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Japonicins A and B from the flowers of *Inula japonica*

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Two new flavonols japonicins, A (**1**) and B (**2**), along with nine known flavonoids were isolated from an antidepressant fraction, which was separated from the 70% alcohol extract of the flowers of *Inula japonica* Thumb. The structures of compounds **1** and **2** were determined as 3,3′,4′,5,9,10-hexahydroxy-12-methylchroman[2,3-*h*]flavone and 8-(1-(3,4-dihydroxyphenyl)ethyl)-3,3′,4′,5,7-pentahydroxyflavone by the analyses of physical constants and spectral data. The special flavonoid structure having the substituent 1-phenylethyl at C-8 position was discovered for the first time.

Keywords: *Inula japonica*; Compositae; Flavonol; Japonicin A; Japonicin B

1. Introduction

The flowers of *Inula japonica* Thumb. are used in traditional Chinese medicine for the treatment of diverse diseases such as tracheitis, bronchitis, hepatitis and alimentary tract carcinoma. Previous phytochemical investigation revealed the sesquiterpene lactones are the main active constituents [1–3] which display cytotoxic [3], anti-inflammatory and anti-ulcer activities [4]. During the course of a screening for antidepressant agents from Chinese herbal medicine, it was found that the 70% alcohol extract of the flowers of *I. japonica* possesses remarkable antidepressive activity. Further fractionation of the extract under the guidance of bioassays led to the discovery of an active fraction that contains mainly flavonoids. Two novel flavonols, named japonicins A (**1**) and B (**2**), along with nine known flavonoids (**3**–**11**) were isolated from this fraction. The special flavonoid structure of compounds **1** and **2** having the substituent 1-phenylethyl at C-8 position was discovered for the first time. In this report, we give the details of the isolation of all compounds and the structural elucidation of compounds **1** and **2** (see figure 1).

2. Results and discussion

Compound **1** was obtained as a yellow solid with mp $>320^{\circ}C$. The molecular formula of $C_{23}H_{16}O_9$ was established by HR-ESIMS (*m/z* 437.0877 $[M + H]^+$). The UV adsorption

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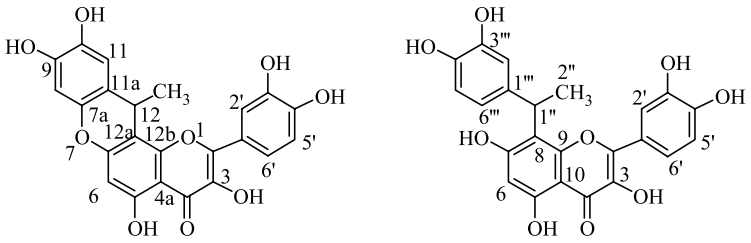


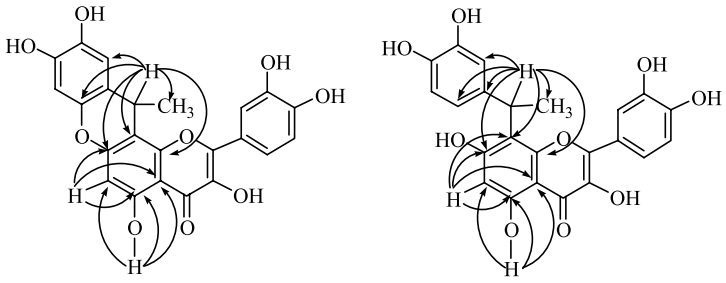
1 **2**

Figure 1. Structure of compounds **1** and **2**.

