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Three new xanthones from the roots of *Polygala japonica* Houtt.

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Three new xanthones from the roots of *Polygala japonica* Houtt.

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Three new xanthones, 3, 6-dihydroxy-1, 2, 7-trimethoxyxanthone (**2**), 3, 7-dihydroxy- 1, 2-dimethoxyxanthone (**4**) and 1, 2, 7-trihydroxy-3-methoxyxanthone (**9**), together with seven known compounds were isolated from the roots of *Polygala japonica* Houtt. Their structures were determined on the basis of spectroscopic data.

Keywords: *Polygala japonica* Houtt; Xanthones; 3, 6-Dihydroxy-1, 2, 7-trimethoxy-xanthone; 3, 7-Dihydroxy-1, 2-dimethoxyxanthone; 1, 2, 7-Trihydroxy-3-methoxy-xanthone

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1. Introduction

Polygala japonica Houtt. is a perennial herbaceous plant which is widely distributed in southern China, and in traditional Chinese medicine it is used as an expectorant, an anti-inflammatory agent for pharyngitis and an anti-bacterial agent. Previous researches on this plant were mainly concerned with the MeOH extract in which a series of triterpenoid saponins had been discovered [1–3]. In the present paper, the EtOAc extract, which exhibited antioxidant bioactivities, was investigated and ten compounds were isolated. These compounds included three new xanthones named 3, 6-dihydroxy-1, 2, 7-trimethoxyxanthone (**2**), 3, 7-dihydroxy-1, 2-dimethoxyxanthone (**4**) and 1, 2, 7-trihydroxy-3-methoxyxanthone (**9**) as well as seven known compounds elucidated as 7-hydroxy-1-methoxy-2, 3-methylenedioxyxanthone (**1**), 7-hydroxy-1, 3-dimethoxyxanthone (**3**), hederagenin (**5**), 3-O-(6'-O-palmitoyl-β-D-glucopyrano-syl)-spinasta-7, 22 (23)-diene (**6**), 3-O-β-D-gluco-pyranosyl-(24R)-stigmast-7, 22(E)-dien-3α-ol (**7**), presenegenin (**8**) and 1, 3, 7-trihydroxy-xanthone (**10**), respectively. All the compounds above were obtained from this plant for the first time.

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2. Results and discussion

The EtOAc parts of the 95% EtOH extract of *P. japonica* Houtt. were subjected to silica gel column chromatography and Sephadex LH-20 column chromatography repeatedly to afford three new xanthones and seven known compounds. On the basis of the spectral data and by comparison with those of literature, the structures of these xanthones were established as follows.

Compound **2** was isolated as an amorphous powder. Based on the HREIMS of **2** indicating $[M]^+$ at *m/z* 318.0730, its molecular formula was deduced to be $C_{16}H_{14}O_7$. The UV spectrum showed absorption maxima at 209, 245, 274 and 315 nm, suggesting the presence of a xanthone skeleton. Furthermore, the UV spectrum was unchanged with the addition of $AlCl_3$, indicating that neither chelated hydroxyl groups at peri-position to the carbonyl function nor di-*ortho*-hydroxyl groups existed in compound **2** [4]. The ^{1}H-NMR spectrum of **2** showed the presence of three methoxyl signals at δ 3.76, 3.81 and 3.85, two singlets of hydroxyl proton at δ10.51 and 10.73, and three singlets of aromatic protons at δ 6.70, 6.82 and 7.43 which were respectively assigned as H-4, H-5 and H-8 by the comparison of NMR data with known compound 2, 3-dihydroxy-1, 6, 7-trimethoxyxanthone [5]. The NOE experiment showed that H-8 was correlated with the methoxyl group at δ3.85 and that two other methoxyl groups were correlated with each other, indicating that three methoxyl groups were respectively linked to C-7, C-1 and C-2. Therefore compound 2 was identified as 3, 6-dihydroxy-1, 2, 7-trimethoxyxanthone. The structure was further confirmed and all signals were assigned by the HMBC spectrum (see figure 1).

