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Tetrahedron Letters 49 (2008) 2799–2803
Tetrahedron Letters

Takaneones A–C, prenylated butylphloroglucinol derivatives from *Hypericum sikokumontanum*

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Received 26 December 2007; revised 17 February 2008; accepted 22 February 2008
Available online 4 March 2008

Abstract

Three new prenylated butylphloroglucinol derivatives, takaneones A–C (**1**–**3**), were isolated from the MeOH extracts of the aerial parts of *Hypericum sikokumontanum* together with two new prenylated phloroglucinol derivatives, takaneols A and B (**4** and **5**). The structures of the isolated compounds were elucidated by exhaustive spectroscopic analysis. The cytotoxicities of the isolated compounds against human cancer cell lines were evaluated. Compounds **2**–**4** showed cytotoxicities against K562/Adr multi-drug resistant (MDR) cancer cells with IC_{50} values ranging from 4.7 to 10.0 μg/mL, which were slightly more potent than doxorubicin. Their potency of cytotoxicities against MDR cancer cell lines (KB-C2 and K562/Adr) were similar to those against sensitive cell lines (KB and K562).

Keywords: *Hypericum sikokumontanum*; Prenylated butylphloroglucinol; Cytotoxicity; Clusiaceae

The recent widespread interest in the antidepressant activity of *Hypericum perforatum* (St. John's Wort) has inspired the investigation of secondary metabolites from *Hypericum* species.[1] The genus *Hypericum*, which is distributed widely in temperate regions, has been used as traditional medicines in various region of the world. *H. sikokumontanum* Makino (Takane-otogiri in Japanese) is a herb which grows on the mountain area more than 1400 m above sea level in Shikoku island in Japan,[2] and until now, no scientific study for the constituents of this plant has been performed. As a part of our study on *Hypericum* species aimed at searching new biologically active natural products,[3] we have examined the MeOH extracts from the aerial parts of *H. sikokumontanum* to isolate three new compounds, designated takaneones A–C (**1**–**3**), which have unique structures containing butylphloroglucinol moiety, together with two new prenylated phloroglucinols, takaneols A and B (**4** and **5**). In this Letter, we report the isolation, structure elucidation, and cytotoxicity against human cancer cell lines of the new compounds.

Dried aerial parts of *H. sikokumontanum*[4] (460 g) were extracted with hot MeOH. The MeOH extract (113 g) was partitioned between *n*-hexane and H_2O, and the *n*-hexane-soluble fraction (23.7 g) was subjected to silica gel chromatography ($CHCl_3$/MeOH) followed by a Toyopearl HW-40 column ($CHCl_3$/MeOH), a silica gel HPLC (*n*-hexane/EtOAc), and an ODS HPLC (CAPCELL PAK C18, MeOH/H_2O) to give **1** (2 mg), **2** (3 mg), **3** (4 mg), **4** (5 mg), and **5** (7 mg).

Takaneone A (**1**)[5] had a molecular formula of $C_{25}H_{34}O_5$ based on the pseudomolecular ion at *m*/*z* 437.2281 $[M+Na]^+$ (calcd 437.2304, $C_{25}H_{34}O_5Na$) in the HRESIMS. The ^1H and ^{13}C NMR spectra showed the presence of a prenyl group, a 2-methylpropanoyl group, an acetyl group, two methine groups, two methylene groups, three methyl

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doi:10.1016/j.tetlet.2008.02.121

Table 1
NMR data (δ) for takaneones A–C (**1**–**3**)