maxima at λ_{max} 259 and 380 nm were ascribed to bands II and I of the flavonoid nucleus. The IR spectrum showed the presence of hydroxyl (3400 cm^{-1}), carbonyl (1650 cm^{-1}) groups and benzene rings (1595, 1560, 1500 cm^{-1}). ^{1}H NMR spectrum (see table 1) showed the presence of characteristic signals for 3,4-dioxygenated flavonol at δ 12.50 (5-OH), 7.80 (H-2′), 7.66 (H-6′) and 6.97 (H-5′). Comparing the ^{13}C NMR spectral data of compound **1** (see table 1) with that of quercetin, the flavone moiety in the molecule was determined as quercetin. The long-range correlations between the proton of 5-OH and C-5, C-4a and a tertiary carbon at δ 97.8 shown in the HMBC spectrum (see figure 2) revealed that the carbon at δ 97.8 and its directly attached proton at δ 6.46 were C-6 and H-6, and the singlet signal of H-6 resulted in the substitution at C-8 position of the quercetin moiety. With the exception of

Table 1. NMR data of compounds **1** and **2** (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR in DMSO-d_6).

	1			***2***	
Position	δ_H *(J in Hz)*	δ_C	*Position*	δ_H *(J in Hz)*	δ_C
2	–	147.4	2	–	147.0
3	–	136.4	3	–	135.4
4	–	176.1	4	–	176.1
4a	–	105.6	5	–	158.4
5	–	158.4	6	6.34 (1H, s)	98.2
6	6.46 (1H, s)	97.8	7	–	161.7
6a	–	155.8	8		110.7
7a	–	142.4	9		153.5
8	6.55 (1H, s)	103.3	10		103.2
9	–	144.9	1′		122.1
10	–	142.0	2′	7.71 (1H, d, 1.7)	115.5
11	6.78 (1H, s)	115.0	3′		144.9
11a	–	114.4	4′		147.6
12	4.39 (1H, q, 6.7)	26.7	5′	6.84 (1H, d, 8.7)	115.0
12a	–	104.5	6′	7.16 (1H, br. s)	119.8
12b	–	152.5	1″	4.76 (1H, q, 6.7)	31.5
1′	–	122.1	2″	1.68 (3H, d, 6.7)	17.9
2′	7.80 (1H, d, 2.2)	115.9	1″	–	135.4
3′	–	145.2	2″	6.68 (1H, br. s)	114.5
4′	–	148.0	3″	–	143.0
5′	6.97 (1H, d, 8.4)	115.1	4″	–	144.7
6′	7.66 (1H, dd, 8.4, 2.2)	120.0	5″	6.66 (1H, d, 8.1)	117.3
12-Me	1.43 (3H, d, 6.7)	25.8	6″	6.63 (1H, br. *d*, 8.1)	115.5
5-OH	12.50 (s)	–	5-OH	12.62 (s)	–



1 **2**

Figure 2. Selected HMBC correlations of compounds **1** and **2**.

the aromatic carbons corresponded with that of quercetin, other three oxygenated aromatic carbons at δ 142.0, 142.4, 144.9 and three non-oxygenated aromatic carbons at δ 103.3, 114.4 and 115.0 were shown in the ^{13}C NMR spectrum, indicating the presence of a phenyl group in the molecule. The phenyl group was determined as 2,4,5-trioxygenated phenyl by the support from HMQC experiment which revealed the carbons at δ 103.3 and 115.0 resonances with two aromatic proton singlets at δ 6.55 and 6.78. In addition, ^{1}H NMR spectrum showed a proton quartet at δ 4.39 coupled to a methyl doublet at δ 1.43, indicating the presence of an ethyl group in the molecule. Carbon signals at δ 26.7 and 25.8 were assigned to the carbons of the ethyl group by the indication from HMQC spectrum. The carbon at δ 26.7 is an aliphatic tertiary carbon, indicating the connection between the ethyl group and two quaternary carbons. Furthermore, HMBC spectrum revealed the long-range correlations between the proton at δ 4.39 and C-6a, 7a, 11, 12b and a carbon at δ 104.5. All evidence mentioned above determined the carbon at δ 26.7 of the ethyl group connected with both the carbon at δ 114.4 (C-11a) of the phenyl group and the carbon at δ 104.5 (C-12a) of the quercetin moiety. A ring exists between the quercetin moiety and the phenyl group by calculating the degree of unsaturation. So it was deduced that the quercetin moiety connected with the phenyl group via the oxygen atom at C-7. Hence, the structure of compound **1** was determined as 3,3′,4′,5,9,10-hexahydroxy-12-methylchroman[2,3-*h*]flavone, designated with the common name japonicin A. Complete assignments of the NMR signals were confirmed by HMQC and HMBC spectral analyses.

