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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.

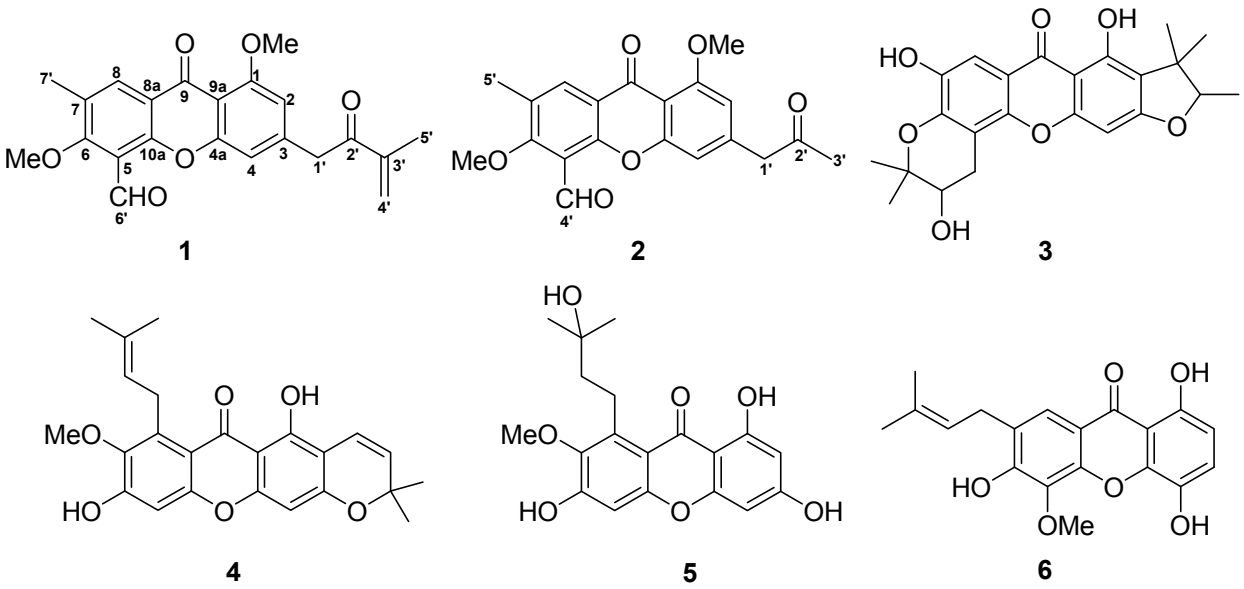


**Figure 1.** Chemical structures of compounds **1**-**6** from *C. pulmonarium*

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]

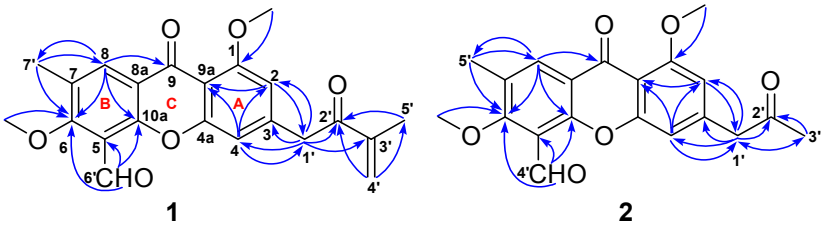


**Figure 2.** Selected HMBC (H ↷ C) correlations of compounds **1** and **2**

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 ($\delta_C$ 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 [$\delta_H$ 6.85 d (1.8), 6.64 d (1.8), and 7.70 s],[12] a 2-oxo-3-methylbut-3-enyl group ($\delta_C$ 38.1 t, 201.1 s, 144.2 s, 123.9 t, and 18.2 q; $\delta_H$ 4.63 s, 5.86, 6.12 brs,

and 2.02 s),[8] an aldehyde group ($\delta_C$ 191.7; $\delta_H$ 9.92),[13] a methyl group ($\delta_C$ 16.6 q; $\delta_H$ 2.39 s), and two methoxy groups ($\delta_C$ 56.1 q and 61.0 q; $\delta_H$ 3.80 s and 3.83 s). The HMBC correlations of H-1′ ($\delta_H$ 4.63) with C-2 ($\delta_C$ 108.3), C-3 ($\delta_C$ 141.3), and C-4 ($\delta_C$ 106.2), and of H-2 ($\delta_H$ 6.85) and H-4 ($\delta_H$ 6.64) with C-1′ ($\delta_C$ 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 ($\delta_H$ 9.92) with C-5 ($\delta_C$ 114.3), C-6 ($\delta_C$ 165.7), and C-10a ($\delta_C$ 152.3). The methyl group at C-7 was supported by HMBC correlations of methyl proton ($\delta_H$ 2.39) with C-6 ($\delta_C$ 165.7), C-7 ($\delta_C$ 119.4), and C-8 ($\delta_C$ 136.2), and of H-8 ($\delta_H$ 7.70) with C-7′ ($\delta_C$ 16.6). Finally, the methoxy groups located at C-1 and C-6 were supported by the HMBC correlations of methoxy proton signals ($\delta_H$ 3.80 and 3.83) with C-1 ($\delta_C$ 161.9) and C-6 ($\delta_C$ 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 ($\delta_C$ 46.1 t, 206.6 s, 30.3 q; $\delta_H$ 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′ ($\delta_H$ 4.41) with C-2 ($\delta_C$ 108.1), C-3 ($\delta_C$ 138.4), and C-4 ($\delta_C$ 106.4), and of H-2 ($\delta_H$ 6.82) and H-4 ($\delta_H$ 6.64) with C-1′ ($\delta_C$ 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** ($\delta$ in ppm, in $CDCl_3$, 500 and 125 MHz)

| No. | **1** | | **2** | |
|---|---|---|---|---|
| | $d\delta_C$ | $\delta_H$ (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 $\mu$M 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 $\mu$M (%) | Compounds | Inhibition rates at 20 $\mu$M (%) |
|---|---|---|---|
| **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 ($\delta$) 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 $\mu$m, Merck, Darmstadt, Germany), and MCI gel (75–150 $\mu$m, 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) $\lambda_{max}$ (log $\varepsilon$) 318 (3.62), 253 (3.56), 210 (4.05) nm; IR (KBr): $\nu_{max}$ 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) $\lambda_{max}$ (log $\varepsilon$) 315 (3.57), 256 (3.56), 210 (4.12) nm; IR (KBr): $\nu_{max}$ 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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