Position	**1**		**2**		**3**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	205.6	—	205.2	—	202.2	—
2	67.1	—	67.6	—	83.5	—
3	204.6	—	203.6	—	206.4	—
4	80.2	—	78.9	—	61.1	—
5	196.7	—	199.6	—	204.1	—
6	74.0	—	73.9	—	73.3	—
7	24.1	2.67 [1H, dd (12.8, 8.4)] 2.30 [1H, dd (12.8, 6.0)]	23.4	2.56 [1H, dd (14.0, 7.6)] 2.46 [1H, dd (14.0, 11.6)]	23.7	2.50 [2H, m]
8	60.5	2.60 [1H, dd (8.4, 6.0)]	63.3	2.81 [1H, dd (11.6, 7.6)]	63.5	2.86 [1H, dd (11.6, 8.0)]
9	208.5	—	207.5	—	207.2	—
10	31.5	2.18 [3H, s]	31.3	2.18 [3H, s]	31.3	2.18 [3H, s]
11	33.0	2.39 [1H, dd (13.6, 10.0)] 1.67 [1H, dd (13.6, 8.4)]	30.4	2.35 [1H, dd (14.4, 10.4)] 1.81 [1H, dd (14.4, 5.6)]	26.0	2.89 [1H, dd (14.8, 6.0)] 2.06 [1H, dd (14.8, 10.0)]
12	50.1	2.87 [1H, dd (10.0, 8.4)]	52.9	2.67 [1H, dd (10.4, 5.6)]	53.4	2.34 [1H, dd (10.0, 6.0)]
13	44.1	—	45.9	—	45.4	—
14	23.7	1.02 [3H, s]	16.9	0.66 [3H, s]	16.8	0.67 [3H, s]
15	24.6	0.91 [3H, s]	27.4	1.13 [3H, s]	27.3	1.16 [3H, s]
16	12.6	1.24 [3H, s]	12.8	1.26 [3H, s]	207.2	—
17	35.4	2.99, 2.86 [each 1H, dd (13.2, 8.0)]	35.6	2.95 [2H, d (8.0)]	40.4	2.66 [1H, sept (6.4)]
18	115.4	4.87 [1H, t (8.0)]	114.9	4.45 [1H, t (8.0)]	19.0	1.18 [3H, d (6.4)]
19	138.2	—	138.6	—	19.9	1.18 [3H, d (6.4)]
20	17.7	1.49 [3H, s]	17.7	1.48 [3H, s]	39.5	2.55, 2.42 [each 1H, m]
21	25.6	1.56 [3H, s]	25.6	1.55 [3H, s]	116.3	4.73 [1H, t (8.0)]
22	208.4	—	209.2	—	137.9	—
23	36.8	3.24 [1H, sept (6.4)]	36.9	3.35 [1H, sept (6.8)]	17.7	1.50 [3H, s]
24	20.2	1.13 [3H, d (6.4)]	20.0	1.13 [3H, d (6.8)]	25.6	1.63 [3H, s]
25	20.1	1.14 [3H, d (6.4)]	20.1	1.13 [3H, d (6.8)]	23.6	1.50 [3H, s]

Measured in $CDCl_3$. Coupling constants given (J, Hz) in parentheses.

groups, and seven quaternary carbons including three ketone groups (Table 1). The analyses of the ^{1}H–^{1}H COSY and HMBC spectra provided the structure of a partial unit A as shown in Figure 1.

The ^{13}C NMR resonances at δ_C 205.6, 204.6, 196.7, 80.2, 74.0, and 67.1 suggested the presence of a cyclohexa-1,3,5-trione moiety. The connections between the partial unit A and the cyclohexa-1,3,5-trione moiety were established from the following long-range correlations in the HMBC spectrum: H_2-11 with C-1, -2, and C-3; H-12 and H_2-7 with C-1, -5, and C-6. The locations of the prenyl, the 2-methylpropanoyl, and the methyl groups were also confirmed to be at C-4, -4, and C-2, respectively, from the HMBC correlations. The relative stereochemistry of **1** was deduced from the NOESY spectrum as shown in Figure 2. NOE correlations of H_3-14 with H-8 and H-11α in the NOESY spectrum revealed that these protons are located on the same side. In contrast, H_3-15 exhibited

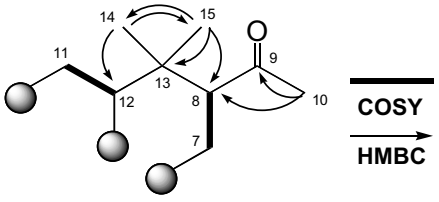

Fig. 1. Key COSY and HMBC correlations of partial unit A.

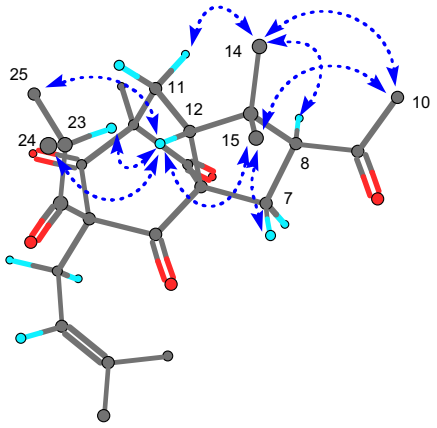

Fig. 2. Key NOE correlations of **1**.

NOE correlation with H-12, which further showed NOE correlations with the protons of 2-methylpropanoyl group, indicating the β-orientations of CH_3-15, H-12, and 2-methylpropanoyl group. Consequently, the structure of **1** was characterized as shown in Figure 3.