Compound **2** was obtained as a yellow solid with mp 156–158°C. The molecular formula of $C_{23}H_{18}O_9$ was established by HR-ESIMS. UV and IR spectra were similar to that of compound **1**, suggesting compound **2** had a similar structure to **1**. Comparing the ^{1}H NMR spectrum of compound **2** (see table 1) with that of compound **1**, similar proton signals were shown except three aromatic proton signals at δ 6.68, 6.66 and 6.63 which were located on the same benzene ring by the analyses of coupling constants and ^{1}H–^{1}H-COSY spectrum. ^{1}H–^{1}H-COSY spectrum also showed the proton at δ 6.68 in the *para*-position with the proton at δ 6.66 as well as the proton at δ 6.63 in the *ortho*-position of the proton at δ 6.66, indicating the presence of 3,4-dihydroxyphenyl group in the molecule. HMQC spectrum revealed the protons at δ 6.63, 6.66 and 6.68 were linked with the carbons at δ 115.5, 117.3 and 114.5, respectively. In addition, the degree of unsaturation indicated that no ring existed between the quercetin moiety and the phenyl group. Accordingly, the structure of compound **2** was the quercetin moiety with the substituent 1-(3,4-dihydroxyphenyl)ethyl at C-8 position. By the

analysis of HMBC spectrum (see figure 2), the structure of compound **2** was determined as 8-(1-(3, 4-dihydroxyphenyl) ethyl)-3,3′,4′,5,7-pentahydroxyflavone, designated with the common name japonicin B. A literature survey showed that the flavonoids having the substituent 1-phenylethyl at the C-8 position were discovered for the first time.

The nine known compounds: onpordin (**3**) [5], 3′-*O*-methylorobol (**4**) [6], nepetin (**5**) [7], patuletin (**6**) [8], apigenin (**7**) [9], luteolin (**8**) [10], quercetin (**9**) [10,11], isoquercitrin (**10**) [12] and nepitrin (**11**) [13] were identified by direct comparison of their spectral data (MS, ^{1}H NMR and ^{13}C NMR) with reported values in the literature.

3. Experimental

3.1 General experimental procedures

Melting points were measured on a Fisher-Johns apparatus and are uncorrected. Optical rotations were recorded with a Perkin–Elmer 341 polarimeter. IR spectra were measured on a Nicolet Manga infrared spectrometer as pressed KBr discs. UV spectra were recorded in MeOH using a Cintra 20 UV–Visible spectrometer. EI-MS spectra were measured on an Zabspec mass spectrometer at 70 eV, ESI-MS spectra were measured on an API-3000 LC/MS/MS system, HRESI-MS spectra were measured on a Micromass LCT system. NMR spectra were measured on a JEOL GX-400 spectrometer operating at a basic frequency of 400 MHz, using TMS as the internal standard.

3.2 Plant material

The flowers of *Inula japonica* Thumb. were purchased from Tongrentang shop, Beijing, China and were identified by Professor Zhongtao Wang, Institute of Botany, Chinese Academy of Sciences (CAS). A voucher specimen (No. 02003) is deposited in the Laboratory of Phytochemistry, Beijing Institute of Pharmacology and Toxicology, China.

3.3 Extraction and isolation

Dried flowers (5 kg) were extracted three times with 70% alcohol at boiling temperature. The combined extracts were filtered and evaporated on a rotary evaporator under reduced pressure to obtain a viscous alcoholic extract (1.06 kg), which was dispersed in 1 L water and partitioned with petroleum ether. Subsequently, the water layer was diluted with 4 L water and the water solution was centrifuged (4000 rpm, 30 min). Then the solution was subjected to column chromatography on AB-8 macroporous resin (7 L). After eluting with 14 L water, the column was eluted with 21 L 70% alcohol and the elution was evaporated and dried *in vacuo* to give the antidepressant fraction (320 g). This fraction was dispersed in 0.3 L water and partitioned with EtOAc to give the EtOAc-soluble fraction (114 g).

