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# Chromone and chromanone glucosides from *Hypericum sikokumontanum* and their anti-*Helicobacter pylori* activities

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ABSTRACT

Chromone glucosides, takanechromones A–C (**1**, **2** and **5**) and chromanone glucosides, named takanechromanones A and B (**3** and **4**), were isolated from the methanolic extracts of *Hypericum sikokumontanum* together with 27 known compounds. Their structures were established based on spectroscopic evidence. The isolated compounds and some chromone derivatives were assayed for antimicrobial activity against *Helicobacter pylori* and cytotoxicity against human cancer cell lines.



## 1. Introduction

The genus *Hypericum* occurs widely throughout temperate regions of the world, and many of its species have been used as traditional medicinal plants in various parts of the world. Naphtodianthrones, phloroglucinols, flavonoids, xanthones, and benzophenones were reported as components of this genus plants, and many of which show bioactivity (Pinaros, 2005). *Hypericum sikokumontanum* is an herb which grows on mountains more than 1400 m above the sea level in Shikoku island in Japan. As a part of a program to discover new bioactive natural products from *Hypericum* species (Matsuhisa et al., 2002; Tanaka et al., 2004, 2005; Tanaka and Takaishi, 2006, 2007; Hashida et al., 2007), we studied for the constituents of the MeOH extracts from the aerial parts of *H. sikokumontanum*. As the results, three new chromone glucosides named takanechromones A–C (**1**, **2** and **5**) and two new chromanone glucosides named takanechromanones A and B (**3** and **4**) were isolated together with 27 known compounds. In this paper, we describe the isolation and structure elucidation of these compounds. The antimicrobial activity against *Helicobacter pylori* and cytotoxicity against human cancer cell lines of the isolated new compounds and their analogues are also reported.

## 2. Results and discussion

Dried aerial parts of *H. sikokumontanum* were extracted with MeOH. The MeOH extract was partitioned successively with *n*-hexane, EtOAc, BuOH, and $H_2O$. Repeated column chromatography of the EtOAc-soluble fraction and the BuOH-soluble fraction afforded five new compounds (**1**–**5**) and 27 known compounds. The known compounds were identified by comparison with the literature data: 5,7-dihydroxy-3-methylchromone (**6**) (Nedialkov and Kitanov, 2002), 5,7-dihydroxy-3-ethylchromone (**7**) (Al-Douri and Dewick, 1988), 5,7-dihydroxy-2-isopropylchromone-8-*C*-β-D-glucoside (**8**) (Wu et al., 1998), 5,7-dihydroxy-2-(1-methylpropyl)chromone-8-*C*-β-D-glucoside (**9**) (Wu et al., 1998), hyperxanthone (Cardona et al., 1990; Chen and Chen, 1985), 1,3,5-trihydroxyxanthone (Frahm and Chaudhuri, 1979), quercetin (Markham et al., 1978), quercetin-3-*O*-β-D-glucoside (Markham et al., 1978), hyperin (Markham et al., 1978), quercetin-3-*O*-(2″-acetyl)-β-D-galactoside (Jürgenliemk and Nahrstedt, 2002), avicularin (Markham et al., 1978), quercitrin (Markham and Ternai, 1976), kaempferol-3-*O*-β-D-glucoside (Markham et al., 1978), isomangiferin (Faizi et al., 2006; Chen and Chen, 1985), 3-*O*-caffeoylquinic acid butyl ester (Zhang et al., 2001), 5-*O*-caffeoylquinic acid butyl ester (Corthout et al., 1992), 5-*O*-caffeoylquinic acid methyl ester (Peng et al., 2000), 5-*O*-*cis*-caffeoylquinic acid methyl ester (Runbero-Sanchez and Vazquez, 1991), 3-*O*-caffeoylquinic acid methyl ester (Zhu et al., 2005), 5-*O*-caffeoylquinic acid (Cheminat

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et al., 1988), betulalbuside A (Morikawa et al., 2004), (6*S*,9*S*)-3-oxo-α-ionol-β-D-glucoside (Lee et al., 2003), 1-*O*-benzoyl β-D-glucoside (Ushiyama et al., 1989), 2-phenylethyl-β-D-glucoside (Piao et al., 2003), vanillic acid 4-*O*-β-D-glucoside (Sakushima et al., 1995), *trans*-*p*-coumaric acid 4-*O*-β-D-glucoside (Foo et al., 2000), *cis*-*p*-coumaric acid 4-*O*-β-D-glucoside (Foo et al., 2000).

Takanechromone A (**1**) was assigned to have the molecular formula of $C_{16}H_{18}O_9$ on the basis of HRESIMS analysis. The IR absorption bands at 1660, 1628, and 1587 $cm^{-1}$ suggested the presence of a chromone skeleton. The $^1H$ NMR spectrum of **1** contained signals for an olefinic proton, a pair of *meta*-coupled aromatic protons, and a vinyl methyl proton, together with an anomeric proton resonance (Table 1). The $^{13}C$ NMR spectrum exhibited signals for a conjugated carbonyl carbon, six aromatic carbons, one double bond, one methyl group, together with resonances for a hexosyl moiety (Table 2). From these data, **1** was presumed to be a methyl chromone glycoside. Enzymatic hydrolysis of **1** with β-glucosidase, together with D-glucose, gave an aglycone, which was identified as 5,7-dihydroxy-3-methylchromone (**6**). The HMBC correlation between H-1′ and C-7 clearly indicated that the glucosyl moiety is connected to the C-7 hydroxyl group, and its β-linkage was concluded from the *J*-value (6.8 Hz) of the anomeric proton signal. Thus, the structure of **1** was elucidated as 5,7-dihydroxy-3methylchromone-7-*O*-β-D-glucopyranoside (Fig. 1).

