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Tomoeones A–H, cytotoxic phloroglucinol derivatives from *Hypericum ascyron*

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ABSTRACT

Phloroglucinol derivatives tomoeones A–H (**1**–**8**) and three known compounds were isolated from leaves of *Hypericum ascyron*. Their structures were established based on spectroscopic analyses. They are all acylphloroglucinol derivatives possessing a spiro skeleton with geminal isoprenyl groups and a monoterpene moiety, and they are stereoisomers to each other at C-4 and C-13. They appear to be a class of phloroglucinol derivatives. Cytotoxicities of the isolated phloroglucinol derivatives against human tumor cell lines, including multidrug-resistant (MDR) cancer cell lines, were evaluated. Tomoeone F (**6**) demonstrated significant cytotoxicity against KB cells with an IC_{50} value of 6.2 μM. Compound **6** was also cytotoxic against MDR cancer cell lines (KB-C2 and K562/Adr), which was more potent than doxorubicin.

1. Introduction

The genus *Hypericum* occurs widely in temperate regions and has been used as a traditional medicinal plant in various parts of the world. *Hypericum perforatum* (St. John's Wort) is used as a herbal medicine for treatment of moderate depression, and this effect has inspired investigations of secondary metabolites from *Hypericum* species (Pinarosa, 2005). As a result, various classes of bioactive compounds were reported. Among others, acylphloroglucinols possessing unique structures were shown to have a variety of bioactivities, and are paid attention as a new drug lead from plant sources (Tada et al., 1991; Verotta et al., 2004; Medina et al., 2006).

Hypericum ascyron is a Chinese natural drug used in treatment of wounds, swelling, headache, nausea and abscesses (Jiang Su New Medical College, 1977). Up to the present, some reports described the constituents of *H. ascyron*, in which flavonoids and xanthones were confirmed as components of this plant (Wang and Wang, 1980; Komissarenko and Levashova, 1992; Zhang et al., 1998; Park et al., 2000). However, there is no report about acylphloroglucinols from this plant.

To discover new bioactive natural products from plants, we are studying the constituents of *Hypericum* plants, and reported some bioactive compounds (Matsuhisa et al., 2002; Tanaka et al., 2004, 2005). As a part of this program, we have examined the MeOH extracts from the leaves of *H. ascyron* and isolated eight new phloroglucinol derivatives named tomoeones A–H (**1**–**8**) together with three known compounds. Some of these compounds showed potent to moderate cytotoxicity against human cancer cell lines. In this paper, we describe the isolation, structure elucidation, and cytotoxicity of isolated compounds.

2. Results and discussion

The MeOH extracts of *H. ascyron* leaves were partitioned between *n*-hexane and H_2O. The *n*-hexane-soluble fraction was subjected to column chromatography repeatedly to give eight new (**1**–**8**) and three known (**9**–**11**) compounds.

Tomoeone A (**1**) had a molecular formula of $C_{30}H_{44}O_6$ on the basis of its HRESIMS. The IR spectrum showed absorption bands of a hydroxyl (3463 cm^{-1}), a carbonyl (1718 cm^{-1}), and a conjugated carbonyl (1670 cm^{-1}) groups. The ^1H NMR spectrum of **1** showed the presence of two isoprenyl groups [δ_H 4.96 (1H, *t*-like), 4.59 (1H, *t*-like), 2.73 (1H, *dd*, *J* = 13.6 and 7.8 Hz), 2.65 (1H, *dd*, *J* = 13.6 and 7.8 Hz), 2.55 (1H, *dd*, *J* = 13.2 and 8.8 Hz), 2.35 (1H, *dd*, *J* = 13.2 and 8.8 Hz), 1.60, 1.57, 1.50, 1.35 (each 3H, *s*)], a 2-methylpropanoyl group [δ_H 3.33 (1H, *sept*, *J* = 6.8 Hz, H-28), 1.23, 1.11 (each 3H, *d*, *J* = 6.8 Hz)], two methines [δ_H 1.67, 1.65 (each 1H, *m*)], four methylenes [δ_H 2.12 (1H, *d*, *J* = 13.6 Hz), 1.89, 1.70 (each 1H, *m*), 1.74, 1.73 (each 2H, *m*), 1.15 (1H, *d*, *J* = 13.6 Hz)], and two tertiary methyls [δ_H 1.35, 0.92 (each 3H, *s*)]. In addition, the characteristic signal of a hydrogen-bonded hydroxyl group was observed at δ_H 18.32 (1H, *s*). This down-fielded resonance is peculiar to acylphloroglucinol derivatives isolated from *Hypericum chinense* (Nagai and Tada, 1987), and bitter acids from hops (Zhao

