Pergamon

PII: S0031–9422(98)0010-6X

*Phytochemistry*, Vol. 49, No. 5, pp. 1417–1420, 1998

Printed in Great Britain
0031–9422/98/$—see front matter

# CHROMONE GLYCOSIDES AND FLAVONOIDS FROM *HYPERICUM JAPONICUM*

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(*Received* 12 *January* 1998; *received in revised form* 12 *January* 1998)

**Key Word Index**—*Hypericum japonicum*; Clusiaceae; chromone glycoside; flavonoid; coagulant activity; 5, 7-dihydroxy-2-(1-methylpropyl) chromone-8-*β*-D-glucoside; 5, 7-dihydroxy-2-isopropylchromone-8-*β*-D-glucoside; 7, 8-(2″, 2″-dimethylpyrano)-5, 3′, 4′-trihydroxy-3-mehoxyflavone; (2R,3R) dihydroquercetin-3, 7-O-*α*-L-dirhamnoside.

**Abstract**—From the aerial parts of *Hypericum japonicum*, two novel chromone glycosides, 5, 7-dihydroxy-2-(1-methylpropyl) chromone-8-*β*-D-glucoside and 5, 7-dihydroxy-2-isopropylchromone-8-*β*-D-glucoside, and two new flavonoids, 7, 8-(2″, 2″-dimethylpyrano)-5, 3′, 4′-trihydroxy-3-mehoxyflavone and (2R, 3R) dihydroquercetin-3, 7-O-*α*-L-dirhamnoside were isolated together with nine known flavonoids. Their structures were deduced from spectroscopic and chemical evidence. Some of the compounds were found to exert an interesting coagulant activity in an *in vitro* test. 

## INTRODUCTION

*Hypericum* (Clusiaceae) is a large genus of herbs or shrubs, which occurs widely in temperate regions of the world. In China there are 55 species and 8 subspecies and half of them have been used in Chinese herbal medicine mainly for the treatment of infectious hepatitis. The recent surge of interest in chemistry of this genus has led to the isolation of of more than 100 components with different biological activities. In particular, extracts of *H. perforatum* are now widely used in Europe as drugs for the treatment of depression [1–3].

*H. japonicum* Thunb. ex Murray is a Chinese medicinal plant [4]. Previous papers reported the isolation of thirteen phloroglucinol derivatives [4–8], five flavonoids [9, 10], a peptide [11], a lactone [12] and two xanthones [10, 13]. As a chemical investigation of this plant, two new chromone glycosides, two novel flavonoids and nine known flavonoids were isolated; some of them showed good coagulant bioactivity *in vitro*.

## RESULTS AND DISCUSSION

The ethanol extract of dried aerial parts was extracted with petrol, $CH_2Cl_2$, EtOAc, $Me_2CO$ and MeOH in a Soxhlet. The EtOAc and $Me_2CO$ fractions were purified and thirteen chromone glycosides and flavonoids were obtained, four of which are new compounds (**1**, **2, 3** and **4**).

Compound **1**, white crystals, mp 80–82°, $[\alpha]_D^{25} = +13.4°$(MeOH), has the molecular formula of $C_{19}H_{24}O_9$ based on EI-MS ($M^+$ *m*/*z* 396), $^1$H NMR and $^{13}$C NMR. Both the UV (MeOH) and IR (KBr) absorption maxima (250, 270 nm and 1660, 1620, 1590, 1430 $cm^{-1}$, respectively) of the compound were typical of a 5, 7-dioxygenated chromone nucleus [14].

In its $^1$H NMR (500 MHz, DMSO-$d_6$) spectrum, the upfield proton signals at $\delta$ 2.63 (1H, m, H-1′), 1.70, 1.56 (each 1H, *m*, H-2′$^{a,b}$), 1.21 (3H, *d*, $J = 6.9$ Hz, Me-1′) and 0.86 ppm (3H, *t*, $J = 7.4$ Hz, H-3′) indicated the presence of 1-methylpropyl group. The aromatic proton singlets at $\delta$ 6.20 (1H), 6.12 ppm (1H) were due to H-6 or H-8 and H-3 [14–16]. The anomeric proton signal at $\delta$ 4.63 (1H, *d*, $J = 10.0$ Hz, H-1″) and carbon signals at $\delta$ 81.5 (C-5″), 78.7 (C-3″), 73.3 (C-1″), 71.2 (C-2″), 70.9 (C-4″), 61.7 (C-6″) in its $^{13}$C NMR (125 MHz, DMSO-$d_6$) showed the presence of *β*-D-glucose moiety. In its HMBC, the proton signals at $\delta$ 4.63 (H-1″) and 6.12 ppm (H-3) corresponding to the carbon signals at $\delta$ 164.2 (C-7), 104.7 (C-8), 156.6 (C-8a) and 39.8 (C-1′) respectively, suggested the location of glucose moiety and 1-methylpropyl to be at C-8 and C-2 (Figure 1). Thus **1** is 5, 7-dihydroxy-2-(1-methylpropyl)chromone-8-*β*-D-glucoside. From the $^1$H-$^1$H COSY and $^{13}$C-$^1$H COSY, all of the carbon signals were assigned (see Experimental).