The $^1H$ and $^{13}C$ NMR spectroscopic data of takanechromone B (**2**) were quite similar to those of **1** (Tables 1 and 2). The observation of the signal for an ethyl group [$\delta_H$ 2.43 (2H, *q*, *J* = 7.2 Hz), 1.17 (3H, *t*, *J* = 7.2 Hz); $\delta_C$ 19.3, 13.4] in **2** instead of the methyl resonance in **1** suggested **2** to be 5,7-dihydroxy-3-ethylchromone-7-*O*-β-glucoside. Enzymatic hydrolysis yielded 5,7-dihydroxy-3-ethylchromone (**7**) and D-glucose. The location and linkage of the glucosyl moiety were elucidated from analysis of the HMBC spectrum and the $^1H$ NMR spectroscopic data. Consequently, the structure of **2** was established as 5,7-dihydroxy-3-ethylchromone-7-*O*-β-D-glucopyranoside (Fig. 1).

Takanechromanone A (**3**) had a molecular formula of $C_{16}H_{20}O_{10}$ based on the pseudmolecular ion at *m*/*z* 371.0997 ($[M-H]^-$ calcd. 371.0978, $C_{16}H_{19}O_{10}$) in the HRESIMS. The $^1H$ NMR spectrum displayed signals for a pair of *meta*-coupled aromatic protons, an oxygen-bearing methine proton, a methine proton, and a secondary methyl proton, together with resonances arising from a sugar moiety (Table 3). In the $^{13}C$ NMR spectrum, the following 16 signals were observed: one ketone, one benzene ring, one acetal methine, one methine adjacent to carbonyl group, one methyl, and one hexosyl group (Table 3). The appearance of a carbonyl carbon resonance at lower field than those of **1** and **2** suggested **3** to be a methyl chromanone glycoside. The structure of the aglycone of **3** was elucidated as 2,5,7-trihydroxy-3-methyl-chromanone by the

**Table 2**
$^{13}C$ NMR spectroscopic data (δ, $CD_3OD$) for **1**–**5**.

| Position | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 2 | 154.8 | 154.7 | 105.9 | 104.7 | 177.5 |
| 3 | 120.3 | 125.8 | 46.2 | 52.9 | 106.6 |
| 4 | 183.8 | 183.5 | 199.5 | 198.9 | 184.5 |
| 5 | 163.0 | 163.1 | 165.3 | 165.2 | 162.9 |
| 6 | 100.9 | 100.8 | 97.5 | 97.5 | 101.1 |
| 7 | 164.6 | 164.6 | 168.4 | 168.4 | 164.8 |
| 8 | 95.8 | 95.8 | 97.0 | 97.0 | 95.9 |
| 9 | 159.4 | 159.5 | 160.4 | 160.2 | 159.4 |
| 10 | 107.4 | 107.6 | 102.0 | 102.4 | 107.0 |
| 1’ | 101.6 | 101.6 | 104.1 | 104.1 | 101.6 |
| 2’ | 74.7 | 74.7 | 75.0 | 75.0 | 74.7 |
| 3’ | 77.8 | 77.8 | 77.9 | 77.9 | 77.8 |
| 4’ | 71.2 | 71.2 | 71.0 | 71.0 | 71.2 |
| 5’ | 78.4 | 78.4 | 78.0 | 78.0 | 78.4 |
| 6’ | 62.4 | 62.4 | 62.5 | 62.5 | 62.4 |
| 3-Me | 10.2 | – | 13.8 | – | – |
| 3-Et | – | 19.3 | – | 23.7 | – |
| | – | 13.4 | – | 11.8 | – |
| 2-*i*Pro | – | – | – | – | 64.5 |
| | – | – | – | – | 20.3 |

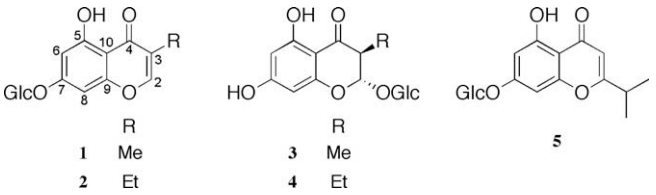



**Fig. 1.** The structures of **1**–**5**.

**Table 3**
$^1H$ NMR spectroscopic data (δ, $CD_3OD$) for **3** and **4**.

| Position | 3 | 4 |
|---|---|---|
| 2 | 5.44 [1H, *d* (2.8)] | 5.58 [1H, *d* (1.6)] |
| 3 | 2.79 [1H, *dq* (7.6, 2.8)] | 2.61 [1H, *dt* (1.6, 7.6)] |
| 6 | 5.89 [1H, *d* (2.0)] | 5.88 [1H, *d* (2.0)] |
| 8 | 5.91 [1H, *d* (2.0)] | 5.89 [1H, *d* (2.0)] |
| 1′ | 4.59 [1H, *d* (7.6)] | 4.58 [1H, *d* (8.0)] |
| 2′–5′ | 3.19–3.35 [4H, *m*] | 3.16–3.38 [4H, *m*] |
| 6′ | 3.78 [1H, *dd* (12.4, 2.0)] | 3.75 [1H, *dd* (12.0, 2.0)] |
| | 3.66 [1H, *dd* (12.4, 4.4)] | 3.64 [1H, *dd* (11.6, 4.0)] |
| 3-Me | 1.27 [3H, *d* (7.6)] | – |
| 3-Et | – | 1.77, 1.66 [each 1H, *dq* (7.6, 7.6)] |
| | – | 1.04 [3H, *t* (7.6)] |

Coupling constants given (*J* in Hz) in parentheses.