Compound **4** was yellow needles from MeOH, whose molecular formula was established as $C_{15}H_{12}O_6$ by HREIMS and showed a $[M]^+$ ion at *m/z* 288.0636. The UV spectrum also suggested the presence of a xanthone skeleton at 240, 284, 313 and 360 nm. Being unchanged with the addition of $AlCl_3$ indicated that compound **4** had no chelated hydroxyl groups or di-*ortho*-hydroxyl groups either. The ^{1}H-NMR spectrum showed two methoxyl groups at δ 3.91 and 4.16, a aromatic proton at δ 7.03 and a ABX spin system at δ8.20 (d, $J = 3.0$ Hz), 7.48 (dd, $J = 9.0$, 3.0 Hz) and 7.43 (d, $J = 9.0$ Hz) indicated substitution at C-7 [6]. Comparison of the ^{13}C NMR spectral data from C-1 to C-4 of compound **4** with those of **2** revealed that **4** and **2** had identical substitution pattern in ring A (see table 1), which can be further identified in NOE experiment exhibiting a correlation between two methoxyl groups. Therefore, the compound **4** was finally identified as 3, 7-dihydroxy-1, 2-dimethoxyxanthone (figure 2).

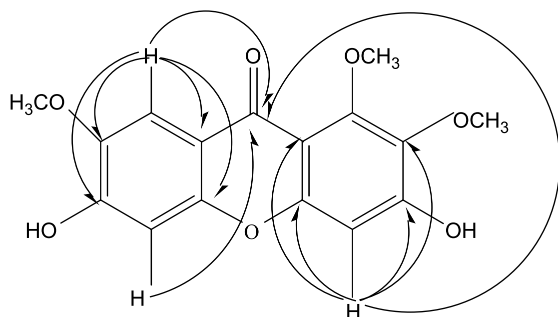

Figure 1. Selected key HMBC correlations of **2**.

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Table 1. ^{13}C NMR data of compounds **2**, **4** and **9** (125 MHz).

Position	***2****	***4*****	***9****
1	153.2	154.3	153.9
2	138.4	139.6	130.5
3	153.6	155.2[a]	153.8
4	99.3	100.4	93.9
4a	156.5	158.7	159.0
4b	153.1	155.3[b]	152.7
5	102.3	118.9	118.9
6	150.5	123.3	124.5
7	145.7	149.2	149.0
8	105.6	110.2	107.7
8a	113.7	124.0	119.9
8b	108.7	110.0	102.1
C=O	172.8	174.9	180.1
OCH_3-1	61.6	62.1	
OCH_3-2	60.8	61.2	
OCH_3-3			59.9
OCH_3-7	55.8		

*Measured in DMSO-d_6, **measured in pyridine-d_5
a and b can be exchanged with each other.

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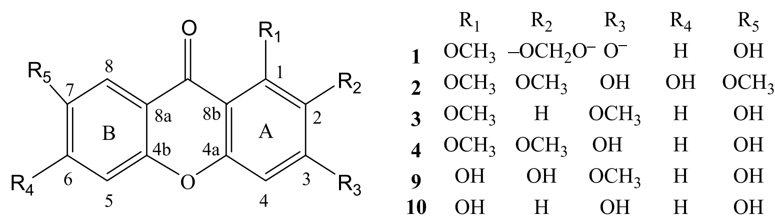
Compound **9**, yellow needles obtained from MeOH, showed a $[M]^+$ ion at *m/z* 274.0476 in the HREIMS which corresponded to the molecular formular $C_{14}H_{10}O_6$. The ^{1}H-NMR spectrum of **9** showed the presence of a H-4 (δ6.45, s), a methoxyl group (δ3.79, s), a chelated hydroxyl group (δ12.95, s) on ring A and a ABX spin system (δ 7.45, d, $J = 9.0$ Hz; δ7.40, d, $J = 3.0$ Hz; δ7.27, dd, $J = 9.0$ Hz, 3.0 Hz) characteristic for 7-hydroxyxanthones. It also exhibited a xanthone skeleton in the UV spectrum at 237, 261, 316, 358 nm. However, a bathochromic shift was induced by addition of $AlCl_3$ at 234, 248 (sh), 273, 326 nm. After addition of HCl, the absorption maximum of **9** was shifted to a short wavelength at 236, 261, 319 nm, which indicated the existence of di-*ortho*-hydroxyl groups in compound **9** [4]. This conclusion can be further confirmed by NOE experiment in which a correlation between H-4 and the methoxyl group was observed, indicating the methoxyl group was linked to C-3. Thus the di-*ortho*-hydroxyl groups were respectively linked to C-1 and C-2. The ^{13}C-NMR spectrum of **9** (see table 1) was basically identical with the one of 1, 3, 7-trihydroxy-2-methoxyxanthone [6]. Considered all the data given above, the structure of compound **9** was determined as 1, 2, 7-trihydroxy-3-methoxyxanthone.



