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Phloroglucinol derivatives from *Hypericum japonicum*

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Abstract

The isolation and identification of a new phloroglucinol derivative (**2**), a diterpenol (**4**), together with the known compounds flavesone (**1**) and sarothralen B (**3**), from the aerial parts of *Hypericum japonicum* are reported. Their structures were established by extensive spectral analysis and the structure of (**3**) has also been confirmed by a single crystal X-ray determination.

Keywords: *Hypericum japonicum*; Guttiferae; Phloroglucinol derivatives; Diterpenol

1. Introduction

Hypericum japonicum is a Chinese herbal medicine used in the treatment of numerous disorders such as several bacterial diseases, infectious hepatitis, gastrointestinal disorder and tumors (Ishiguro, Yamaki, Kashihara & Takagi, 1986). *H. japonicum* is a prolific producer of secondary metabolites and was previously reported to contain phloroglucinol derivatives (Ishiguro, Yamaki, Kashihara, Takagi, Yamagata & Tomita, 1985; Ishiguro et al., 1986; Ishiguro, Yamaki, Kashihara & Takagi, 1987; Ishiguro, Yamaki, Kashihara, Takagi & Isoi, 1990a; Ishiguro, Nagata, Fukumota, Yamaki, Takagi & Isoi, 1990b; Gu, Feng & Wang, 1988), flavonoids (Ishiguro, Nagata, Fukumota, Yamaki, Takagi & Isoi, 1991a, 1991b; Ishiguro, Nagata, Fukumota, Yamaki, Takagi, Isoi & Yoshiaki, 1993), xanthonoids (Ishiguro et al., 1993; Ishiguro, Nagareya, Suitani & Fukumoto, 1997; Wu, Wang, Du, Yang & Xiao, 1998a, 1998b), chromone glycosides (Wu et al., 1998a, 1998b), a peptide (Ishiguro et al., 1991a, 1991b), and a lactone (Ishiguro et al., 1990a, 1990b). As a part of our investigation on Chinese *Hypericum*, we previously reported several novel polyprenylated benzoylphloroglucinol derivatives from *H. sampsonii* (Hu & Sim, 1998, 1999) and several xanthones from *H. ascyron* (Hu, Yip & Sim, 1999). In this paper, the isolation and characterisation of three phloroglucinol derivatives, together with a diterpenol, from the aerial parts of *H. japonicum* are reported.

2. Results and discussion

The acetone soluble fraction of the hexane extract of dry aerial parts of *H. japonicum* was fractionated on a silica gel column, affording eight major fractions. Fraction 2 yielded three phloroglucinol derivatives (**1**–**3**) and a diterpenol (**4**) after further extensive chromatographic purification.

Compound **1**, obtained as a yellow oil, showed the $[M]^+$ at m/z 252.13915 in the HREIMS, which corresponds to $C_{14}H_{20}O_4$ (calculated 252.13615). The IR spectrum showed a broad absorption in the 3461 cm^{-1} region and this, coupled with intense peaks at 1610–1710 cm^{-1}, suggested the presence of an enolic 1,3-diketo system or 2-hydroxyaryl ketone. The very low field (δ 18.96 ppm) signal in the ^{1}H-NMR spectrum of **1** further suggests the presence of an enolizable β-triketone system. Careful examination of its ^{1}H- and ^{13}C-NMR data established compound **1** as flavesone, pre-

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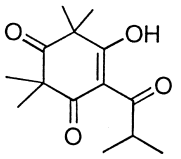

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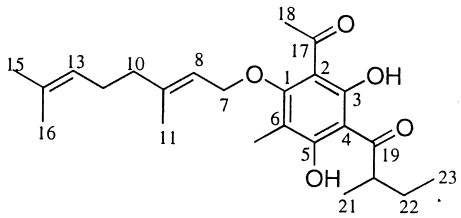

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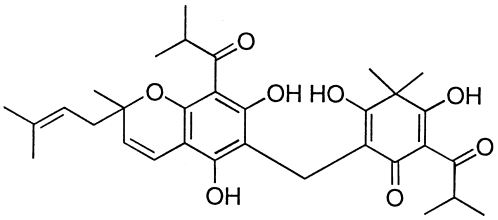

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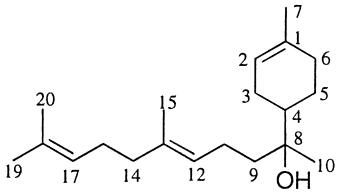

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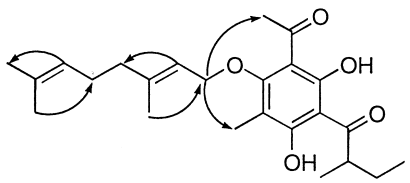
viously reported as a main antimicrobial component in the essential oil from *Leptospermum scoparium* (van Klink, Brophy, Perry & Weavers, 1999).