**Table 1**
$^1H$ NMR spectroscopic data (δ, $CD_3OD$) for **1**, **2**, and **5**.

| Position | 1 | 2 | 5 |
|---|---|---|---|
| 2 | 7.95 [1H, *s*] | 7.93 [1H, *s*] | – |
| 3 | – | – | 6.12 [1H, *s*] |
| 6 | 6.46 [1H, *d* (2.0)] | 6.46 [1H, *d* (2.0)] | 6.49 [1H, *d* (2.0)] |
| 8 | 6.62 [1H, *d* (2.0)] | 6.63 [1H, *d* (2.0)] | 6.69 [1H, *d* (2.0)] |
| 1’ | 5.02 [1H, *d* (6.8)] | 5.02 [1H, *d* (7.6)] | 5.06 [1H, *d* (7.2)] |
| 2’–5’ | 3.37-3.52 [4H, *m*] | 3.37-3.52 [4H, *m*] | 3.40 - 3.58 [4H, *m*] |
| 6’ | 3.90 [1H, *dd* (12.0, 2.0)] | 3.90 [1H, *dd* (12.0, 2.0)] | 3.93 [1H, *dd* (12.0, 2.0)] |
| | 3.70 [1H, *dd* (12.0, 5.6)] | 3.70 [1H, *dd* (12.0, 5.6)] | 3.73 [1H, *dd* (12.0, 5.6)] |
| 3-Me | 1.94 [3H, *s*] | – | – |
| 3-Et | – | 2.43 [2H, *q* (7.2)] | – |
| | – | 1.17 [3H, *t* (7.2)] | – |
| 2-*i*Pro | – | – | 2.91 [1H, *sept* (6.8)] |
| | – | – | 1.33 [6H, *d* (6.8)] |

Coupling constants given (*J* in Hz) in parentheses.

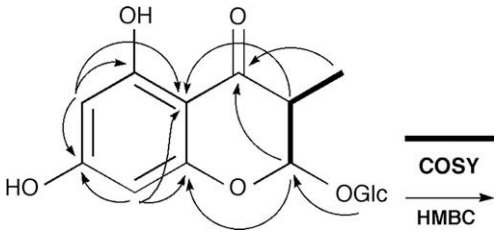
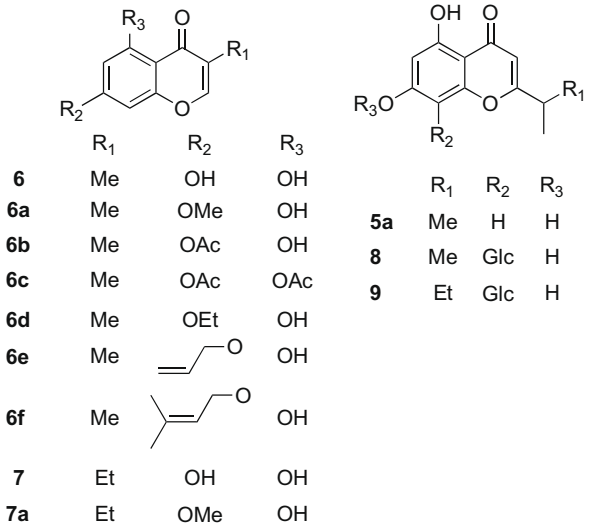



**Fig. 2.** Key COSY and HMBC correlations for **3**.

analysis of the COSY and the HMBC spectra (key correlations shown in Fig. 2). On enzymatic hydrolysis, **3** liberated D-glucose and an aglycone, which was readily converted to **6**; the expected aglycone initially formed was not detected. Production of **6** can be explained by the C-2 hydroxyl group of 2,5,7-trihydroxy-3-methyl-chromanone readily undergoing dehydration. The location of the glucosyl moiety at C-2 was established from the HMBC correlation between the anomeric proton and C-2; its β-linkage was shown by the *J*-value of the anomeric proton signal. The configurations of C-2 and C-3 were elucidated by analysis of the NOESY spectrum. Thus, the anomeric-H showed NOESY correlations with H-2 and H-3, while 3-$CH_3$ exhibited NOESY cross-peaks with H-2 and H-3. Considering the small coupling constants between H-2 and H-3 ($J$ = 2.8 Hz), the configurations of 3-$CH_3$ and 2-OH groups were concluded to be *trans*. Consequently, **3** was characterized as 2α,5,7-trihydroxy-3β-methylchromanone-2-*O*-β-D-glucopyranoside.

The molecular formula of takanechromanone B (**4**) was elucidated as $C_{17}H_{22}O_{10}$ based on HRESIMS analysis. The $^1H$ and $^{13}C$ NMR spectroscopic data of **4** were quite similar to those of **3**, and the signal of an ethyl group in **4** [$\delta_H$ 1.77, 1.66 (each $^1H$, *dq*, $J$ = 7.6, 7.6 Hz), 1.04 (3H, *t*, $J$ = 7.6 Hz); $\delta_C$ 23.7, 11.8] was easily discerned instead of the methyl resonance observed in **3**. This observation suggested that the 3-methyl group in **3** was replaced with an ethyl group in **4**. The structure of **4** was verified from the HMBC correlations between the methylene protons of the ethyl group and C-2, C-3, and C-4. In addition, enzymatic hydrolysis of **4** gave D-glucose and **7**; the latter was considered to be the dehydrated aglycone of **4** as noted for hydrolysis of **3**. The NOESY correlations similar to those observed in **3** indicated that the relative stereostrucutre of **4** was the same as **3**. Accordingly, the structure of **4** was 2α,5,7-trihydroxy-3β-ethylchromanone-2-*O*-β-D-glucopyranoside (Fig. 1).