	R_1	R_2	R_3	R_4	R_5
1	OCH_3	$-OCH_2O-$	O^-	H	OH
2	OCH_3	OCH_3	OH	OH	OCH_3
3	OCH_3	H	OCH_3	H	OH
4	OCH_3	OCH_3	OH	H	OH
9	OH	OH	OCH_3	H	OH
10	OH	H	OH	H	OH

Figure 2. Chemical structure of xanthones in *Polygala japonica* Houtt.

3. Experimental

3.1 General experimental procedures

Melting points were determined on an XT-4 micro-melting point apparatus and are uncorrected. IR spectra were recorded on an IMPACT 400 spectrometer as KBr pellets. UV spectra were obtained on a shimadzu UV-260 spectrophotometer. NMR spectra were run on Varian INOVA-500 spectrometer using TMS as internal standard. ESIMS were measured on Agilent 1100 series LC/MSD Trap SL mass spectrometer and Autospee-Ultima ETOF, respectively. Silica gel (100–200, 200–300 mesh, Qingdao) was used for column chromatography and silica gel GF-254 (Qingdao) for TLC.

3.2 Plant material

The roots of *Polygala japonica* Houtt. were collected on June 2003 in Jiangxi Province, China. And this plant was identified by Professor Yongming Luo, Jiangxi College of Traditional Chinese Medicine. A voucher specimen is deposited in Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing.

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3.3 Extraction and isolation

Dried roots of *P. japonica* Houtt. (5.0 kg) were extracted three times with 95% EtOH for 2 h each time, obtained 0.839 kg residue on removal of the solvent under reduced pressure. This residue was subjected to silica-gel column chromatography (100–200 mesh), eluted with $CHCl_3$, EtOAc, EtOAc:Acetone (1:1), EtOAc:Acetone (1:3), Acetone, Acetone:EtOH (1:1), EtOH and MeOH, successively. The EtOAc extract (30.5 g) was subjected to silica-gel column chromatography (100–200 mesh), eluting with a gradient of $CHCl_3$–MeOH (100:1 → 100:25) to yield 61 fractions. Fractions 19–20 were separated by Sephadex LH-20 column chromatography ($CHCl_3$–MeOH 2:1) and recrystallized from MeOH to give **1** (17 mg) and **2** (13 mg). Fractions 21–22, in the same way, yielded **3** (10 mg) and **9** (12 mg). Fractions 23–25 were subjected to silica-gel column chromatography (200–300 mesh) and eluted with a gradient of Me_2CO in petroleum ether (1:3 → 1:1) to yield **4** (9 mg) and **10** (4 mg). Fraction 30 was rechromatographed on a silica-gel column chromatography (200–300 mesh) also and eluted with a gradient of Me_2CO in petroleum ether (1:4 → 1:3) to yield **5** (67 mg). Compounds **6** (12 mg) and **7** (70 mg) were yielded from fraction 33 and fraction 45, respectively. Fractions 49–54 were separated by Sephadex LH-20 column chromatography ($CHCl_3$–MeOH 2:1) and then purified by silica-gel column chromatography (200–300 mesh, $CHCl_3$–MeOH 9:1) to give **8** (40 mg).

3.3.1 3, 6-Dihydroxy-1, 2, 7-trimethoxyxanthone (2). light yellow needles (MeOH); mp 257–258°C; UV (MeOH) λ_{max} (nm) (log ε): 209 (4.35), 245 (4.50), 274 (4.00), 315 (4.27); ($AlCl_3$) λ_{max} 209, 245, 274, 315. IR (KBr) ν_{max} (cm^{-1}): 3141, 2852, 1620, 1570, 1479; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 10.73 (1H, s, OH-3/OH-6), 10.51 (1H, s, OH-3/OH-6), 7.43 (1H, s, H-8), 6.82 (1H, s, H-5), 6.70 (1H, s, H-4), 3.85 (3H, s, OCH_3-7), 3.81 (3H, s, OCH_3-1), 3.74 (3H, s, OCH_3-2); ^{13}C-NMR data, see table 1; ESIMS *m/z*: 319 $[M + H]^+$; HREIMS *m/z* 318.0730 $[M]^+$ (calcd for $C_{16}H_{14}O_7$, 318.0740).