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et al., 2005). The ^{13}C NMR spectrum suggested the presence of 30 carbons including three carbonyl carbons, one oxygenated sp^2 carbon, three sp^2 quaternary carbons, two sp^2 methines, two oxygenated sp^3 quaternary carbons, two sp^3 quaternary carbons, three sp^3 methines, six sp^3 methylenes, and eight methyls. Based on these data, **1** was considered as an acylphloroglucinol derivative having two isoprenyl groups, one 2-methylpropanoyl group, and a partial unit constituted of 10 carbons. The plane structure of the partial unit was elucidated by the analysis of its 2D NMR spectra including COSY, HSQC, and HMBC spectra (key correlations were shown in Fig. 1). The phloroglucinol moiety consisted of six quaternary carbons including two ketones (δ_C 207.4 and 197.4), an enol (δ_C 198.1 and 113.1), and two sp^3 carbons (δ_C 65.3 and 61.8). The HMBC correlations of H_2-7 and H_2-14 with C-3, C-4, and C-5 indicated that C-7 and C-14 in the partial unit were connected to C-4 (Fig. 2). The positions of the 2-methylpropanoyl group and two isoprenyl groups were determined to be C-2, C-6, and C-6, respectively, from the following HMBC correlations: OH-1 with C-1, C-2, C-6, and C-27; H_2-17 and H_2-22 with C-1, C-5, and C-6 (Fig. 2). Thus, compound **1** was established as shown in Fig. 4.

The relative stereochemistry of **1** was elucidated by the NOESY spectroscopic analysis as shown in Fig. 3. The NOE correlations of H-7ax/H-14ax and H-12/H-14ax indicated their axial orientations. Therefore, the cyclohexane ring, consisted of C-4, C-7, C-8, and C-12 to C-14, adopts a chair conformation. In addition, β-orientations of CH_3-15 and CH_3-16 were noted from the NOE correlations of H-8/H_3-15, and H-8/H_3-16. On the other hand, the methine proton signal and one of the methyls due to the 2-methyl propanoyl group at C-2 displayed NOESY correlations with H_3-15 and H-14eq, respectively, indicating that the 2-methyl propanoyl group is present on the upper side of the cyclohexane ring. Consequently, the structure of **1** was established as illustrated in Fig. 4.

The same molecular formula of tomoeone B (**2**) as that of **1** was established from its HRESIMS. The ^{13}C NMR spectroscopic data were similar to those of **1**, except for the signals of C-4, C-7, C-14, C-15 (Table 1). Detailed analyses of the COSY and the HMBC spectra indicated that **2** was a stereoisomer of **1**. The relative stereochemistry of C-7 to C-16 in **2** was decided to be the same as that of **1** from the following NOE correlations in the NOESY spectrum: H-8 with H_3-15, and H_3-16; H-12 with H-14ax; H_3-15 with H-14eq (Fig. 5). In addition, H_3-15 and H-14eq exhibited NOE correlations with an olefinic proton signal due to one of the isoprenyl groups. This observation indicated that the isoprenyl group at C-6 is located on the upper side of the cyclohexane ring. Accordingly, the structure of **2** was elucidated as shown in Fig. 4.

