

Xanthenes from Stems of *Hypericum chinense*

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Six new xanthenes, 4,6-dihydroxy-2,3-dimethoxyxanthone (**1**), 2,6-dihydroxy-3,4-dimethoxyxanthone (**2**), 6-hydroxy-2,3,4-trimethoxyxanthone (**3**), 3,6-dihydroxy-1,2-dimethoxyxanthone (**4**), 4,7-dihydroxy-2,3-dimethoxyxanthone (**5**), and 3,7-dihydroxy-2,4-dimethoxyxanthone (**6**) were isolated from the stems of *Hypericum chinense*, together with four known xanthenes. Their structures were established based on spectroscopic studies.

Key words *Hypericum chinense*; xanthone; Clusiaceae

The family Clusiaceae is a rich source of xanthenes,^{1,2} which show various bioactivities: anti-methicillin-resistant *Staphylococcus aureus* (MRSA),^{3–5} anti-vancomycin-resistant *Enterococci* (VRE),⁶ anti-malarial,⁷ tumor-promoting inhibition,⁸ selective cyclooxygenase-2 inhibition,⁹ inhibitory effects on PAF-induced hypotension.¹⁰ The genus *Hypericum* belonging to Clusiaceae are distributed widely in temperate regions, and have been used as traditional medicines in various parts of the world. In Japan, *H. chinense* is used as a folk medicine for treatment of female disorders.¹¹ Anti-bacterial acylphloroglucinols and spiro-lactones were also isolated from this species.^{11–14} In the course of our search for bioactive metabolites from plants, we became interested in the *Hypericum* plants and began to study their chemical constituents. As a part of this program, we studied the constituents of this plant, and isolated six new and four known xanthenes. We report herein the isolation and the structure elucidation of these compounds.

The MeOH extracts of *H. chinense* stems were partitioned with *n*-hexane, EtOAc, and H₂O. From the EtOAc soluble fraction, six new (**1–6**) and four known (**7–10**) xanthenes were isolated.

Compound **1** showed a pseudomolecular ion at *m/z* 289.0728, [M+H]⁺ (Calcd for C₁₅H₁₂O₆, 289.0712) in its HR-ESI-MS, suggesting the molecular formula of C₁₅H₁₂O₆. The ¹H-NMR spectrum of **1** showed the presence of a 1,2,4-trisubstituted benzene ring [δ_{H} 8.58 (1H, d, *J*=8.8 Hz), 7.19 (1H, dd, *J*=8.8, 1.6 Hz), 7.08 (1H, d, *J*=1.6 Hz)], one aromatic proton [δ_{H} 7.64 (1H, s)], and two methoxyl groups [δ_{H} 3.96, 3.79 (each 3H, s)]. Its ¹³C-NMR revealed the presence of 15 carbons including one conjugated carbonyl carbon, 12 aromatic carbons, and two methoxyl carbons. These data suggested that **1** was a xanthone derivative having two hydroxyl groups and two methoxyl groups. In the HMBC spec-

trum, the following key long-range correlations were observed: H-8 (δ_{H} 8.58) with C-9 (δ_{C} 175.8); H-1 (δ_{H} 7.64) with C-2 (δ_{C} 150.9), C-3 (δ_{C} 142.9), C-4a (δ_{C} 143.1), C-9a (δ_{C} 118.5), and C-9; OMe-2 (δ_{H} 3.79) with C-2; OMe-3 (δ_{H} 3.96) with C-3. The NOE correlation between H-1 with OMe-2 was observed in its NOESY spectrum. On the basis of these data, two hydroxyl groups and two methoxyl groups could be placed on C-4, 6, 2, and 3, respectively. Thus, the structure of **1** was determined as 4,6-dihydroxy-2,3-dimethoxyxanthone (Fig. 1).

Compound **2** has the same molecular formula of C₁₅H₁₂O₆ as **1**. Comparison of the ¹³C-NMR chemical shifts of **2** and **1** showed good agreement on C-5—8, 9, 9a, and 10a. In its HMBC spectrum, the following correlations were observed: H-1 (δ_{H} 8.08) with C-2 (δ_{C} 149.1), C-3 (δ_{C} 148.0), C-4a (δ_{C} 145.0), and C-9 (δ_{C} 175.5); OMe-3 (δ_{H} 4.08) with C-3; OMe-4 (δ_{H} 4.05) with C-4 (δ_{C} 142.3). From these data, **2** was determined as 2,6-dihydroxy-3,4-dimethoxyxanthone (Fig. 1).