The $^{13}C$ NMR spectroscopic data for takanechromone C (**5**), $C_{18}H_{22}O_9$, were similar to that of **1** except for the chemical shifts of C-2 to C-4. The $^1H$ NMR spectrum of **5** indicated the presence




| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| **6** | Me | OH | OH |
| **6a** | Me | OMe | OH |
| **6b** | Me | OAc | OH |
| **6c** | Me | OAc | OAc |
| **6d** | Me | OEt | OH |
| **6e** | Me |  | OH |
| **6f** | Me |  | OH |
| **7** | Et | OH | OH |
| **7a** | Et | OMe | OH |

| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| **5a** | Me | H | H |
| **8** | Me | Glc | H |
| **9** | Et | Glc | H |

**Fig. 3.** The structures of **6–9**, **5a**, **6a–6f** and **7a**.

**Table 4**
Anti-*H. pylori* activities of compounds isolated from *H. sikokumontanum* (**1**, **3–9**) and derivatives from **5**, **6**, and **7** (**5a**, **6a–6f**, and **7a**).

| Compounds (100 μg/disc) | Inhibition zone in diameter (mm)[b] | |
|---|---|---|
| | *H. pylori* SS-1 | *H. pylori* ATCC43504 |
| **1** | – | – |
| **3** | 10 | 14 |
| **4** | 16 | 16 |
| **5** | 8 | – |
| **5a** | 18 | 23 |
| **6** | 26 | 28 |
| **6a** | – | – |
| **6b** | 16 | 19 |
| **6c** | 16 | 19 |
| **6d** | – | – |
| **6e** | 13 | 12 |
| **6f** | – | – |
| **7** | 19 | 20 |
| **7a** | 23 | 27 |
| **8** | – | – |
| **9** | – | – |
| AMPC[a] | 32 | 34 |

[a] Amoxicillin (250 ng/ disc).
[b] Disc: 6 mm.

**Table 5**
Cytotoxicity of **1**, **3–7** against human tumor cell lines.

| Compounds | KB[a] | KB-C2[b] | KB-C2 (+Col.)[c] | MCF-7[d] | K562[e] | K562/Adr[f] | COLO205[g] |
|---|---|---|---|---|---|---|---|
| **1** | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **3** | >100 | >100 | >100 | >100 | >100 | 58.8 ± 3.9 | >100 |
| **4** | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| **5** | >100 | >100 | >100 | >100 | >100 | 25.0 ± 5.1 | >100 |
| **6** | 61.9 ± 3.3[h] | 69.7±3.4 | 73.4 ± 2.3 | >100 | >100 | 11.0 ± 2.3 | >100 |
| **7** | 39.1 ± 1.6 | 44.5 ± 1.6 | 46.4 ± 0.6 | >100 | >100 | 22.3 ± 5.8 | 80.7 ± 6.2 |
| Doxorubicin | 0.22 ± 0.01 | >100 | –[i] | 0.33 ± 0.02 | 0.45 ± 0.01 | 15.2 ± 0.43 | 0.50 ± 0.01 |

[a] Human epidermoid carcinoma.
[b] Multidrug-resistant KB cells.
[c] 2.5 μM colchicine.
[d] Breast carcinoma.
[e] Leukemia.
[f] Doxorubicin-resistant K562 cells.
[g] Colon carcinoma.
[h] Mean ± SE.
[i] Not tested.

of an isopropyl group instead of a methyl group in **1**. Based on these data, **5** was assumed to be a choromone glucoside having two hydroxyl and one isopropyl groups. Enzymatic hydrolysis of **5** gave 5,7-dihydroxy-2-isopropylchromone (**5a**) (Bohlmann et al., 1984) and D-glucose. The HMBC correlation of H-1′ with C-7, and the coupling constant ($J$ = 7.2 Hz) of the anomeric proton indicated that the β-glucosyl moiety was attached at C-7. Thus, **5** was established as 5,7-dihydroxy-2-isopropylchromone-7-*O*-β-D-glucopyranoside.

As a part of our ongoing study to discover the anti-*Helicobacter pylori* compounds from plants (Ochi et al., 2005), the isolated chromone and chromanones (**1**, **3**–**9**), together with **5a** and derivatives (**6a**–**6f** and **7a**) of **6** and **7** were evaluated for their anti-*H. pylori* activity by the disk diffusion method (Fig. 3, Table 4). Among the evaluated compounds, **6**, **7**, and **7a** showed inhibitory activity against *H. pylori* growth. 5,7-Dihydroxy-3-ethylchromone (**7**, lathodoratin) and its 7-*O*-methyl ether (**7a**, methyl-lathodoratin) were reported as phytoalexins of *Lathyrus odoratus* (Robeson et al., 1980) possessing fungitoxicity. The cytotoxicity of **1**, **3**–**7** against human cancer cell lines were also evaluated. Compounds **6** and **7** showed cytotoxicities against multi-drug resistant cancer cell lines (KB-C2 and K562/Adr) comparable to those of doxorubicin. However, only weak or no cellular toxicities were observed against sensitive cancer cell lines (Table 5).