Takaneone B (**2**)[6] had the same molecular formula as **1**. The ^{1}H NMR spectral data of **2** was similar to that of **1**, and the analysis of ^{1}H–^{1}H COSY and HMBC spectra of **2** provided the same plane structure as **1**. The carbon resonances of C-4 to C-8 and C-11 to C-15 in the ^{13}C NMR spectrum were slightly different from those of **1**, and there-

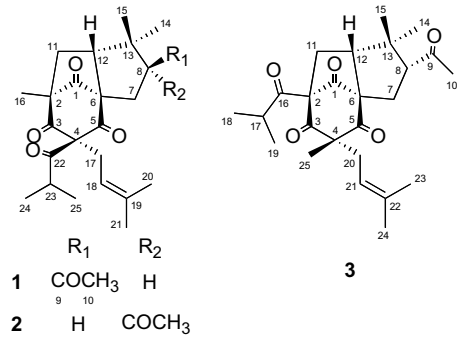

Fig. 3. Takaneones A–C (**1**–**3**).

fore, they were considered to be stereoisomers. The NOESY spectrum of **2** exhibited similar correlations with those of **1**, but difference was found in the observation of an NOESY correlation of H-8 with H_3-15 instead of that of H-8 with H_3-14 seen in **1**. Accordingly, **2** was concluded to be a 8-epimer of **1**, and its structure was elucidated as illustrated (Fig. 3).

Takaneone C (**3**)[7] also had the same molecular formula ($C_{25}H_{34}O_5$) as **1** and **2**. The ^{1}H and ^{13}C NMR spectral analyses of **3** revealed that the structure of **3** is closely correlated with **1** and **2**, and the locations of the functional groups were presumed to be different (Table 1). The HMBC correlations between H_3-25 and C-3, -4, -5, and C-20 indicated that the methyl group and the prenyl group were attached to C-4. The ^{13}C resonance of C-2 (δ_C 83.5), which was shifted to downfield as compared with those of **1** and **2**, implied the position of the 2-methylpropanoyl group at C-2. The configurations of C-8 and C-12 in **3** were concluded to be the same as **2** from the similar NOESY correlations those observed in **2**. The β-orientation of CH_3-25 was judged from the NOE correlation of H_3-25 with H-12 in the NOESY spectrum. Thus, the structure of **3** was elucidated (Fig. 3).

Takaneol A (**4**)[8] had a molecular formula of $C_{31}H_{46}O_5$ on the basis of the HRESIMS. A hydroxyl (3510 cm^{-1}) and carbonyl (1732 and 1699 cm^{-1}) absorption bands were observed in the IR spectrum. Analyses of ^{1}H and ^{13}C NMR data revealed the presence of three prenyl groups, one 2-methyl propanoyl group, two tertiary methyl groups, two carbonyl carbons, two quaternary carbon, one oxygen-bearing quaternary carbon, one oxygen-bearing methine, and two methylens (Table 2). In addition, HMBC correlations of H_3-10 with C-8, -9, and C-11, combined with ^{1}H–^{1}H COSY correlations of H_2-7–H-8; H_2-11–H_2-12–H-13, provided a 3,7-dimethyloct-6-ene-2,3-diol structure, which contains one prenyl moiety. These spectroscopic evidence suggested that **4** is a phloroglucinol derivative with a 5-hydroxycyclohex-4-ene-1,3-dione structure possessing one 2-methylpropanoyl group, two prenyl groups, one methyl group, and the 3,7-dimethyloct-6-ene-2,3-diol moiety. HMBC correlations of H_2-7 with C-1, -2, and C-3 indicated the location of the 3,7-dimethyloct-6-ene-2,3-diol moiety at C-2, and the presence of an ether linkage between C-3 and C-8 was argued from the HMBC correlation of H-8 with C-3. The positions of the methyl group and one prenyl group at C-6 were elucidated from the HMBC correlations between H_3-31 and C-1, -5, -6, and C-26. The HMBC correlations of H_2-17 with C-3, -4, -5, and C-22 disclosed that the 2-methylpropanoyl group and one prenyl group are attached at C-4. The prenyl group on C-4 and CH_3-31 was concluded to be mutually located on the same side from the NOE correlation between H_3-31 and H-18 in the NOESY spectrum. Therefore, the structure of **4** was elucidated as shown in Figure 4.