Compound **2**, a colourless oil, has the molecular formula, $C_{24}H_{34}O_5$, which was established by HREIMS (found: 402.24203; calculated: 402.24063). The IR spectrum suggested the presence of hydroxyl group (3440 cm^{-1}), and conjugated carbonyl group (1619, 1595 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra suggested a structure for **2** related to a phloroglucinol derivative. Furthermore, the NMR, ^{1}H–^{1}H COSY, NOESY and HMQC spectra revealed the presence of chelated phenolic hydroxyl group (δ_H 9.45 brs), one aromatic methyl [δ_H: 2.12 (3H, *s*, H-24)], one acetyl [δ_H: 2.16 (3H, *s*, H-18)], and two proton-coupled systems. One was a 2-methylbutyryl side chain [δ_H: 3.78 (1H, *m*, H-20), 1.18 (3H, *d*, J = 6.9 Hz, H-21), 1.85 (1H, *q*, J = 6.8 Hz, H-22), 1.41 (1H, *q*, J = 6.8 Hz, H-22), 0.91 (3H, *t*, J = 6.9 Hz, H-23)]. The other was a geranyl side chain [δ_H: 4.32 (2H, *d*, J = 7.0 Hz, H-7), 5.55 (1H, *t*, J = 7.0 Hz, H-8), 2.10 (2 H, *m*, H-10), 1.68 (3H, *s*, H-11), 2.12 (2 H, *m*, H-12), 5.10 (1 H, t, J = 6.3 Hz, H-13), 1.68 (3 H, s, H-15), 1.61 (3 H, *s*, H-16)]. The geminal protons H_2C-7 of the geranyl moiety appeared at a lower field indicating that this chain had to be linked to one of the oxygen atoms of the phloroglucinol system. There were no aromatic proton peaks in the ^{1}H-NMR spectrum and the substitution pattern of **2** was deduced from NOE results (Fig. 1). Irradiation of H_2C-7 at δ 4.32 produced enhancements of the aromatic methyl at δ 2.12 and the acetyl methyl at δ 2.16. Thus, **2** was established as 2-acetyl-3,5-di-



2

Fig. 1. NOE-difference correlations of **2**.

Table 1
NMR data for **2**

Position	^{1}H[a]	^{13}C[b]	DEPT	HMBC[c]
1		61.50	C	
2		106.79	C	
3		157.94	C	
4		108.82	C	
5		157.94	C	
6		108.82	C	
7	4.32 (2H, *d*, J = 7 Hz)	69.73	CH_2	1, 8, 9
8	5.55 (1H, *m*)	119.37	CH	10, 11
9		141.64	C	
10	2.10 (2H, *m*)	39.50	CH_2	8, 9, 12, 13
11	1.68 (3H, *m*)	16.37	CH_3	8, 10
12	2.12 (2H, *m*)	26.22	CH_2	9, 10, 13
13	5.10 (1H, *m*)	123.66	CH	12, 14, 15
14		131.77	C	
15	1.68 (3H, *s*)	25.57	CH_3	13, 14
16	1.61 (3H, *s*)	17.58	CH_3	13, 14, 15
17		205.60	C	
18	2.16 (3H, *s*)	30.80	CH_3	17
19		211.24	C	
20	3.78 (1H, *m*)	46.28	CH	19, 21, 22, 23
21	1.18 (3H, *d*, J = 6.9 Hz)	16.56	CH_3	19, 20, 22
22	1.85 (1H, *m*)	26.85	CH_2	19, 20, 21, 23
	1.41 (1H, *m*)			19, 20, 21, 23
23	0.91 (3H, *t*, J = 7.3 Hz)	11.87	CH_3	20, 22
24	2.12 (3H, *s*)	8.57	CH_3	
OH	9.45 (1H, *s*)			

[a] Recorded in $CDCl_3$ at 300 MHz.
[b] Recorded in $CDCl_3$ at 75 MHz.
[c] Carbons that correlate with the proton resonance.

hydroxy-1-geranoxy-6-methyl-4-(2-methyl)butyryl-benzene, which was further confirmed by the HMBC spectrum (Table 1). In contrast to C-prenylation, *O*-prenylation is seldom found in phenolic natural products and it is interesting to note that besides compound **2**, another phloroglucinol derivative, sarothralin, isolated earlier from the same plant (Ishiguro et al., 1985) possesses a prenoxyl group. The geranoxyl group occurs very rarely in natural products and compound **2** seems to be the first metabolite with this functional group to be found in *Hypericum* species.

