucher specimen (Ynni-12-09-63) has been deposited in our Laboratory.

Extraction and Isolation. The air-dried and powdered *C. pulmonarium* (1.5 kg) were extracted four times with 70% aqueous acetone (4 × 2.0 L) at room temperature and filtered. The filtrate was evaporated under reduced pressure, and the crude extract (86.3 g) was decolorized by MCI. The 90% MeOH part (22.5 g) was chromatographed on a silica gel column 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 B (9:1, 22.5 g) by silica gel column chromatography, eluted with petroleum ether-acetone (9:1–1:2), yielded mixtures B1–B7. Fraction B2 (8:2, 1.21 g) was subjected to silica gel column chromatography using petroleum ether-acetone and semi-preparative HPLC (70% MeOH-H_2O, flow rate 12 mL/min) to give **1** (8.5 mg) and **2** (11.9 mg). Fraction B3 (7:3, 1.52 g) was subjected to silica gel column chromatography using petroleum ether-acetone and semi-preparative HPLC (63% MeOH-H_2O, flow rate 12 mL/min) to give **3** (15.2 mg), **4** (21.6 mg), **5** (13.9 mg), and **6** (12.2 mg).

Anti-TMV Assays. The Anti TMV activities were tested using the half-leaf method,[16] and ningnanmycin, a commercial product for plant disease in China, was used as a positive control.

Pulmonarxanthone A (**1**)**:** $C_{22}H_{20}O_6$, pale yellow gum; UV (MeOH) λ_{max} (log ε) 318 (3.62), 253 (3.56), 210 (4.05) nm; IR (KBr): ν_{max} 2958, 2860, 1685, 1650, 1600, 1537, 1462, 1357, 1226, 1048, 953, 862 cm^{-1}; ^1H and ^{13}C NMR data (500 and 125 MHz), see Table 1; ESIMS *m/z* 403; HRESIMS *m/z* 403.1150 $[M + Na]^+$ (calcd for $C_{22}H_{20}O_6Na$, 403.1158).

Pulmonarxanthone B (2): $C_{20}H_{18}O_6$, pale yellow gum; UV (MeOH) λ_{max} (log ε) 315 (3.57), 256 (3.56), 210 (4.12) nm; IR (KBr): ν_{max} 2946, 2851, 1682, 1643, 1602, 1557, 1472, 1432, 1360, 1254, 1072, 903, 847 cm^{-1}; ^1H and ^{13}C NMR data (500 and 125 MHz), see Table 1; ESIMS *m/z* 397; HRESIMS *m/z* 377.1008 $[M + Na]^+$ (calcd for $C_{20}H_{18}O_6Na$, 377.1001).

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