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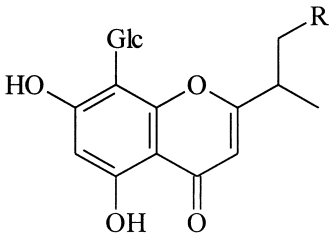
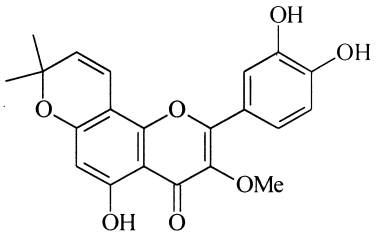
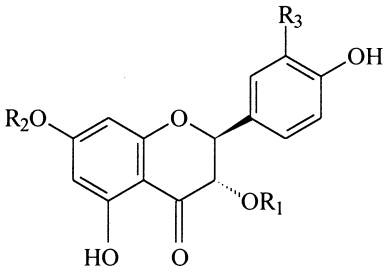


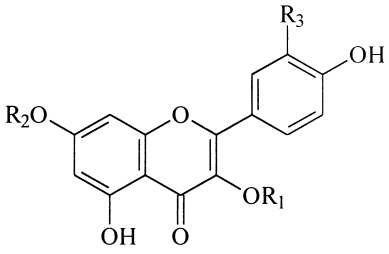

**1** R=Me
**2** R=H




**3**




**4** $R_1$=$R_2$=Rha, $R_3$=OH
**5** $R_1$=H, $R_2$=Rha, $R_3$=OH
**6** $R_1$=$R_2$=H, $R_3$=OH
**7** $R_1$=$R_3$=H, $R_2$=Rha




**8** $R_1$=Glc, $R_2$=H, $R_3$=OH
**9** $R_1$=H, $R_2$=Rha, $R_3$=OH
**10** $R_1$=Rha (2→1) Rha, $R_2$=H, $R_3$=OH
**11** $R_1$=$R_2$=H, $R_3$=OH
**12** $R_1$=$R_2$=$R_3$=H
**13** $R_1$=$R_3$=H, $R_2$=Rha

Compound **2**, gave white powder, mp 152–154°, $[\alpha]_D^{25} = +9.8°$(MeOH), with molecular formula of $C_{18}H_{22}O_9$ based on EI-MS ($M^+$ *m/z* 382), $^1$H NMR and $^{13}$C NMR. The bands at 250, 270 in its UV (MeOH) and 1655, 1620, 1430 $cm^{-1}$ in its IR (KBr) showed presence of 5, 7-dioxygenated chromone nucleus [14].

The $^1$H NMR and $^{13}$C NMR spectra of **2** closely resembled those of **1** except for upfield proton and carbon signals. In its $^1$H NMR (500 MHz, DMSO-$d_6$) spectrum, the upfield proton signals at $\delta$ 2.88 (1H, m, H′) and 1.26, 1.25 (each 3H, *d*, *J*=6.6 Hz, 1′-Me's) indicated the presence of isopropyl group at C-2. The aromatic proton singlets at $\delta$ 6.25 (1H), 6.14 ppm (1H) were due to H-6 and H-3 [14–16], respectively. The anomeric proton signal at $\delta$ 4.64 (1H, *d*, *J*=9.8 Hz, H1″) and carbon signals at $\delta$ 81.5 (C-5″), 78.6 (C-3″), 73.1 (C-1″), 71.2 (C-2″), 70.8 (C-4″), 61.8 (C-6″) in its $^{13}$C NMR (125 MHz, DMSO-$d_6$) showed the presence of $\beta$-D-glucose moiety at C-8. Thus taking into consideration all mentioned above data and analyses, **2** was established as 5, 7-dihydroxy-2-isopropyl-chromone-8-$\beta$-D-glucoside, which is in agreement with its $^{13}$C NMR spectral data (see experimental).