Compound **3**, C₁₆H₁₄O₆, revealed the presence of four aromatic protons [δ_{H} 8.55 (1H, d, *J*=8.4 Hz), 7.78 (1H, s), 7.27 (1H, d, *J*=2.0 Hz), 7.22 (1H, dd, *J*=8.4, 2.0 Hz)] and three methoxyl groups [δ_{H} 4.02, 4.00, 3.76 (each 3H, s)] in its ¹H-NMR spectrum. Its ¹³C-NMR spectrum showed the presence of one conjugated carbonyl carbon, 12 aromatic carbons, and three methoxyl carbons. From these data, **3** was considered as tetrasubstituted xanthone derivative by one hydroxyl and three methoxyl groups. The ¹³C-NMR chemical shifts of **3** were compared with those of **1**, and they showed good agreement on the chemical shifts of C-5—8, 8a, 9, and 10a. So, the hydroxyl group could be placed on C-6. The positions of methoxyl groups were determined by the analysis of long-range correlations in its HMBC spectrum shown in Fig. 2. This fact was supported by the NOE correlation between H-1 (δ_{H} 7.78) and OMe-2 (δ_{H} 3.76). Thus, the structure of **3** was decided as 6-hydroxy-2,3,4-trimethoxyxanthone (Fig. 1).

The ¹H-NMR spectral data of **4** was similar to that of **1** except for the chemical shifts of singlet aromatic protons (δ_{H}

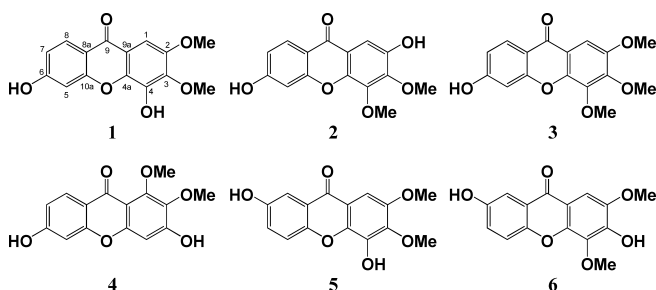


Fig. 1. Structures of New Xanthenes Isolated from *H. chinense*

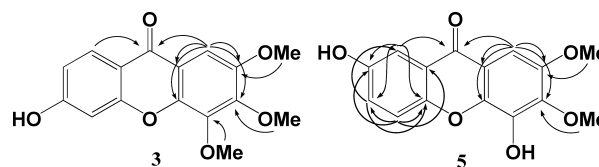


Fig. 2. The Key Long-Range Correlations of **3** and **5**

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7.00 in **4**; δ_{H} 7.64 in **1**). This fact suggested that the singlet aromatic proton could not be placed at C-1. In its HMBC spectrum, the following correlations were observed: H-4 (δ_{H} 7.00) with C-2 (δ_{C} 139.7), C-3 (δ_{C} 158.3), C-4a (δ_{C} 155.2), and C-9a (δ_{C} 110.4); OMe-1 (δ_{H} 4.20) with C-1 (δ_{C} 154.6); OMe-2 (δ_{H} 3.92) with C-2 (δ_{C} 139.7). These data indicated that one hydroxyl group and two methoxyl groups could be placed on C-3, C-1, and C-2, respectively. Thus, the structure of **4** was elucidated as 3,6-dihydroxy-1,2-dimethoxyxanthone (Fig. 1).