Compound **3** was isolated as yellow plates (m.p. 93.5–94.0°C) from acetone. The IR spectrum suggested the presence of an enolic 1,3-diketo system or 2-hydroxyaryl ketone (3300–3100 1640 cm^{-1}). The ^{1}H-NMR spectra (in $CDCl_3$ or acetone-d_6) of **3** showed pairs of peaks (*ca.* 4:1), characteristic of the existence of two tautomers in solution. The spectral data were similar to sarothralen B (Ishiguro et al., 1986) which had been reported with a higher melting point (m.p. 116–119°C). The structure of the major tautomer **3** was unambiguously confirmed by a single-crystal X-ray determination (Fig. 2). The molecular structure showed the two rings of the chromene system are nearly coplanar with an interplanar angle of 6.2° and that the acylfilicinic acid mioety and the phenyl ring of the chromene system are linked together by the methylene bridge C(7) such that, their planes with a dihedral angle of 54.5° are fixed rigidly by four OH...O intramolecular hydrogen bonds as shown in Fig. 2. This particular molecular conformation is similar to that of sarothralin (Ishiguro et al., 1985).

Compound **4**, $C_{20}H_{34}O$, obtained as an optically active colourless oil, was characterised as a diterpenol **4** by detailed analysis of its ^{1}H-, ^{13}C-NMR, ^{1}H–^{1}H COSY, NOESY, HMQC and HMBC spectral data (Table 2). This compound was previously isolated from the roots of *Helichrysum nudifolium* (L) and assigned the structure **4** based mainly on its ^{1}H-NMR spectral data and its similarity to the parent hydrocarbon (Jakupovic, Kuhnke, Schuster, Metwally & Bohlmannn, 1986). The relative stereochemistry at C-4 and C-8 could not be established. However, the C_6 methylene at δ 1.98 showed NOE interactions with the C_4 methine proton at δ 1.54, but no NOE interaction with the C_{10} methyl protons at δ 1.14 indicating that the C_4 methine proton at δ 1.54 is axially oriented. Similarly, the NOESY data indicated that the geometric configuration at C_1–C_2 and C_{12}–C_{13} is Z and E, respectively, as expected. The occurrence of this diterpenol (9-geranyl- $\propto$ -terpineol or prenyl- $\propto$ -bisabolol) is interesting chemotaxonomically, as there are only a handful of known monocyclic diterpenes with the prenylbisabolane skeleton, whereas the corresponding monocyclic monoterpenoids with the *p*-menthane skeleton and monocyclic sequiterpenoids with the bisabolane skeleton are very common.

3. Experimental

3.1. General

EIMS were determined on a Micromass VG 7035 mass spectrometer at 70 eV. NMR spectra were recorded on Bruker ACF 300 [300 MHz (^{1}H) and 75 MHz (^{13}C)] and AMX 500 [500 MHz (^{1}H) and 125 MHz (^{13}C)] instruments using $CDCl_3$ and acetone-d_6 solutions with TMS as an internal standard. IR spectra

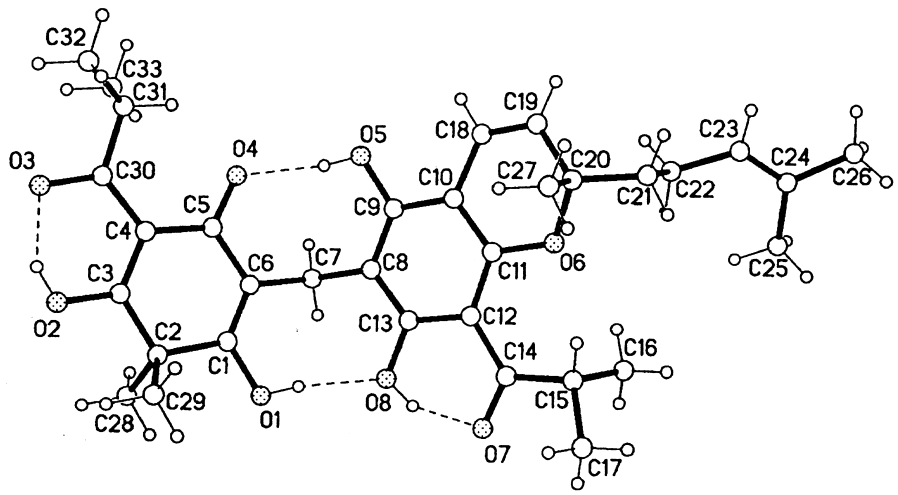


Fig. 2. X-ray structure of sarothralen B (**3**).

were recorded on a Bio-Rad FTIR spectrophotometer and UV spectra were recorded on a Hewlett–Packard 8452A diode array spectrophotometer. Chromatographic separations were carried out on silica gel 60 (40–63 μm), silica gel 60 RP-18 (40–63 μm) and LiChroprep DIOL (40–63 μm).