Compound **3**, pale yellow crystals, mp 190–192°, has the molecular formula, $C_{21}H_{18}O_7$, which was established by its EI-MS ($M^+$ *m/z* 382), $^1$H NMR and $^{13}$C NMR spectra. The UV (MeOH) bands at 248, 282sh, 324 nm suggested a flavonol skeleton. The $^1$H NMR (300 MHz, DMSO-$d_6$) spectrum of **3** showed the aromatic proton signals at $\delta$ 7.54 (1H, *d*, *J*=2.0 Hz), 7.42 (1H, *dd*, *J*=2.0, 8.5 Hz), 6.90 (1H, *d*, *J*=8.5 Hz) due to H-2′, H-6′ and H-5′ of B-ring. The AB-type doublets at $\delta$ 6.60 and 5.78 (each 1H, *d*, *J*=10.0 Hz, H-4″ and H-3″) together with a six-proton singlet at $\delta$ 1.43 (2″-Me's) indicated a 2, 2-dimethylpyran attached to A-ring. In addition, its $^1$H NMR spectrum revealed a methoxyl singlet at $\delta$ 3.79. To confirm the substitution pattern, a NOE difference experiment was conducted. Irradiation of the methoxyl signal resulted in significant enhancement of two aromatic proton signals at $\delta$ 7.54 (H-2′) and 7.42 (H-6′) indicating the methoxyl group at C-3. The aromatic singlet at $\delta$ 6.46 was due to C-6 or C-8. In its $^{13}$C NMR (75 MHz, DMSO-$d_6$) spectrum, the downfield shift (*ca.* 10.3 ppm) for C-8 suggested the prenylated substitution at C-8 [17, 18] and its HMBC (Figure 2) confirmed the structure elucidation as **3** which is in agreement with its $^{13}$C NMR spectral data (see experimental). This is only a second report of a prenylated flavonoid from the family [10].

Compound **4**, a white crystals, mp 247–249°, $[\alpha]_D^{25} = -44.8°$(MeOH), gave a molecular ion peak *m/z* 595 [M-H] in the negative FAB mass spectrum and a

molecular ion peak of aglycone $m/z$ 304 in its EI-MS. The UV (MeOH) bands at 274sh, 286 and 340sh nm suggested a dihydroflavonol structure. In the $^1$H NMR (500 MHz, DMSO-$d_6$) spectrum of **4**, the aromatic proton signals at $\delta$ 6.88 (1H, *brs*), 6.74 (2H, *m*.), 6.17, 6.13 (each 1H, *d*, $J=2.1$ Hz) and aliphatic signals at $\delta$ 5.30 and 4.71 (each 1H, *d*, $J=10.3$ Hz) suggested the presence of dihydroquercetin as the aglycone of **4**, and should be described to H-2′, H-5′, 6′, H-8, H-6, H-2 and H-3, respectively [9]. A *trans*-diaxial relationship between the protons at C-2 and C-3 in **4** was evident from the 10.3 Hz coupling constant. Acidic hydrolysis of **4** yielded L-rhamnose and an aglycone, which was spectroscopically ($^{13}$C NMR) identical with the known (2*R*, 3*R*) dihydroquercetin (**6**). In its $^{13}$C NMR (125 MHz, DMSO-$d_6$), the characteristic glycosylation shifts $+4.0$ and $-2.2$ ppm were observed for C-3 and C-7, indicating the locations of the rhamnose moieties to be the C-3 and C-7 [19]. The anomeric protons at $\delta$ 5.45 (1H, brs, 7 Rha H-1) and 4.00 (1H, brs, 3 Rha H-1) in the $^1$H NMR established the α-configuration of rhamnose. Thus **4** is (2*R*, 3*R*) dihydroquercetin- 3, 7-O-α-L-dirhamnoside. From the $^1$H-$^1$H COSY and $^{13}$C-$^1$H COSY of **4**, all of the carbon signals were assigned (see Experimental).

Compounds **5-13** were identified as (2*R*, 3*R*) dihydroquercetin-7-O-α-L rhamnoside (**5**) [9], (2*R*, 3*R*) dihydroquercetin (**6**) [18], 2, 3-*trans*-dihydro-3, 5, 4′-trihydroxyflavonol 7-*O*-α-L-rhamnoside (**7**) [20], quercetin 3-O-β-D-glucoside (**8**) [8, 21], quercetin -7-*O*-α-L-rhamnoside (**9**) [9], quercetin-3-*O*-α-L-rhamnosyl(1→2)-*O*-α-L-rhamnoside (**10**) [22], quercetin (**11**), Kaempferol (**12**) [23] and Kaempferol-7-*O*-α-L-rhamnoside (**13**) [24], respectively, by spectroscopic analysis.