Takaneol B (**5**)[9] had the same molecular formula $C_{31}H_{46}O_5$ as that of **4**. The ^{1}H and ^{13}C NMR spectral data of **5**, which were quite correlated with those of **4**, revealed the presence of the same functional groups as found in **4**, but differences were found in the carbon resonances for the phloroglucinol moiety in the ^{13}C NMR spectrum (Table 2). Analysis of the HMBC spectrum indicated that a methyl group and a prenyl group are connected to C-6, while a 2-methylpropanoyl group and a prenyl group are attached at C-4. The ^{13}C NMR chemical shifts of C-8 (δ_C 91.0) and C-1 (δ_C 178.0), and the HMBC correlations of H_2-7 with C-1, -2, and C-3; H-8 and H_3-31 with C-1 revealed a connectivity between C-8 and C-1 through an ether bond. The relative stereochemistry of C-4 and C-6 were elucidated from the NOE correlation between H_3-31 and H-18. Accordingly, the structure of **5** was determined as shown in Figure 4.

Takaneones A–C (**1**–**3**) contain a unique ring system with a characteristic C_4 alkyl moiety (C-7 to C-10) at the C-6 position, together with common prenyl groups and a 2-methyl propanoyl group seen in general acylphloroglucinols. These compounds appeared to be a new class of acylphloroglucinols, designated as butylphloroglucinols.

The cytotoxicities of the new compounds (**1**–**5**) against human cancer cell lines were evaluated (Table 3). Compounds **2**–**4** demonstrated relatively potent to moderate cytotoxicities against K562/Adr multi-drug resistant (MDR) cancer cells with IC_{50} values ranging from 4.7 to 10.0 μg/mL, which were slightly more potent than doxorubicin.[10] Their potency of cytotoxicities against MDR cancer cell lines (KB-C2 and K562/Adr) was similar to those against sensitive cell lines (KB and K562).

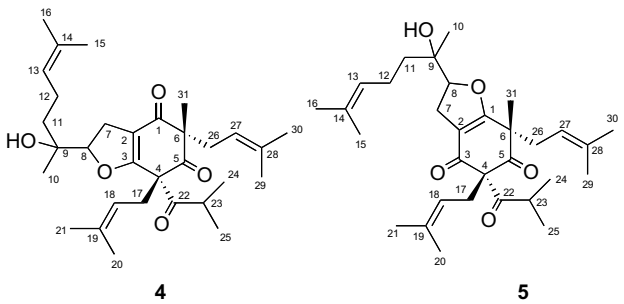

Fig. 4. Takaneols A and B (**4** and **5**).

Table 2
NMR data (δ) for takaneols A and B (**4** and **5**)

Position	**4**		**5**	
	^{13}C	^{1}H	^{13}C	^{1}H
1	193.1	—	178.0	—
2	117.7	—	115.0	—
3	170.4	—	188.7	—
4	70.3	—	78.1	—
5	206.4	—	208.5	—
6	60.3	—	50.7	—
7	26.8	3.11 [1H, dd (14.8, 6.8)] 2.91 [1H, dd (14.8, 10.4)]	27.3	3.04 [1H, dd (15.2, 10.4)] 2.95 [1H, dd (15.2, 8.0)]
8	91.2	4.71 [1H, dd (10.4, 6.8)]	91.0	4.79 [1H, dd (10.4, 8.0)]
9	73.7	—	73.7	—
10	23.4	1.31 [3H, s]	21.8	1.53 [3H, s]
11	36.5	1.59, 1.44 [each 1H, m]	36.7	1.54 [2H, m]
12	21.9	2.11, 2.04 [each 1H, m]	21.8	2.19, 2.11 [each 1H, m]
13	123.8	5.13 [1H, t (6.8)]	123.5	5.13 [1H, t (6.8)]
14	132.0	—	132.6	—
15	17.5	1.64 [3H, s]	17.6	1.62 [3H, s]
16	25.6	1.70 [3H, s]	25.8	1.69 [3H, s]
17	32.2	3.33 [1H, dd (13.2, 7.8)] 2.79 [1H, dd (13.2, 7.8)]	35.1	2.90 [1H, dd (14.0, 7.6)] 2.77 [1H, m]
18	116.7	4.76 [1H, t (7.8)]	118.3	4.85 [1H, t (7.6)]
19	137.0	—	135.2	—
20	17.7	1.62 [3H, s]	17.7	1.52 [3H, s]
21	25.9	1.62 [3H, s]	25.8	1.59 [3H, s]
22	208.2	—	206.4	—
23	36.1	3.41 [1H, sept (6.8)]	40.4	2.54 [1H, sept (6.4)]
24	19.6	0.98 [3H, d (6.8)]	20.7	0.99 [3H, d (6.4)]
25	20.6	1.13 [3H, d (6.8)]	20.3	1.00 [3H, d (6.4)]
26	37.9	2.40 [1H, dd (14.4, 8.4)] 2.33 [1H, dd (14.4, 6.0)]	36.3	2.80 [1H, m] 2.44 [1H, dd (14.0, 7.2)]
27	117.8	4.70 [1H, t (8.4)]	118.1	4.95 [1H, t (7.2)]
28	135.5	—	136.0	—
29	17.7	1.49 [3H, s]	17.7	1.65 [3H, s]
30	25.7	1.57 [3H, s]	25.8	1.64 [3H, s]
31	20.5	1.18 [3H, s]	22.5	1.30 [3H, s]

Measured in $CDCl_3$. Coupling constants given (J, Hz) in parentheses.