Compound **5** had a molecular formula of $\text{C}_{15}\text{H}_{12}\text{O}_6$ on the basis of HR-ESI-MS. The ^{13}C -NMR spectrum of **5** showed the signals of one conjugated carbonyl carbon, 12 aromatic carbons, and two methoxyl carbons. The presence of the 1,2,4-trisubstituted benzene ring [δ_{H} 8.25 (1H, d, $J=1.6$ Hz), 7.52 (1H, dd, $J=9.2, 1.6$ Hz), 7.48 (1H, d, $J=9.2$ Hz)], one aromatic proton [δ_{H} 7.59 (1H, s)], and two methoxyl groups [δ_{H} 3.98, 3.77 (each 3H, s)] were revealed in its ^1H -NMR spectrum. From these data, **5** was considered as tetrasubstituted xanthone derivative having two hydroxyl and two methoxyl groups. The ^{13}C -NMR chemical shifts of **5** were compared with those of **1**, and they showed good agreement on the chemical shifts of C-1—4, 4a, and 9a. So, the positions of two methoxyl groups and one hydroxyl group were supposed to be at C-2, 3, and 4, respectively. This fact was supported by the NOE correlation between H-1 (δ_{H} 7.59) with OMe-2 (δ_{H} 3.77). The position of the remaining hydroxyl group was decided by the analysis of long-range correlations in its HMBC spectrum shown in Fig. 2. Thus, the structure of **5** was elucidated as 4,7-dihydroxy-2,3-dimethoxyxanthone (Fig. 1).

Compound **6**, $\text{C}_{15}\text{H}_{12}\text{O}_6$, was a xanthone derivative having two hydroxyl and two methoxyl groups on the basis of its ^1H - and ^{13}C -NMR spectrum. Comparison of the ^{13}C -NMR chemical shifts of **6** and **5** showed good agreement on C-5—8, 8a, 9, and 10a. In the NOESY spectrum of **6**, the proton signal of H-1 (δ_{H} 7.80) was correlated with the proton signal of OMe-2 (δ_{H} 4.12). The HMBC spectrum of **6** showed the following long-range correlations: H-1 with C-2 (δ_{C} 147.4), C-3 (δ_{C} 147.2), C-4a (δ_{C} 148.7), and C-9 (δ_{C} 176.0); OMe-2 (δ_{H} 4.12) with C-2; OMe-4 (δ_{H} 3.75) with C-4 (δ_{C} 136.4). From these data, the structure of **6** was decided as 3,7-dihydroxy-2,4-dimethoxyxanthone (Fig. 1).

The following known compounds were identified by comparison with literature data; 3-hydroxy-2-methoxyxanthone (**7**),¹⁵ 1,5-dihydroxy-3-methoxyxanthone (**8**),¹⁶ 3,4-dihydroxy-2-methoxyxanthone (**9**),¹⁷ 1,5,6-trihydroxy-3-methoxyxanthone (**10**).¹⁸

Experimental

General Experimental Procedures NMR experiments were run on a Bruker ARX-400 instrument, ^1H -NMR: 400 MHz, ^{13}C -NMR: 100 MHz, using TMS as int. stand. MS were obtained on a Waters LCT Premier. Chromatography column: silica gel 60 (Merck, 63—210 mm), Sephadex LH-20 (pharmacia), and Toyopearl HW-40 (TOSOH); HPLC: GPC (Shodex H-2001, 2002, CHCl_3 ; Asahipak, GS-310 2G, MeOH), silica gel HPLC (YMC-Pack SIL-06 SH-043-5-06, 250×20 mm). IR spectra were recorded on a 1720 Infrared Fourier Transform spectrometer (Perkin-Elmer). Optical rotations were measured with a JASCO DIP-370 digital polarimeter.

Plant Material The aerial parts of *Hypericum chinense* were collected in October 2002 in Tokushima Prefecture, Japan, and identified by Dr. Kotaro Murakami (Sojo University). Herbarium specimens were deposited in the botanical garden of the University of Tokushima (specimen number: UTP98008).

Table 1. ^{13}C -NMR Spectral Data (δ , Measured in Pyridine- d_5) for New Xanthones (**1**—**6**)

Position	1	2	3	4	5	6
1	96.9	106.1	102.0	154.6	96.5	101.2
2	150.9	149.1	150.8 ^{a)}	139.7	150.9	147.4
3	142.9	148.0	148.6	158.3	143.1	147.2
4	141.5	142.3	142.4	100.6	141.5	136.4
4a	143.1	145.0	146.3	155.2	143.5	148.7
10a	158.9	158.8	158.9	157.7	150.5	150.0 ^{a)}
5	103.0	103.3	103.4	102.5	119.7	119.8
6	165.0	165.5	165.5	164.5	124.7	124.2
7	114.9	114.9	115.3	114.3	155.4	155.4
8	128.9	128.7	128.9	128.8	109.9	110.0
8a	115.1	114.8	114.9	116.0	122.7	122.9
9	175.8	175.5	176.1	174.4	176.6	176.0
9a	118.5	118.9	118.9	110.4	117.7	113.6
1-OMe	—	—	—	62.2	—	—
2-OMe	55.9	—	56.3	61.3	55.9	55.1
3-OMe	60.8	61.8	61.6	—	60.8	—
4-OMe	—	61.1	62.2	—	—	61.2

a) Overlapped with solvent.