Tomoeones C (**3**) and D (**4**) also had the same molecular formula as those of **1** and **2** as established by HRESIMS. The ^1H and ^{13}C NMR, HMBC spectra indicated that **3** and **4** were stereoisomers of **1** and **2**. Although the ^{13}C NMR spectra of **3** and **4** were closely correlated with those of **1** and **2**, respectively, the resonances due to C-12 to C-15 were different from those found in **1** and **2** in each case. Since the ^{13}C resonance for C-13 was significantly shifted to downfield (*ca.* 12 ppm) as compared to those of **1** and **2**, the configuration of C-13 in **3** and **4** was considered to be different from that of **1** and **2**. A chair conformation of the cyclohexane ring, consisted of C-4, C-7, C-8, and C-12 to C-14, as well as a β-orientation of CH_3-16 in **3** and **4**, were shown to be the same as those of **1** and **2** by

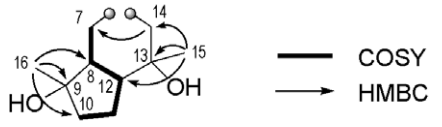


Fig. 1. The key COSY and HMBC correlations of the partial unit of **1**.

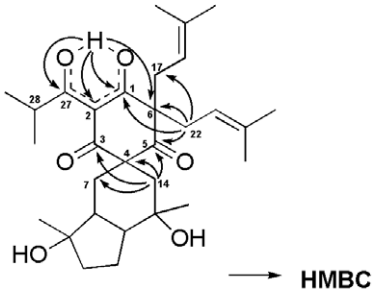


Fig. 2. The key HMBC correlations of **1**.

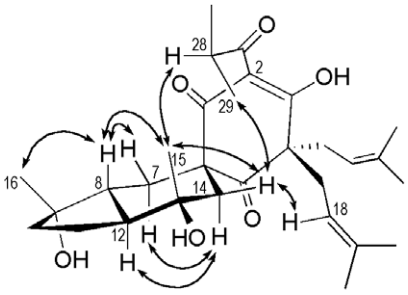
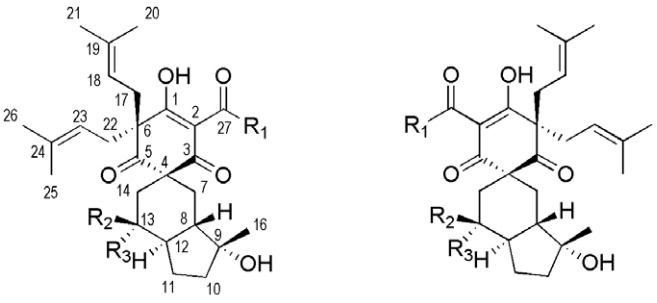


Fig. 3. Key NOE correlations of **1**.



	R_1	R_2	R_3		R_1	R_2	R_3
1	isopropyl	CH_3	OH	**2**	isopropyl	CH_3	OH
3	isopropyl	OH	CH_3	**4**	isopropyl	OH	CH_3
5	isobutyl	CH_3	OH	**6**	isobutyl	CH_3	OH
7	isobutyl	OH	CH_3	**8**	isobutyl	OH	CH_3

Fig. 4. Structures of tomoeones A–H (**1**–**8**).

NOESY correlations shown in Figs. 6 and 7. The equatorial orientation of CH_3-15 in **3** and **4** was also demonstrated by NOESY correlations of H_3-15 with H-12, H-14ax, and H-14eq in both cases. Furthermore, the configurations of C-4 in **3** and **4**, which were considered to be opposite to each other, were elucidated from the following NOE correlations. Thus, NOESY correlations of H-14eq with H_3-29 and H-18 were observed in the case of **3** similar to those seen in **1**. In contrast, H-14eq exhibited an NOESY correlation only with H-18, which is the same as that found in **2**. Accordingly, the structures for **3** and **4** were concluded as illustrated.

Tomoeones E (**5**) and F (**6**) gave the closely correlated ^1H and ^{13}C NMR spectra, which also quite resembled with those of **1** and **2**, respectively. In addition, the presence of a 3-methylbutanoyl group in both **5** and **6** was deduced in each case instead of the 2-methylpropanoyl group from the following ^1H and ^{13}C resonances; [δ_H

Table 1
^{13}C NMR spectroscopic data for **1**–**8** (δ, ppm, at 100 MHz) in $CDCl_3$