3.3.2 3, 7-Dihydroxy-1, 2-dimethoxyxanthone (4). yellow needles (MeOH); mp 250–252°C; UV (MeOH) λ_{max} (nm) (log ε): 240 (4.73), 285 (4.14), 314 (4.29), 360 (3.99); ($AlCl_3$) λ_{max} 240, 285, 314, 361 nm; IR (KBr) ν_{max} s(cm^{-1}): 3365, 1608, 1568, 1475; ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 8.20 (1H, d, $J = 2.5$ Hz, H-8), 7.48 (1H, dd, $J = 9.0$, 2.5 Hz, H-6), 7.43 (1H, d, $J = 9.0$ Hz, H-5), 7.03(1H, s, H-4), 4.16 (3H, s, OCH_3-1), 3.91 (3H, s, OCH_3-2); ^{13}C-NMR data, see table 1; ESIMS *m/z*: 289 $[M + H]^+$; HR-EIMS *m/z* 288.0636 $[M]^+$ (calcd for $C_{15}H_{12}O_6$, 288.0633).

3.3.3 1, 2, 7-Trihydroxy-3-methoxyxanthone (9). yellow needles (MeOH); mp 245–246°C; UV (MeOH) λ_{max} (nm) (log ε): 237 (4.15), 261 (4.08), 316 (3.78), 358 (2.99); ($AlCl_3$) λ_{max} 234, 248(sh), 273, 326; ($AlCl_3$/HCl) λ_{max} 236, 261, 319; IR (KBr) ν_{max} (cm^{-1}): 3384, 2920, 1653, 1612, 1581, 1477; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 12.95 (1H, s, OH-1), 7.45 (1H, d, $J = 9.0$ Hz), 7.40 (1H, d, $J = 3.0$ Hz), 7.27 (1H, dd, $J = 9.0$ Hz, 3.0 Hz), 6.45 (1H, s, H-4), 3.79 (3H, s, OCH_3); ^{13}C-NMR data, see table 1; ESIMS *m/z*: 273 $[M - H]^+$; HR-EIMS *m/z* 274.0476 $[M]^+$(calcd for $C_{14}H_{10}O_6$, 274.0477).

7-Hydroxy-1-methoxy-2, 3-methylenedioxyxanthone (**1**): light yellow amorphous power; ^{1}H-NMR, ^{13}C-NMR and EIMS data were identical to literature [7].

7-Hydroxy-1, 3-dimethoxyxanthone (**3**): white needles (MeOH); ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 12.00 (1H, s, OH-1), 8.21 (1H, d, $J = 2.5$ Hz, H-8), 7.48 (1H, dd, $J = 9.0$, 2.5 Hz, H-6), 7.45 (1H, d, $J = 9.0$ Hz, H-5), 6.60 (1H, d, $J = 2.5$ Hz, H-4), 6.47 (1H, d, $J = 2.5$ Hz, H-2), 3.82 (3H, s, OCH_3-3/OCH_3-7), 3.78 (3H, s, OCH_3-3/OCH_3-7); ^{13}C-NMR (pyridine-d_5, 125 MHz) δ: 174.6 (C=O), 165.1 (C-1), 162.4 (C-3), 160.2 (C-4a), 155.4 (C-4b), 149.0 (C-7), 124.6 (C-6, C-8a), 118.8 (C-5), 110.5 (C-8, C-8b), 95.5 (C-4), 93.3 (C-2), 56.2 (OCH_3-3/OCH_3-7), 55.8 (OCH_3-3/OCH_3-7). ESIMS *m/z*: 567 $[2M + Na]^+$.

Hederagenin (**5**): white amorphous power; ^{1}H-NMR and ^{13}C-NMR data were identical to literature [8]; ESIMS *m/z*: 495 $[M + Na]^+$.

3-O-[6'-O-palmitoyl-β-D-glucopyranosyl]-spinasta-7, 22(23)-diene (**6**): colourless crystals ($CHCl_3$); all spectroscopic data were identical to literature [9].

3-O-β-D-glucopyranosyl-(24R)-stigmast-7, 22(E)-dien-3α-ol (**7**): white amorphous power; ^{1}H-NMR and ^{13}C-NMR data were identical to literature [10]; FABMS *m/z*: 572 $[M]^+$.

Presenegenin (**8**): white amorphous power; ^{1}H-NMR and ^{13}C-NMR were identical to literature [11]; ESIMS *m/z*: 541 $[M + Na]^+$.

1, 3, 7-Trihydroxyxanthone (**10**): light yellow amorphous power; all spectroscopic data were identical to literature [12].

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