Table 3
Cytotoxicity (μg/mL) of **1**–**5** against human cancer cell lines

	1	**2**	**3**	**4**	**5**
KB[a]	14.3 ± 0.5[g]	9.9 ± 0.7	15.8 ± 0.4	22.0 ± 0.8	32.1 ± 0.5
KB-C2[b]	22.4 ± 2.1	15.6 ± 0.8	21.5 ± 1.3	18.6 ± 0.8	25.8 ± 0.5
MCF7[c]	32.2 ± 1.4	18.1 ± 0.3	25.2 ± 1.0	16.1 ± 0.3	24.7 ± 0.4
K562[d]	93.9 ± 12.4	18.3 ± 0.7	18.3 ± 0.4	27.1 ± 0.4	31.1 ± 0.6
K562/Adr[e]	15.9 ± 0.7	4.7 ± 0.5	8.7 ± 0.4	10.0 ± 0.8	22.5 ± 0.3
COLO205[f]	27.6 ± 1.9	18.9 ± 0.8	15.9 ± 0.4	28.2 ± 1.2	20.4 ± 2.3

[a] Human epidermoid carcinoma.
[b] Multidrug-resistant KB cells.
[c] Breast carcinoma.
[d] Leukemia.
[e] Doxorubicin-resistant K562 cells.
[f] Colon carcinoma.
[g] Mean ± SE.

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4. The aerial parts of *Hypericum sikokumontanum* were corrected in August 2005 in Tokushima prefecture, Japan. Identification was carried out by Dr. K. Murakami of the Sojo University, Japan. Herbarium specimens were deposited in the botanical garden of the University of Tokushima (specimen number: UTP980011).
5. Takaneone A (**1**): colorless oil; $[\alpha]_D$ +30.0 (*c* 0.2, MeOH); IR (KBr): ν_{MAX} cm^{-1} 2976, 2935, 1765, 1698, 1450, 1377, 1248, 1151, 1095; HRESIMS: *m*/*z* 437.2281, $[M+Na]^+$ (calcd for $C_{25}H_{34}O_5Na$, 437.2304).
6. Takaneone B (**2**): colorless oil; $[\alpha]_D$ +24.0 (*c* 0.3, MeOH); IR (KBr): ν_{MAX} cm^{-1} 2976, 2935, 1765, 1697, 1450, 1377, 1248, 1151; HRESIMS: *m*/*z* 437.2276, $[M+Na]^+$ (calcd for $C_{25}H_{34}O_5Na$, 437.2304).
7. Takaneone C (**3**): colorless oil; $[\alpha]_D$ +61.9 (*c* 0.4, MeOH); IR (KBr): ν_{MAX} cm^{-1} 2976, 2935, 1763, 1716, 1699, 1454, 1377, 1244, 1203, 1173; HRESIMS: *m*/*z* 437.2293, $[M+Na]^+$ (calcd for $C_{25}H_{34}O_5Na$, 437.2304).
8. Takaneol A (**4**): colorless oil; $[\alpha]_D$ −16.0 (*c* 0.4, MeOH); IR (KBr): ν_{MAX} cm^{-1} 3510, 2974, 2933, 1732, 1699, 1639, 1450, 1408, 1379, 1234, 1184, 1097; HRESIMS: *m*/*z* 521.3217, $[M+Na]^+$ (calcd for $C_{31}H_{46}O_5Na$, 521.3243).
9. Takaneol B (**5**): colorless oil; $[\alpha]_D$ +21.6 (*c* 0.6, MeOH); IR (KBr): ν_{MAX} cm^{-1} 3687, 2976, 2931, 1732, 1701, 1628, 1456, 1410, 1379, 1238, 1188, 1093; HRESIMS: *m*/*z* 421.3225, $[M+Na]^+$ (calcd for $C_{31}H_{46}O_5Na$, 521.3243).
10. The cytotoxicities (μg/mL) for doxorubicin, used as a positive control, against human cancer cell lines, KB: 0.22 ± 0.01; KB-C2: >100; MCF: 0.33 ± 0.02; K562: 0.45 ± 0.01; K562/Adr: 15.2 ± 0.43; COLO: 0.50 ± 0.01.