## 3. Concluding remarks

As a part of our ongoing study on *Hypericum* plants to discover new bioactive natural products, we studied the constituents of the MeOH extracts from the aerial parts of *H. sikokumontanum*. This has resulted in the isolation and structure elucidation of three new chromone glucosides, takanechromones A–C (**1**, **2** and **5**), and two new chromanone glucosides, takanechromanones A and B (**3** and **4**), together with 27 known compounds. This is the first example of the isolation of 2-hydroxychromanone derivatives from natural source to the best of our knowledge. Transformation of 2-hdroxy-3,7-substituted chromanone derivatives to chromones in the presence of alkali formats and alkali–alkoxide was reported (Borbély and Szabó, 1981). Our result showed that 2,5,7-trihydroxy-3-substituted chromanone was unstable changing readily to chromone derivatives. In contrast, the existence of 3-substituted-chromones has been so far found in *Hypericum annulatum* (Nedialkov and Kitanov, 2002), *H. japonicum* (Wu et al., 1998) (Guttiferae), *Lathyrus odoratus* (Papilionoaceae) (Robeson et al., 1980), and *Baeckea frutescens* (Myrtaceae) (Satake et al., 1999), and this is the first report for the isolation of *O*-glucosyl-3-substituted-chromones.

In addition, the isolated compounds and some chromone derivatives were assayed for antimicrobial activity against *Helicobacter pylori* and cytotoxicity against human cancer cell lines including multi-drug resistant (MDR) cancer cell lines. Anti-*H. pylori* activity was observed in 5,7-dihydroxy-3-methylchromone (**6**) and 3-ethyl-5-methoxy-7-hydroxychromone (**7a**), while **6** and 5,7-dihydroxy-3-ethylchromone (**7**) showed cytotoxicities against MDR cancer cell lines, especially against K562/Adr, which comparable to those of doxorubicin.

## 4. Experimental

### 4.1. General experimental procedures

NMR experiments were run on a Bruker ARX-400 instrument, $^{1}$H 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 N (Merck), Toyopearl HW-40 (TOSOH), Sephadex LH-20 (Amersham Pharmacia Biotech AB), MCI gel CHP-20P (Mitsubishi Chemical), and ODS-A (YMC). The high-performance liquid chromatography (HPLC) consisted of GPC (Shodex, GS-310 2G, MeOH), silica gel HPLC (Mightysil Si 60, 250 × 20 mm), ODS HPLC (CAPCELL PAK C18, Shiseido). IR spectra were recorded on a 1720 infrared Fourier transform spectrometer (Perkin–Elmer). Optical rotations were measured with a JASCO DIP-370 digital polarimeter.

### 4.2. Plant material

The aerial parts of *Hypericum sikokumontanum* were corrected in August 2005 in Tokushima prefecture, Japan. Herbarium specimens were deposited in the botanical garden of the University of Tokushima (specimen number: UTP980011).

### 4.3. Extraction and isolation

Dried aerial parts of *H. sikokumontanum* (460 g) were extracted with MeOH (5 L × 3), and the MeOH ext. (113 g) was suspended in $H_2O$ (600 ml), and partitioned successively with *n*-hexane (600 ml × 3), EtOAc (600 ml × 3), and BuOH (600 ml × 3). The EtOAc-soluble fraction (13.7 g) was subjected to silica gel CC eluted with different solvents of increasing polarity (*n*-hexane → EtOAc → MeOH) to give 9 fractions (Fr. e1–9). Fr. e2 (672 mg) was separated by Toyopearl HW-40 CC ($CHCl_3$–MeOH, 1: 1), and purified by GPC on HPLC (MeOH) to give **6** (171 mg) and **7** (55 mg). Fr. e3 (230 mg) was subjected to GPC on HPLC (MeOH) and ODS-HPLC (MeOH–$H_2O$, 6: 4, v/v) to give hyperxanthone (2 mg) and 1,3,5-trihydroxyxanthone (4 mg). Crystallization of Fr. e4 (700 mg) with MeOH gave quercetin (350 mg), whereas Fr. e8 (8.69 g) gave a pure sample of hyperin (4.5 g) on crystallization. The mother liquor of Fr. E8 was subsequently applied to Sephadex LH-20 CC with MeOH to give three fractions (Fr. e8.1–3). Fr. e8.1 was loaded onto a silica gel column ($CHCl_3$–acetone, 8:2, 6:4, and 0: 10, v/v) to give four fractions (Fr. e8.1.1–4). Fr. e8.1.3 was subjected to GPC on HPLC with MeOH to give four fractions (Fr. e8.1.3.1–4) and quercetin-3-*O*-β-D-glucoside (50 mg, Fr. e8.1.3.5). Fr. e8.1.3.4 was separated by ODS-HPLC (MeOH–$H_2O$, 1:1, v/v) to give quercetin-3-*O*-(2″-acetyl)-β-D-galactoside (14 mg) and kaempferol-3-*O*-β-D-glucoside (4 mg). Fr. e8.2 was loaded onto a MCIgel CHP20P column eluted a gradient of MeOH–$H_2O$ (3: 7, v/v) to MeOH (100%) to afford six fractions (fr. e8.2.1–6). Fr. e8.2.4 was separated by a GPC on HPLC with MeOH to give six fractions (fr. e8.2.4.1–6) including one resulting in a pure sample of avicularin (35 mg, fr. e8.2.4.3). Fr. e8.2.4.2 was purified by ODS-HPLC HPLC (MeOH–$H_2O$, 1: 1, v/v) to give quercitrin (8 mg).