3.2. Plant material

The whole plant of *Hypericum japonicum* was collected from Suzhou, Jiangsu Province, P.R. China in August 1997. A voucher specimen (No. 97007) is deposited at the herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, P.R. China.

3.3. Extraction and isolation

The whole air-dried ground plants (5.0 kg) were extracted at room temperature with 25 l of hexane for seven days. The extract was concentrated in vacuo and the concentrate was stirred with acetone and filtered. Concentration of the acetone-soluble fraction afforded the crude extract (140 g), which was subjected to silica gel column chromatography, eluting with hexane–ethyl acetate step gradient (1:0, 50:1, 25:1, 10:1, 5:1, 2:1, 1:1, 0:1), to give eight fractions. All the fractions contain complex, mostly intractable mixtures and only fraction 2 has been examined in detail. A portion of fraction 2 (3.0 g) was subjected RP-18 column chromatography (85% acetone–water) to fractions 2a–2d. Further DIOL (hexane–ethyl acetate 10:1) chromatographic purification of fraction 2b (30 mg) afforded **1** (2.4 mg). Further DIOL (hexane–ethyl acetate 6:1) and PTLC (hexane–acetone 10:1) chromatographic purification of fraction 2c (260 mg) afforded **2**(6.7 mg) and **4** (5.2 mg). Fraction 2d (320 mg) was subjected to DIOL column chromatography, eluting with hexane–ethyl acetate (4:1) to give **3** (52.4 mg).

3.3.1. Compound *2*

Colourless oil, $[\alpha]_D^{31.2} - 7.02$ (*c*, 0.057, MeOH). EI-HRMS: *m/z* 402.24203, $C_{24}H_{34}O_5$ requires 402.24063. EI-MS *m/z*: 402, 372, 357, 315, 238, 181, 69, 41. IR (KBr) ν_{max} 3440, 1619, 1595, 1457, 1420, 1138, 1121 cm^{-1}. UV (MeOH) λ_{max} (log ε) 210 (3.91), 282 (2.77), 332 (2.37). ^{1}H- and ^{13}C-NMR, Table 1.

3.3.2. Compound *3* (sarothralen B)

Yellow plates; m.p. (93.5–94.0°C. EI-MS *m/z*: 566, 483, 413, 330, 247, 193, 69, 43. ^{1}H-NMR (acetone-d_6) 300 MHz: 18.65 (*s*), 18.43 (*s*), 16.40 (*s*), 16.36 (*s*), 11.46 (*s*), 11.44 (*s*), 10.00 (*s*), 9.90 (*s*), 6.73 (*d*, *J* = 10.1 Hz), 6.72 (*d*, *J* = 10.1 Hz), 5.60 (*d*, *J* = 10.1 Hz),

Table 2
NMR data for **4**

Position	^{1}H[a]	^{13}C[b]	DEPT	HMBC[c]	NOESY
1		133.74	C		
2	5.40 (1H, *m*)	120.69	CH	4, 6, 7	3, 7
3	a 2.07 (2H, *m*)	26.00	CH_2	1, 2, 9	2, 3b, 4
	b 1.88 (1H, *m*)			2, 9	2, 3a, 4, 10
4	1.54 (1H, *m*)	43.25	CH	5, 8, 10	3a, 3b, 5a, 5b, 6, 10
5	a 1.81 (1H, *m*)	23.91	CH_2	1, 3, 6	4, 5b, 10
	b 1.28 (1H, *m*)			4, 6	4, 5a, 6
6	1.98 (2H, *m*)	30.98	CH_2	2, 4	4, 5a, 5b, 7
7	1.65 (3H, *s*)	23.21	CH_3	1, 2, 6	2, 6
8		74.25	C		
9	1.52 (2H, *m*)	39.24	CH_2	4, 8, 10, 12	10, 11, 12
10	1.14 (3H, *s*)	23.91	CH_3	4, 8, 9	3b, 4, 5a, 9, 11
11	2.07 (2H, *m*)	22.11	CH_2	8, 9, 12, 13	9, 10, 12, 15
12	5.19 (1H, *t*, *J* = 1.0 Hz)	124.37	CH	9, 11, 14, 15	9, 11, 14
13		135.26	C		
14	1.98 (2H, *m*)	39.61	CH_2	13, 15, 16, 17	12, 14, 16, 17
15	1.62 (3H, *s*)	15.91	CH_3	12, 13, 14	11, 14
16	2.07 (2H, *m*)	26.58	CH_2	14, 17, 18	14, 17, 20
17	5.14 (1H, *t*, *J* = 1.1 Hz)	124.18	CH	19, 20	14, 16, 19
18		131.32	C		
19	1.68 (3H, *s*)	25.58	CH_3	17, 18, 20	17, 20
20	1.60 (3H, *s*)	17.58	CH_3	17, 18, 20	16, 19