Position	1	2	3	4	5	6	7	8
1	198.1	196.5	198.0	196.1	196.9	196.4	196.8	195.8
2	113.1	112.4	113.1	112.6	114.4	113.6	114.4	113.7
3	197.4	196.4	197.6	195.7	197.4	196.1	197.6	195.6
4	65.3	62.8	64.5	62.4	65.1	62.7	64.3	62.3
5	207.4	208.8	207.5	208.3	207.3	208.9	207.5	208.3
6	61.8	61.2	61.8	61.1	61.6	61.1	61.6	61.0
7	24.0	26.9	24.2	26.5	24.2	26.9	24.4	25.9
8	48.0	47.7	47.7	47.1	48.0	47.7	47.7	47.2
9	79.2	78.9	78.9	78.4	79.2	78.9	78.9	78.3
10	39.7	40.6	39.8	40.5	39.8	40.5	39.9	40.5
11	21.6	21.5	22.0	22.6	21.6	21.6	22.5	22.7
12	51.9	51.2	46.3	45.6	52.0	51.3	46.2	45.8
13	73.2	72.7	85.0	85.5	73.1	72.8	85.1	85.3
14	50.2	44.5	44.5	38.4	50.1	44.6	44.4	38.3
15	20.3	23.1	16.5	19.9	20.9	22.9	17.0	19.1
16	26.7	26.5	26.5	26.5	26.8	26.5	26.6	26.5
17	34.4	37.0	34.6	36.6	34.6	36.8	34.8	36.6
18	119.2	118.1	119.0	118.5	119.2	118.1	119.2	118.4
19	135.6	137.2	135.9	136.6	135.7	137.2	135.9	136.6
20	17.8[a]	17.9	17.8	17.9	17.9	17.9	17.9	17.9
21	25.8	26.0	25.8	26.0	25.9	26.0	25.9	26.0
22	41.0	38.1	40.9	38.9	40.7	38.2	40.7	38.5
23	116.9	117.9	117.0	117.4	117.0	117.8	117.0	117.4
24	137.9	137.3	137.9	137.6	137.8	136.8	137.8	137.5
25	17.6	17.8	17.6	17.8	17.7	17.8	17.7	17.8
26	25.8	26.0	25.8	25.9	25.9	26.0	25.9	26.0
27	205.1	207.7	205.4	207.6	201.1	203.1	201.4	203.0
28	34.1	35.2	24.3	35.1	46.1	47.1	46.5	46.9
29	17.9[a]	19.0	17.6	18.8	25.5	26.1	25.6	25.6
30	21.0	19.5	21.0	19.3	22.4	22.6	22.1	22.5
31	–	–	–	–	22.8	22.7	22.8	22.6

[a] Signals may be exchangeable.

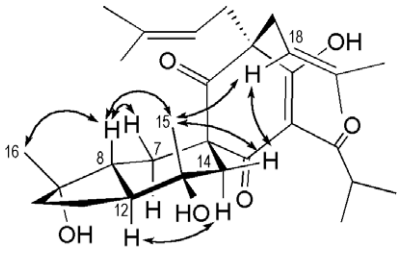


Fig. 5. Key NOE correlations of **2**.

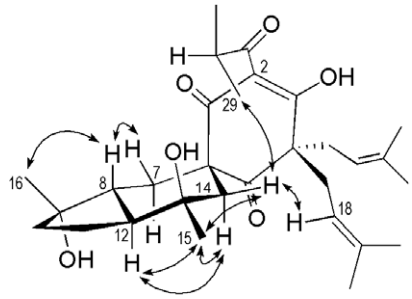


Fig. 6. Key NOE correlations of **3**.

2.78 (1H, *dd*, *J* = 14.8 and 6.4 Hz, H-28a), 2.72, 2.18 (each 1H, *m*), 0.98, 0.96 (each 3H, *d*, *J* = 6.4 Hz); δ_C 203.1, 47.1, 26.1, 22.7, 22.6]. These NMR observations suggested that the 2-methylpropanoyl group at C-2 described in **1** and **2** was replaced by a 3-methylbutanoyl group in **5** and **6**. The molecular formula of $C_{31}H_{46}O_6$ for **5** and