*H. japonicum* has been used for treatment of internal haemorrhage as a folk medicine in some regions of China [25]. So the isolated compounds were tested for their coagulant activity *in vitro* system. The results indicated that **8**, and **10** showed anticoagulation of APTT (Activated Partial Thromboplastin Time Reagent, purchased from Pacific Homostasis, USA), and **5**, **9** and **11** showed promoting coagulation of APTT. Whereas **9**, **10** and **11** were found to exert promoting coagulation of PT (Prothrombin Time Reagent, purchased from Pacific Homostasis, USA). Notably **9**, a flavonol glycoside with high concentration in *H. japonicum*, exhibited an excellent bioactivity. It can promote the coagulation of APTT and PT with dose-effect relationship and the effective concentration is to $10^{-5}$ g/L.

## EXPERIMENTAL

### *General*

All mps are uncorr. $^1$H, $^{13}$C NMR and 2D NMR spectra were recorded with TMS as int. standard. Chromatography separation were carried out on silica gel, polyamide and Sephadex LH-20, TLC on silica gel G and polyamide film.

### *Plant material*

Aerial parts of *Hypericum japonicum* Thunb. ex Murray were collected from Anhui province, China, in Sept., 1994. Voucher specimens are deposited in Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing, P. R. China.

### *Extraction and isolation*

Air-dried aerial part (12 Kg) were extracted with 95% EtOH three times. The ethanol extract (1.1 Kg) was re-extracted successively with petrol, $CH_2Cl_2$, EtOAc, $Me_2CO$ and MeOH in soxhlet extractor. The EtOAc fr. (200 g) were chromatographed on silica gel column using a step-gradient $CHCl_3$-MeOH and 1000 ml fr. was collected. Those frs. containing similar component as judged by TLC were combined and got ten frs. 1–10. Fr. 2 was repeatedly rechromatographed on polyamide (MeOH-$H_2O$ and petrol-$CHCl_3$-MeOH) followed by purification on Sephadex LH-20 (MeOH) to give **3** (60 mg). Fr. 4 was rechromatographed repeatedly (silica gel and polyamide, $CHCl_3$-MeOH) and **6** (120 mg) and **12** (80 mg) were obtained. After recrystallization, **11** (2000 mg) was obtained from fr. 6. Fr. 8 was rechromatographed on polyamide ($CHCl_3$-MeOH) and followed by gel filtration on Sephadex LH-20 (MeOH) to give **7** (120 mg) and **13** (60 mg). The acetone fr. (300 g) were chromatographed on silica gel column using a step-gradient $CHCl_3$-MeOH and got fifteen frs. 1–15. Rechromatography of fr. 7 on polyamide ($CHCl_3$-MeOH) and Sephadex LH-20 (MeOH) gave **5** (150 mg) and **9** (500 mg). Fr. 9 was repeatedly rechromatographed on polyamide ($CHCl_3$-MeOH) and Sephadex LH-20 (MeOH) to give **4** (90 mg), **8** (200 mg), **10** (130 mg) and a mixture **1** and **2**. The mixture was further chromatographed on polyamide (*n*-BuOH-MeOH-$H_2O$-AcOH) and **1** (30 mg) and **2** (20 mg) were obtained.

5, 7-dihydroxy-2-(1-methylpropyl) chromone-8-β-D-glucoside (**1**), $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 13.0 (1H, *br*, chelated H-5), 6.20 (1H, *s*, H-6), 6.12 (1H, *s*, H-3), 4.63 (1H, *d*, $J=10.0$ Hz, H-1″), 2.63 (1H, *m*, H-1′), 1.70, 1.56 (each 1H, *m*, 2′-H′*s*), 1.21 (3H, *d*, $J=6.9$ Hz, Me-1′), 0.86 (3H, *t*, $J=7.4$ Hz, H-3′); $^{13}$C NMR (125 MHz, DMSO-$d_6$): $\delta$ 181.9 (C-4), 173.0 (C-2), 164.2 (C-7), 160.4 (C-5), 156.6 (C-8a), 106.2 (C-3), 104.7 (C-8), 103.3 (C-4a), 98.7 (C-6), 81.5 (C-5″), 78.7 (C-3″), 73.3 (C-1″), 71.2 (C-2″), 70.9 (C-4″), 61.7 (C-6″), 39.1 (C-1′), 27.0 (C-2′), 17.4 (Me-1′), 11.4 (C-3′).