Extraction and Isolation The stems of *H. chinense* (4.54 kg, dried) were crushed and extracted (3×18 l) with MeOH at 60 °C for 4 h. The MeOH extracts were concentrated *in vacuo* to give a residue (548 g), which was partitioned between *n*-hexane, EtOAc, and H₂O. The EtOAc soluble fraction (96.8 g) was subjected to a silica gel CC eluted with solvents of increasing polarity (*n*-hexane–EtOAc–MeOH) to give 13 fractions (fr. 1—13). Fr. 7 (4.54 g) was separated by a Sephadex LH-20 column with MeOH to give four fractions (fr. 7.1—4). Fr. 7.4 was subjected to a silica gel column eluted with solvents of increasing polarity (CHCl_3 –MeOH) to give four fractions (fr. 7.4.1—4). Fr. 7.4.1 was purified by GPC with MeOH to give **7** (8 mg) and **8** (4 mg). Fr. 9 (8.85 g) was chromatographed on a Sephadex LH-20 column with MeOH to give four fractions (fr. 9.1—4). Fr. 9.4 was separated by a silica gel column chromatography with solvents of increasing polarity (CHCl_3 –MeOH) to give six fractions (fr. 9.4.1—6). Fr. 9.4.3 was purified by a GPC (CHCl_3) and a silica gel HPLC with CHCl_3 –acetone (3 : 1) to give **5** (5 mg) and **6** (9 mg). Fr. 9.4.4 was applied to a Sephadex LH-20 column to give two fractions (fr. 9.4.4.1, 2). Fr. 9.4.4.1 was loaded to GPC (MeOH) to give ten fractions (fr. 9.4.4.1.1—10). Fr. 9.4.4.1.1 was recrystallized from MeOH–H₂O (85 : 15) to give **4** (10 mg). Fr. 9.4.4.1.5 was purified by prep. TLC with CHCl_3 –MeOH–H₂O (85 : 15 : 0.1) to give **2** (10 mg). Fr. 9.4.4.1.6 and 7 was recrystallized from MeOH–H₂O (7 : 3) to give **1** (15 mg) and **9** (6 mg), respectively. Fr. 9.4.4.1.10 was separated by prep. TLC with CHCl_3 –MeOH–H₂O (85 : 15 : 0.1) to give **10** (5 mg). Fr. 8 (3.35 g) was subjected to a silica gel chromatography with solvents of increasing polarity (CHCl_3 –MeOH) to give seven fractions (fr. 8.1—7). Fr. 8.3 was chromatographed on a Toyopearl HW-40 with CHCl_3 –MeOH (1 : 1) and purified by a GPC (MeOH) to give **3** (5 mg).

4,6-Dihydroxy-2,3-dimethoxyxanthone (**1**): Pearl white powder. IR (KBr) ν_{max} cm^{-1} : 2934, 1614, 1458, 1390, 1254, 1167, 1134, 1111; ESI-MS m/z : 289.0728, $[\text{M}+\text{H}]^+$ (Calcd for $\text{C}_{15}\text{H}_{13}\text{O}_6$, 289.0712); ^1H -NMR (pyridine- d_5) δ : 8.58 (1H, d, $J=8.8$ Hz, H-8), 7.64 (1H, s, H-1), 7.19 (1H, dd, $J=8.8, 1.6$ Hz, H-7), 7.08 (1H, d, $J=1.6$ Hz, H-5), 3.96 (3H, s, OMe-3), 3.79 (3H, s, OMe-2); ^{13}C -NMR: see Table 1.