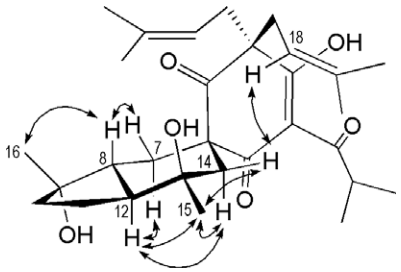


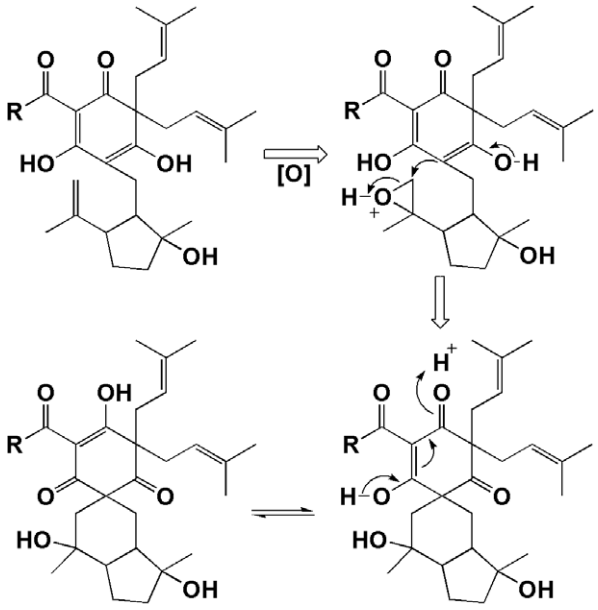
Fig. 7. Key NOE correlations of **4**.

6, confirmed by the HRESIMS in each case, was also consistent with these observations. Analyses of 2D-NMR spectroscopic data for **5** and **6** suggested that they are stereoisomers as noted for **1** and **2**. The relative stereochemistries of **5** and **6** were elucidated from the NOESY correlations. Thus, **5** displayed NOESY cross-peaks quit similar to those observed in **1**, while NOESY correlations in **6** resembled with those of **2**. Thus, the structures of **5** and **6** were elucidated as shown in Fig. 4.

Tomoeones G (**7**) and H (**8**) also had the same molecular formula as those of **5** and **6** based on their HRESIMS data. The NMR spectroscopic data of **7** and **8** were closely correlated with those of **5** and **6**, indicating that they possessed the same overall structure as found in **5** and **6**. In contrast, the ^{13}C resonances of **7** and **8** were in close agreement with those of **3** and **4**, respectively, except for the resonances arising from the 2-methylpropanoyl group. These spectroscopic observations suggested that **7** and **8** are considered to be C-13 isomers of **5** and **6**, and had the same relative stereochemistry as for **3** and **4**, respectively. The NOESY data of **7** and **8** were also consistent with their stereostructures being the same as **3** and **4**, respectively.

The following known compounds were identified by comparison with the literature data; 3,5-dihydroxy-4-{[(1*R*,2*S*,5*S*)-2-hydroxy-2-methyl-5-(1-methylethenyl)cyclopentyl]methyl}-2-(3-methylbutanoyl)-6,6-bis(3-methylbut-2-enyl)cyclohexa-2,4-dien-1-one (**9**), hypercalin C (**10**), hypercalin B (**11**) (Decosterd et al., 1989).

Compounds (**1**–**8**) are considered to be closely related biosynthetically with **9**–**11**, and our postulated biosynthetic pathway of



Scheme 1. Possible biosynthesis pathway of tomoeones.

Table 2
^{1}H NMR spectroscopic data (δ, at 400 MHz) for **1**–**8** in $CDCl_3$[a]