[a] Recorded in $CDCl_3$ at 300 MHz.
[b] Recorded in $CDCl_3$ at 75 MHz.
[c] Carbons that correlate with the proton resonance.

5.58 (*d*, *J* = 10.1 Hz), 5.13 (*m*), 4.19 (*m*), 3.99 (*m*), 3.74 (*s*), 3.54 (*s*), 2.10 (*m*), 1.84 (*m*), 1.63 (*s*), 1.55 (*s*), 1.48 (*s*), 1.20 (*d*, *J* = 7.1 Hz), 1.17 (*d*, *J* = 6.9 Hz). ^{1}H-NMR ($CDCl_3$) 300 MHz: 18.67 (*s*), 18.42 (*s*), 16.38 (*s*), 16.18 (*s*), 11.41 (*s*), 10.62 (*s*), 10.02 (*s*), 9.94 (*s*), 6.76 (*d*, *J* = 10.4 Hz), 6.75 (*d*, *J* = 10.4 Hz), 5.42 (*d*, *J* = 10.7 Hz), 5.39 (*d*, *J* = 10.5 Hz), 5.09 (*m*), 4.21 (*m*), 3.91 (*m*), 3.54 (*m*), 2.12 (*m*), 1.83 (*m*), 1.66 (*s*), 1.58 (*s*), 1.57 (*s*), 1.43 (*s*) 1.22 (*d*, *J* = 6.7 Hz), 1.18 (*d*, *J* = 6.5 Hz).

3.3.3. Crystal data for **3**

$C_{33}H_{42}0_8$, M = 566.66, triclinic, space group P-1, a = 10.4114(6), b = 11.9610(7), c = 14.6434(8) Å, $\alpha = 107.893(1)^\circ$, $\beta = 103.921(1)^\circ$, $\gamma = 103.516(1)^\circ$, V = 1588.2(2) Å^3 ($\lambda = 0.71073$ A), Z = 2, D_{calc} = 1.183 g cm^3, $\mu = 0.084$ mm^{-1}. Frame data were collected at 293(2) K in the θ range 2.18–25.00° ($-12 \leq h \leq 12$; $-14 \leq k \leq 14$; $-17 \leq l \leq 17$) on a Bruker Axs SMART CCD system and processed. The processed *hkl* data were absorption corrected using the programme SADABS. Anisotropic thermal parameters were refined for all the non-hydrogen atoms. All the hydrogen atoms were located in the difference Fourier routines. The positional and isotropic thermal parameters were refined for all the hydrogen atoms. In the least squares-refinement cycles on F^2, the model converged R_1 = 0.0998, wR_2 = 0.2485 and GOF = 1.097 for 3349 reflections with $F_0 > 4\sigma$ (F_0) and 372 parameters. In the final Fourier synthesis, the electron density fluctuates in the range 0.317–0.357 e Å^{-3}. Atomic coordinates, bond lengths and angles and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC No. 137251).

3.4. Compound **4**

Colourless oil, $[\alpha]_D^{31.2} - 51.30$ (*c*, 0.046, MeOH). EI-HRMS: *m*/*z* 290.26145 $[M]^+$, $C_{20}H_{34}O$ requires 290.26096; 272.24857 $[M-18]^+$; $C_{20}H_{32}$ requires 272.25040; 213.16517 $[M-18-69]^+$, $C_{16}H_{21}$ requires 213.16432. EI-MS *m*/*z*: 290, 272, 213, 95, 69. IR (KBr) ν_{max} 3423, 1449, 1377, 1105 cm^{-1}. UV (MeOH) λ_{max} (log ε) 210 (3.91), 282 (2.77), 332 (2.37). ^{1}H- and ^{13}C-NMR, Table 2.

Acknowledgements

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