The BuOH soluble-fraction (31.9 g) was subjected to silica gel CC ($CHCl_3$–MeOH, 95:5, 9:1, 8:2, 6:4, and 0:10, v/v) to give nine fractions (fr. b1–9). Fr. b5 (4.9 g) was applied to MCIgel CHP20P CC eluted with a gradient of MeOH–$H_2O$ (1: 4, v/v) to MeOH (100%) to give seven fractions (fr. b5.1–7). Fr. b5.2 was reapplied to a Sephadex LH-20 column with MeOH–$H_2O$ (1:1, v/v) to give three fractions (fr. b5.2.1–3). of Frs. b5.2.2 and b5.2.3 were subjected to GPC-HPLC (MeOH) and ODS-HPLC (MeOH–$H_2O$, 1: 1, v/v) gave betulalbuside A (44 mg), 1-*O*-benzoyl β-D-glucoside (4 mg), and 5-*O*-caffeoylquinic acid (26 mg), respectively. Fr. b5.4 was loaded onto a Sephadex LH-20 column with MeOH–$H_2O$ (1: 1, v/v) to give three fractions (fr. b5.4.1–3). Fr. b5.4.1 was subjected to GPC-HPLC (MeOH), and then purified by ODS-HPLC (MeOH-$H_2O$, 1: 1, v/v) to afford (6*S*, 9*S*)-3-oxo-α-ionol-β-D-glucoside (13 mg) and 2-phenylethyl-β-D-glucoside (4 mg). Fr. b5.4.2 was applied to GPC on HPLC with MeOH to give three fractions (fr. b5.4.2.1–3) including **1** (156 mg, fr. b5.4.2.3). Fr. b5.4.2.1 and 2 were purified by ODS-HPLC (MeOH–$H_2O$, 1:1, v/v) to give **4** (281 mg), **8** (18 mg), **9** (4 mg), 5-*O*-*cis*-caffeoylquinic acid methyl ester (20 mg), and 3-*O*-*cis*-caffeoylquinic acid methyl ester (35 mg). Fr. b5.5 was applied to a Sephadex LH-20 column with MeOH–$H_2O$ (1:1, v/v) to give five fractions (fr. b5.5.1–5). Fr. b.5.5.2 was purified by GPC-HPLC (MeOH) and ODS-

HPLC (MeOH–$H_2O$, 1: 1, v/v) to give **2** (5 mg) and **5** (18 mg). Compounds 3-*O*-caffeoylquinic acid butyl ester (18 mg), 5-*O*-caffeoylquinic acid butyl ester (13 mg), and 5-*O*-caffeoylquinic acid methyl ester (346 mg) were isolated from fr. b5.5.3 by GPC on HPLC (MeOH) and an ODS-HPLC (MeOH–$H_2O$, 4: 6, v/v). Fr. b7 (13.6 g) was subjected to a MCIgel CHP20P eluted a gradient of MeOH–$H_2O$ (0:1, v/v) to MeOH (100%) to give eight fractions (fr. b7.1–8). Fr. b7.3 was loaded onto an ODS column (MeOH–$H_2O$, 1:4 to 6: 4, v/v) to give vanillic acid 4-*O*-β-D-glucoside (204 mg). Fr. b7.5 was successively applied to a Sephadex LH-20 column (MeOH–$H_2O$, 1:1, v/v), GPC on HPLC (MeOH), and finally purified by ODS-HPLC (MeOH–$H_2O$, 1:1, v/v) to give *trans*-*p*-coumaric acid 4-*O*-β-D-glucoside (32 mg) and *cis*-*p*-coumaric acid 4-*O*-β-D-glucoside (5 mg). Fr. b7.6 was separated by Sephadex LH-20 CC with MeOH–$H_2O$ (1:1, v/v), and then purified by ODS-HPLC with MeOH–$H_2O$ (1:1, v/v) to give **3** (891 mg) and isomangiferin (17 mg).

### 4.4. 5,7-dihydroxy-3-methylchromone-7-O-β-D-glucoside (**1**, takanechromone A)

Amorphous powder; $[\alpha]_D$: -81.5 (*c* 0.3 MeOH); IR (KBr): $\nu_{MAX}$ 3410, 2922, 2893, 1660, 1628, 1587, 1502, 1454, 1367, 1587, 1502, 1454, 1367, 1304, 1188 $cm^{-1}$; HRESIMS: *m/z* 377.0816, $[M+Na]^+$ (calcd for $C_{16}H_{18}O_9Na$, 377.0849); For $^1H$ NMR (400 MHz) and $^{13}C$ NMR (100 MHz) spectroscopic data, see Tables 1 and 2.

### 4.5. 5,7-dihydroxy-3-ethylchromone-7-O-β-D-glucoside (**2**, takanechromone B)

Amorphous powder; $[\alpha]_D$: -86.0 (*c* 0.1 MeOH); IR (KBr): $\nu_{MAX}$ 3388, 2927, 2897, 1662, 1624, 1581, 1498, 1302, 1180, 1078 $cm^{-1}$; HRESIMS: *m/z* 391.0989, $[M+Na]^+$ (calcd for $C_{17}H_{20}O_9Na$, 391.1005); For $^1H$ NMR (400 MHz) and $^{13}C$ NMR (100 MHz) spectroscopic data, see Tables 1 and 2.

### 4.6. 2α,5,7-trihydroxy-3β-methylchromanone-2-O-β-D-glucoside (**3**, takanechromanone A)

Amorphous powder; $[\alpha]_D$: +111.7 (*c* 0.9 MeOH); IR (KBr): $\nu_{MAX}$ $cm^{-1}$ 3398, 2924, 2881, 1637, 1606, 1469, 1389, 1363, 1281, 1171; HRESIMS: *m/z* 371.0997, $[M-H]^-$ (calcd for 371.0978, $C_{16}H_{19}O_{10}$); For $^1H$ NMR (400 MHz) and $^{13}C$ NMR (100 MHz) spectroscopic data, see Tables 2 and 3.