Position	1	2	3	4	5	6	7	8
1-OH	18.32 [1H, *s*]	18.36 [1H, *s*]	18.35 [1H, *s*]	18.42 [1H, *s*]	18.20 [1H, *s*]	18.27 [1H, *s*]	18.23 [1H, *s*]	18.25 [1H, *s*]
7ax	1.70 [1H, *m*]	1.72 [1H, *m*]	1.68 [1H, *m*]	1.71 [1H, *m*]	1.64 [1H, *m*]	1.68 [1H, *m*]	1.60 [1H, *m*]	1.67 [1H, *m*]
7eq	1.89 [1H, *m*]	1.81 [1H, *m*]	1.86 [1H, *m*]	1.88 [1H, *m*]	1.95 [1H, *m*]	1.87 [1H, *m*]	1.88 [1H, *m*]	1.82 [1H, *m*]
8	1.65 [1H, *m*]	1.62 [1H, *m*]	1.7 [1H, *m*]	1.56 [1H, *m*]	1.55 [1H, *m*]	1.77 [1H, *m*]	1.74 [1H, *m*]	1.57 [1H, *m*]
10	1.74 [2H, *m*]	1.76 [2H, *m*]	1.74 [2H, *m*]	1.77 [2H, *m*]	1.77 [2H, *m*]	1.79 [2H, *m*]	1.76 [2H, *m*]	1.70 [2H, *m*]
11	1.73 [2H, *m*]	1.77 [2H, *m*]	1.70 [2H, *m*]	1.73, 1.35 [each 1H, *m*]	1.74 [2H, *m*]	1.80 [2H, *m*]	1.77 [2H, *m*]	1.68, 1.41 [each 1H, *m*]
12	1.67 [1H, *m*]	1.80 [1H, *m*]	1.91 [1H, *m*]	1.95 [1H, *m*]	1.69 [1H, *m*]	1.83 [1H, *m*]	1.92 [1H, *m*]	1.94 [1H, *m*]
14ax	1.15 [1H, *d*, 13.6]	1.60 [1H, *m*]	1.44 [1H, *d*, 13.6]	1.70 [1H, *d*, 14.4]	1.16 [1H, *d*, 13.6]	1.55 [1H, *d*, 14.4]	1.45 [1H, *d*, 13.2]	1.78 [1H, *d*, 14.4]
14eq	2.12 [1H, *d*, 13.6]	2.03 [1H, *d*, 14.4]	2.25 [1H, *d*, 13.6]	2.08 [1H, *d*, 14.4]	2.11 [1H, *d*, 13.6]	2.01 [1H, *d*, 14.4]	2.24 [1H, *d*, 13.2]	2.05 [1H, *d*, 14.4]
15	0.92 [3H, *s*]	1.08 [3H, *s*]	0.97 [3H, *s*]	1.13 [3H, *s*]	0.94 [3H, *s*]	1.07 [3H, *s*]	1.00 [3H, *s*]	1.15 [3H, *s*]
16	1.35 [3H, *s*]	1.26 [3H, *s*]	1.35 [3H, *s*]	1.26 [3H, *s*]	1.36 [3H, *s*]	1.25 [3H, *s*]	1.36 [3H, *s*]	1.27 [3H, *s*]
17	2.73 [1H, *dd*, 13.6, 7.8]	2.72 [1H, *dd*, 14.0, 8.8]	2.74 [1H, *dd*, 13.6, 8.0]	2.71 [1H, *dd*, 13.4, 7.6]	2.75 [1H, *dd*, 14.0, 7.8]	2.74 [1H, *m*]	2.76 [1H, *dd*, 13.6, 7.8]	2.72 [1H, *m*]
	2.65 [1H, *dd*, 13.6, 7.8]	2.52 [1H, *m*]	2.65 [1H, *dd*, 13.6, 8.0]	2.56 [1H, *dd*, 13.4, 7.6]	2.65 [1H, *dd*, 14.0, 7.8]	2.52 [1H, *dd*, 13.6, 6.4]	2.65 [1H, *dd*, 13.6, 7.8]	2.55 [1H, *dd*, 13.6, 6.4]
18	4.96 [1H, *t*-like]	4.9 [1H, *t*-like]	4.97 [1H, *t*-like]	4.91 [1H, *t*-like]	4.97 [1H, *t*-like]	4.89 [1H, *t*-like]	4.99 [1H, *t*-like]	4.92 [1H, *t*-like]
20	1.57 [3H, *s*]	1.52 [3H, *s*]	1.57 [3H, *s*]	1.53 [3H, *s*]	1.58 [3H, *s*]	1.47 [3H, *s*]	1.58 [3H, *s*]	1.53 [3H, *s*]
21	1.60 [3H, *s*]	1.61 [3H, *s*]	1.62 [3H, *s*]	1.60 [3H, *s*]	1.61 [3H, *s*]	1.55 [3H, *s*]	1.64 [3H, *s*]	1.61 [3H, *s*]