2,6-Dihydroxy-3,4-dimethoxyxanthone (**2**): Yellow powder. IR (KBr) ν_{max} cm^{-1} : 3446, 1612, 1516, 1464, 1438, 1387, 1286, 1246, 1084, 1030; ESI-MS m/z : 311.0563, $[\text{M}+\text{Na}]^+$ (Calcd for $\text{C}_{15}\text{H}_{12}\text{O}_6\text{Na}$, 311.0532); ^1H -NMR (pyridine- d_5) δ : 8.51 (1H, d, $J=8.8$ Hz, H-8), 8.08 (1H, s, H-1), 7.27 (1H, d, $J=2.0$ Hz, H-5), 7.19 (1H, dd, $J=8.0, 2.0$ Hz, H-7), 4.08 (3H, s, OMe-3), 4.05 (3H, s, OMe-4); ^{13}C -NMR: see Table 1.

6-Hydroxy-2,3,4-trimethoxyxanthone (**3**): Pearl white powder. IR (KBr) ν_{max} cm^{-1} : 3430, 1610, 1568, 1467, 1431, 1381, 1248, 1182, 1132, 1107; ESI-MS m/z : 303.0883, $[\text{M}+\text{H}]^+$ (Calcd for $\text{C}_{16}\text{H}_{15}\text{O}_6$, 303.0869); ^1H -NMR (pyridine- d_5) δ : 8.55 (1H, d, $J=8.4$ Hz, H-8), 7.78 (1H, s, H-1), 7.27 (1H, d, $J=2.0$ Hz, H-5), 7.22 (1H, dd, $J=8.4, 2.0$ Hz, H-7), 4.02 (3H, s, OMe-4), 4.00 (3H, s, OMe-3), 3.76 (3H, s, OMe-2); ^{13}C -NMR: see Table 1.

3,6-Dihydroxy-1,2-dimethoxyxanthone (**4**): Pearl white needle. IR (KBr) ν_{max} cm^{-1} : 3442, 1616, 1573, 1466, 1429, 1360, 1294, 1265, 1080; ESI-MS m/z : 289.0715, $[\text{M}+\text{H}]^+$ (Calcd for $\text{C}_{15}\text{H}_{13}\text{O}_6$, 289.0712); ^1H -NMR (pyridine- d_5) δ : 8.50 (1H, d, $J=8.8$ Hz, H-8), 7.14 (1H, dd, $J=8.8, 2.4$ Hz, H-7),

7.06 (1H, d, $J=2.4$ Hz, H-5), 7.00 (1H, s, H-4), 4.20 (3H, s, OMe-1), 3.92 (3H, s, OMe-2); $^{13}\text{C-NMR}$: see Table 1.

4,7-Dihydroxy-2,3-dimethoxyxanthone (**5**): Yellow powder. IR (KBr) ν_{max} cm^{-1} : 3194, 1614, 1589, 1469, 1390, 1356, 1248, 1203, 1132; ESI-MS m/z : 289.0696, $[\text{M}+\text{H}]^+$ (Calcd for $\text{C}_{15}\text{H}_{13}\text{O}_6$, 289.0712); $^1\text{H-NMR}$ (pyridine- d_5) δ : 8.25 (1H, d, $J=1.6$ Hz, H-8), 7.59 (1H, s, H-1), 7.52 (1H, dd, $J=9.2$, 1.6 Hz, H-6), 7.48 (1H, d, $J=9.2$ Hz, H-5), 3.98 (3H, s, OMe-3), 3.77 (3H, s, OMe-2); $^{13}\text{C-NMR}$: see Table 1.

3,7-Dihydroxy-2,4-dimethoxyxanthone (**6**): Yellow powder. IR (KBr) ν_{max} cm^{-1} : 3430, 1610, 1574, 1479, 1462, 1398, 1335, 1296, 1240, 1207, 1132; ESI-MS m/z : 311.0534, $[\text{M}+\text{Na}]^+$ (Calcd for $\text{C}_{15}\text{H}_{12}\text{O}_6\text{Na}$, 311.0532); $^1\text{H-NMR}$ (pyridine- d_5) δ : 8.26 (1H, d, $J=2.4$ Hz, H-8), 7.80 (1H, s, H-1), 7.64 (1H, d, $J=8.8$ Hz, H-5), 7.56 (1H, dd, $J=8.8$, 2.4 Hz, H-6), 4.12 (3H, s, OMe-2), 3.75 (3H, s, OMe-4); $^{13}\text{C-NMR}$: see Table 1.

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