### 4.7. 2α,5,7-trihydroxy-3β-ethylchromanone-2-O-β-D-glucoside (**4**, takanechromanone B)

Amorphous powder; $[\alpha]_D$: +93.0 (*c* 0.8 MeOH); IR (KBr): $\nu_{MAX}$ $cm^{-1}$ 3398, 2933, 2879, 1647, 1510, 1468, 1379, 1304, 1275, 1169; HRESIMS: *m/z* 385.1146, $[M-H]^-$ (calcd for $C_{17}H_{21}O_{10}$, 385.1135); For $^1H$ NMR (400 MHz) and $^{13}C$ NMR (100 MHz) spectroscopic data, see Tables 2 and 3.

### 4.8. 5,7-dihydroxy-2-isopropylchromone-7-O-β-D-glucoside (**5**, takanechromone C)

Amorphous powder; $[\alpha]_D$: −99.1 (*c* 0.1 MeOH); IR (KBr): $\nu_{MAX}$ $cm^{-1}$ 3367, 2922, 2885, 1662, 1620, 1574, 1502, 1417, 1377, 1294, 1261, 1205, 1180, 1051; HRESIMS: *m/z* 405.1196, $[M+Na]^+$ (calcd for $C_{18}H_{22}O_9Na$, 405.1162); For $^1H$ NMR (400 MHz) and $^{13}C$ NMR (100 MHz) spectroscopic data, see Tables 1 and 2.

### 4.9. Enzymatic hydrolysis of **1**, **3**–**5**

A solution of **1** (15 mg), **3** (13 mg), **4** (14 mg), and **5** (10 mg) in $H_2O$ (1 ml) were separately treated with β-glucosidase (14 mg, SIGMA) at 37 °C for 60 h. The reaction mixture was diluted with $H_2O$ (2 ml), and extracted with EtOAc (2 ml × 3). The EtOAc layer was dried over $Na_2SO_4$, and concentrated under reduced pressure to give a residue which was purified by silica gel column chromatography to afford an aglycone [**6** from **1** and **3** (5.0 mg and 6.0 mg, respectively); **7** (4.0 mg) from **4**; **5a** (5.0 mg) from **5**]. The aqueous layer was concentrated to a residue, which was subjected to silica gel CC [$CHCl_3$–MeOH–$H_2O$ (7:3:0.5)] to furnish D-glucose.

### 4.10. Methylation of **6**

A solution of **6** (11.8 mg) in MeOH (2 ml) was treated with trimethylsilyldiazomethane ($TMSCHN_2$, 1.5 ml, 0.6 M, in hexane) at room temperature for 1 h with stirring. After removal of solvent by evaporation, the residue was purified by prep. TLC (silica gel, $CHCl_3$–MeOH, 97:3) to afford **6a** (11.3 mg). 5-Hydroxy-7-methoxy-3methylchromone (**6a**): Amorphous powder. HRESIMS: *m/z* 207.0673, $[M+H]^+$ (calcd for $C_{11}H_{11}O_4$, 207.0657); $^1H$ NMR (acetone-$d_6$): $\delta_H$ 12.89 (1H, *s*), 8.03 (1H, *d*, *J* = 1.2 Hz), 6.46 (1H, *d*, *J* = 2.4 Hz), 6.30 (1H, *d*, *J* = 2.4 Hz), 3.89 (3H, *s*), 1.93 (3H, *d*, *J* = 1.2 Hz).

### 4.11. Methylation of **7**

Methylation of **7** (6.7 mg) was carried out as for **6** yielded 5-Hydroxy-7-methoxy-3-ethylchromone (**7a**, 6.0 mg). 5-Hydroxy-7-methoxy-3-ethylchromone (**7a**): Amorphous powder. HRESIMS: *m/z* 221.0815, $[M+H]^+$ (calcd for $C_{12}H_{13}O_4$, 207.0814); $^1H$ NMR (acetone-$d_6$): $\delta_H$ 12.89 (1H, *s*), 8.00 (1H, *brd*), 6.48 (1H, *d*, *J* = 2.0 Hz), 6.31 (1H, *d*, *J* = 2.0 Hz), 3.90 (3H, *s*), 2.43 (2H, *dq*, *J* = 7.8, 0.8 Hz), 1.17 (3H, *t*, *J* = 7.8 Hz).

### 4.12. 12. Acetylation of **6**

A mixture of **6** (23.6 mg) in $Ac_2O$ (1 ml) and pyridine (1 ml) was kept standing at room temperature overnight. The mixture was concentrated to dryness to give a residue, which was applied to prep. TLC (silica gel, $CHCl_3$–MeOH, 97: 3) to afford **6b** (13 mg) and **6c** (10 mg).

7-Acethoxy-5-hydroxy-3-methylchromone (**6b**): Amorphous powder. HRESIMS: *m/z* 257.0440, $[M+Na]^+$ (calcd for $C_{12}H_{10}O_5Na$, 257.0426); $^1H$ NMR (acetone-$d_6$): $\delta_H$ 7.90 (1H, *s*), 6.74 (1H, *d*, *J* = 2.4 Hz), 6.57 (1H, *d*, *J* = 2.4 Hz), 2.30 (3H, *s*), 1.84 (3H, *d*, *J* = 2.4 Hz). 5,7-Diacethoxy-3-methylchromone (**6c**): Amorphous powder. HRESIMS: *m/z* 299.0523, $[M+Na]^+$ (calcd for $C_{14}H_{12}O_6Na$, 299.0532); $^1H$ NMR (acetone-$d_6$): $\delta_H$ 8.05 (1H, *q*, *J* = 1.2 Hz), 7.27 (1H, *d*, *J* = 2.4 Hz), 6.92 (1H, *d*, *J* = 2.4 Hz), 2.33, 2.32 (each 3H, *s*), 1.89 (3H, *d*, *J* = 1.2 Hz).