5,7-dihydroxy-2-isopropyl chromone-8-β-D-glucoside (**2**), $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 13.0 (1H, *br*, chelated H-5), 6.25 (1H, *s*, H-6), 6.14 (1H, *s*, H-3), 4.64 (1H, *d*, $J=9.8$ Hz, H-1″), 2.88 (1H, *m*, H-1′), 1.26, 1.25 (each 3H, *d*, $J=6.6$ Hz, 1′-Me′*s*); $^1$H NMR (300 MHz, $CD_3OD$): $\delta$ 6.24 (1H, *s*, H-6), 6.08

(1H, *s*, H-3), 4.90 (buried in solvent, H-1″), 2.93 (1H, *m*, H-1′), 1.35, 1.34 (each 3H, *d*, $J=6.6$ Hz, 1′-Me′*s*); $^{13}$C NMR(125 MHz, DMSO-$d_6$): $\delta$ 182.2 (C-4), 174.4 (C-2), 162.8 (C-7), 160.4 (C-5), 156.5 (C-8a), 105.0 (C-3), 104.1 (C-8), 102.0 (C-4a), 98.5 (C-6), 81.5 (C-5″), 78.6 (C-3″), 73.1 (C-1″), 71.2 (C-2″), 70.8 (C-4″), 61.8 (C-6″), 32.7 ((C-1′), 20.0, 19.5 (1′-Me′s).

7, 8-(2″, 2″-dimethylpyrano)-5, 3′, 4′-trihydroxy-3-methoxylflavone (**3**), $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 13.07 (chelated OH-5), 7.54 (1H, *d*, $J=2.0$ Hz, H-2″), 7.42 (1H, *dd*, $J=8.5$, 2.0 Hz, H-6′), 6.90 (1H, *d*, $J=8.5$ Hz, H-5′), 6.60 (1H, *d*, $J=10.0$ Hz, H-4″), 6.46 (1H, *s*, H-6), 5.78 (1H, *d*, $J=10.0$ Hz, H-3″), 3.79 (3H, *s*, 3-OMe), 1.43 (6H, s, 2″-Me′*s*); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 177.5 (C-4), 158.0 (C-7), 155.4 (C-2), 154.8 (C-5), 154.7 (C-9), 148.3 (C-4′), 144.6 (C-3′), 137.2 (C-3), 128.5 (C-3″), 120.0 (C-6′, 1′), 115.1 (C-2′), 115.0 (C-5′), 113.9 (C-4″), 104.6 (C-10), 103.8 (C-8), 94.1 (C-6), 77.4 (C-2″), 59.1 (3-OMe), 27.2 (2″-Me's); EI-MS *m*/*z* (%): 382 [M$^+$] (31.2), 267 [M-Me]$^+$ (100), 352 (12.4), 324 (6.0), 251 (4.2), 184 (9.8), 167 (9.2), 153 (7.9), 139 (8.8).

(2R, 3R) dihydroquercetin-3, 7-O-α-L-dirhamnoside (**4**), $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 6.88 (1H, *brs*, H-2′), center at 6.74 (2H, *m*, H-5′, 6′), 6.17 (1H, *d*, $J=2.1$ Hz, H-8), 6.13 (1H, *d*, $J=2.1$ Hz, H-6), 5.30 (1H, *d*, $J=10.3$ Hz, H-2), 4.71 (1H, *d*, $J=10.3$ Hz, H-3), 5.45 (1H, *brs*, 7Rha H-1), 4.00 (1H, *brs*, 3Rha H-1), 1.10 (3H, *d*, $J=6.2$ Hz, 3Rha-Me), 1.04 (3H, *d*, $J=6.4$ Hz, 7Rha-Me); $^{13}$C NMR (125 MHz, DMSO-$d_6$): $\delta$ 195.1 (C-4), 164.2 (C-7), 162.8 (C-5), 161.9 (C-9), 145.9 (C-4′), 145.1 (C-3′), 126.7 (C-1′), 118.6 (C-6′), 115.3 (C-2′), 114.7 (C-5′), 102.5 (C-10),100.0 (3 Rha C-1), 98.3 (7 Rha C-1), 96.5 (C-6), 95.4 (C-8), 81.6 (C-2), 75.7 (C-3), 70.2 (3 Rha C-2), 70.4 (3 Rha C-3), 71.6 (3 Rha C-4), 69.7 (3 Rha C-5), 70.0 (7 Rha C-2), 70.2 (7 Rha C-3), 71.5 (7 Rha C-4), 68.9 (7 Rha C-5), 17.7 (3 Rha C-6), 17.5 (3 Rha C-6); EI-MS *m*/*z* (%): 304 [M-rha-rha]$^+$ (16.9), 286 (21.5), 153 (59.1), 123 (100); FAB-MS (*m*/*z*): 595 [M-H]$^+$, 449 [M-Rha-H]$^+$, 303 [M-Rha-rha-H]$^+$.

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