The EtOAc-soluble fraction was subjected to column chromatography on silica gel and eluted with $CHCl_3/CH_3OH$ (100:0 → 8:2). Six fractions were obtained: Fr-A (100:0), Fr-B (98:2), Fr-C (95:5), Fr-D (90:10), Fr-E (85:15) and Fr-F (80:20). Fraction A after separation by column chromatography on polyamide with $CHCl_3/CH_3OH$ (99:1 → 94:6) afforded

3 (20 mg). Fraction B gave **4** (253 mg), **5** (78 mg), **6** (52 mg) and **7** (24 mg) by polyamide column chromatography with $CHCl_3/CH_3OH$ (94:6 → 85:15) and were purified by silica gel column chromatography and recrystallisation. **8** (122 mg) and **9** (487 mg) were obtained from fraction C by polyamide column chromatography with $CHCl_3/CH_3OH$ (85:15 → 80:20). Fraction D was subjected to column chromatography on polyamide and eluted with $CHCl_3/CH_3OH$ (8:2), to give **9** (53 mg) and **1** (29 mg). Fraction E was chromatographed on a polyamide column eluted with $CHCl_3/CH_3OH$ (8:2 → 7:3), to give **10** (45 mg) and **11** (18 mg), and a fraction which afforded **2** (14 mg) after polyamide column chromatography with $CHCl_3/CH_3OH$ (8:2) and repeated Sephadex LH-20 column chromatography with CH_3OH.

3.3.1 Japonicin A (1). Yellow solid (MeOH), mp > 320°C, $[\alpha]_D^{20}$ -9.59 (*c* 0.146, MeOH). UV λ_{max}^{MeOH} nm (log ε): 203 (4.64), 259 (4.27), 276 sh, 334 (3.98), 380 (4.14). IR ν_{max}^{KBr} cm^{-1}: 3400, 1650, 1595, 1560, 1500, 1450, 1400, 1340, 1260, 1180, 1110, 670. (+) HRESI-MS *m/z*: 437.0877 $[M + H]^+$ (calcd for $C_{23}H_{17}O_9$, *m/z* 437.0873). (−) ESI-MS *m/z*: 435.3 $[M - H]^-$. (+) ESI-MS *m/z*: 437.3 $[M + H]^+$. EIMS *m/z* (%): 436 $[M]^+$(5), 421 (48), 368 (3), 313 (2), 283 (3), 271 (6), 239 (5), 208 (9), 168 (11), 152 (16), 139 (14), 125 (16), 111 (28), 98 (34), 84 (100), 69 (45). ^{1}H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz) data, see table 1.

3.3.2 Japonicin B (2). Yellow solid (MeOH), mp 156–158°C, $[\alpha]_D^{20}$ -2.93 (*c* 0.376, MeOH). UV λ_{max}^{MeOH} nm (log ε): 204 (4.90), 260 (4.46), 272 sh, 336 sh, 376 (4.37). IR ν_{max}^{KBr} cm^{-1}: 3400, 1650, 1580, 1410, 1020, 1000, 640. (+) HRESI-MS *m/z*: 439.1030 $[M + H]^+$ (calcd for $C_{23}H_{19}O_9$, *m/z* 439.1029). (−) ESIMS *m/z*: 437.3 $[M - H]^-$, 327.2. (+) ESI-MS *m/z*: 461.2 $[M + Na]^+$, 439.3 $[M + H]^+$. EI-MS *m/z* (%): 438 $[M]^+$(3), 358 (4), 328 (4), 302 (100), 285 (3), 274 (6), 273 (7), 257 (5), 246 (5), 245 (6), 231 (6), 229 (5), 228 (4), 200 (2), 171 (2), 153 (8), 137 (15), 136 (13), 128 (7), 123 (11), 110 (38), 81 (80), 64 (14). ^{1}H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz) data, see table 1.

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