### 4.13. General procedure for preparation of 3-O-alkyl derivatives of **6** (**6d**–**6f**)

A mixture of **6** (21–6 mg), an appropriate alkylbromide (0.4 ml), and $K_2CO_3$ (50 mg) in dry acetone (15 ml) was refluxed for 2 h with stirring. After removal of inorganic salts by filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel CC (benzene–acetone, 98:2) to afford a derivative.

7-Ethoxy-5-hydroxy-3-methylchromone (**6d**): (22 mg, 60% yield). Amorphous powder. HRESIMS: *m/z* 221.0827, $[M+H]^+$ (calcd. for $C_{12}H_{13}O_4$, 221.0814); $^1H$ NMR (acetone-$d_6$): $\delta_H$ 12.82 (1H, *s*), 8.02 (1H, *d*, *J* = 1.2 Hz), 6.43 (1H, *d*, *J* = 2.4 Hz), 6.28 (1H, *d*, *J* = 2.4 Hz), 4.15 (1H, *q*, *J* = 7.2 Hz), 1.93 (3H, *d*, *J* = 1.2 Hz), 1.39 (3H, *t*, *J* = 7.2 Hz). 7-Allyloxy-5-hydroxy-3-methylchromone (**6e**): (21 mg, 90% yield). Amorphous powder. HRESIMS: *m/z* 255.0650, $[M+Na]^+$ (calcd. for $C_{13}H_{12}O_4Na$, 255.0633); $^1H$ NMR (acetone-$d_6$): $\delta_H$ 12.82 (1H, *s*), 8.00 (1H, *s*), 6.45 (1H, *d*, *J* = 2.0 Hz), 6.31 (1H, *d*,

$J$ = 2.0 Hz), 6.08 (1H, *ddd*, $J$ = 17.2, 10.8, 5.2 Hz), 5.45 (1H, *dd*, $J$ = 17.2, 1.2 Hz), 5.29 (1H, *dd*, $J$ = 10.8, 1.2 Hz), 4.67 (2H, *d*, $J$ = 5.2 Hz), 1.93 (3H, *s*). 7-Dimethylallyloxy-5-hydroxy-3-methylchromone (**6f**): (26 mg, 65% yield). Amorphous powder. HRESIMS: *m/z* 283.0927, $[M+Na]^+$ (calcd for $C_{15}H_{16}O_4Na$, 283.0946); $^1H$ NMR (acetone-$d_6$): $\delta_H$ 12.83 (1H, *s*), 8.03 (1H, *d*, $J$ = 0.8 Hz), 6.47 (1H, *d*, $J$ = 1.2 Hz), 6.31 (1H, *d*, $J$ = 1.2 Hz), 5.47 (1H, *td*, $J$ = 6.8, 0.8 Hz), 4.66 (2H, *d*, $J$ = 6.8 Hz), 1.94 (3H, *d*, $J$ = 0.8 Hz), 1.79 (3H, *d*, $J$ = 6.8 Hz), 1.77 (3H, *s*).

### 4.14. Anti-Helicobacter pylori activity

The disc-diffusion method was used to screen compounds against *H. pylori* ATCC43504 and *H. pylori* SS-1 as described previously (Ochi et al., 2005).

### 4.15. Cell Lines and Cell Culture

KB (human epidermoid carcinoma of the nasopharynx), MCF-7 (breast carcinoma), COLO205 (colon carcinoma), K-562 (leukemia), and K562/Adr (multidrug-resistant human erythromyelogenous leukemia) cells were obtained from the Cell Resource Center for Biomedical Research (Tohoku University). Multidrug-resistant human epidermoid carcinoma KB-C2 cells were kindly provided by Prof. Shin-ichi Akiyama (Kagoshima University, Japan). KB cells were cultured in Dulbecco's modified Eagles medium (DMEM) with 10% fetal bovine serum (FBS). KB-C2 cells were maintained in DMEM medium in the presence of 10% FBS and 2 μg/ml colchicine. MCF-7, COLO205, and K-562 cells were cultured in RPMI1640 supplemented with 10% FBS. K-562/Adr (doxorubicin-resistant K562 cell line) cells were cultured in RPMI1640 medium containing 10% FBS and 0.5 μM doxorubicin. All cells were incubated at 37 ℃ in a humidified atmosphere with 5% $CO_2$ to 95% air.

### 4.16. Cytotoxicity Assays

Cells in exponential growth were trypsinized, dispersed in a single cell suspension, and dispensed in 100 μl volumes into 96-well plates. For each assay, 1 × 104 cells/well for K562 and K562/Adr, 5 × 103 cells /well for KB and KB-C2, or 5 × 103 cells for MCF-7, and COLO205 were inoculated in 100 μl medium containing 10% FBS and incubated for 24 h. Test samples were dissolved in small amounts of DMSO and diluted in the appropriate culture medium (final concentration of DMSO <0.5%). After removal of pre-incubated culture medium, 100 μl of medium containing various concentrations of each test compound were added and further incubated for 48 h. Cell proliferation was determined by a 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. $IC_{50}$ values are defined as the concentration of each test samples that reduced absorbance to 50% of vehicle-treated controls.

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