22	2.55 [1H, *dd*, 13.2, 8.8]	2.65 [1H, *m*]	2.57 [1H, *dd*, 13.2, 8.0]	2.62 [1H, *dd*, 13.4, 8.4]	2.59 [1H, *dd*, 13.2, 8.0]	2.68 [1H, *m*]	2.60 [1H, *dd*, 13.2, 8.4]	2.65 [1H, *dd*, 13.2, 8.0]
	2.35 [1H, *dd*, 13.2, 8.8]	2.50 [1H, *m*]	2.37 [1H, *dd*, 13.2, 8.0]	2.46 [1H, *dd*, 13.4, 8.4]	2.36 [1H, *m*]	2.46 [1H, *dd*, 13.6, 7.6]	2.37 [1H, *m*]	2.47 [1H, *dd*, 13.2, 8.0]
23	4.59 [1H, *t*-like]	4.74 [1H, *t*-like]	4.59 [1H, *t*-like]	4.68 [1H, *t*-like]	4.60 [1H, *t*-like]	4.72 [1H, *t*-like]	4.61 [1H, *t*-like]	4.70 [1H, *t*-like]
25	1.35 [3H, *s*]	1.47 [3H, *s*]	1.35 [3H, *s*]	1.44 [3H, *s*]	1.38 [3H, *s*]	1.52 [3H, *s*]	1.38 [3H, *s*]	1.48 [3H, *s*]
26	1.50 [3H, *s*]	1.56 [3H, *s*]	1.50 [3H, *s*]	1.52 [3H, *s*]	1.53 [3H, *s*]	1.61 [3H, *s*]	1.51 [3H, *s*]	1.55 [3H, *s*]
28	3.33 [1H, *sept*, 6.8]	3.57 [1H, *m*]	3.34 [1H, *sept*, 6.8]	3.52 [1H, *sept*, 6.8]	2.95 [1H, *dd*, 15.2, 6.8]	2.78 [1H, *dd*, 14.8, 6.4]	2.97 [1H, *dd*, 15.2, 6.4]	2.84 [1H, *dd*, 14.8, 6.8]
	-	-	-	-	2.37 [1H, *m*]	2.72 [1H, *m*]	2.38 [1H, *m*]	2.68 [1H, *m*]
29	1.11 [3H, *d*, 6.8]	1.15 [3H, *d*, 6.8]	1.11 [3H, *d*, 6.8]	1.16 [3H, *d*, 6.8]	2.26 [1H, *m*]	2.18 [1H, *m*]	2.37 [1H, *m*]	2.20 [1H, *m*]
30	1.23 [3H, *d*, 6.8]	1.19 [3H, *d*, 6.8]	1.23 [3H, *d*, 6.8]	1.19 [3H, *d*, 6.8]	1.01 [3H, *d*, 6.4]	0.98 [3H, *d*, 6.4]	0.95 [3H, *d*, 6.4]	0.98 [3H, *d*, 6.8]
31	–	–	–	–	0.95 [3H, *d*, 6.4]	0.96 [3H, *d*, 6.4]	1.01 [3H, *d*, 6.4]	0.99 [3H, *d*, 6.8]

[a] Coupling constant given (*J* in Hz).

these new compounds is shown in Scheme 1. Thus, epoxidation of exomethylene of **9**–**11**, followed by intramolecular cyclization gave **1**–**8**. The epoxide-mediated spirocyclization yields theoretically four stereoisomers in each case. In this point of view, we isolated all the possible stereoisomers having a 2-methylpropanoyl or a 3-methylbutanoyl group in the molecule. Since biosynthetic precursors **9**–**11** are closely correlated structurally with chinesin II (Aramaki et al., 1995), whose absolute stereochemistry was determined by chemical examinations, the configurations at C-8, C-9, and C-12 in compounds **1**–**8** are presumed to be the same as those in chinesin II.

Some phloroglucinol derivatives are known to have cytotoxicity (Arisawa et al., 1990; Ito et al., 2000; Ishii et al., 2003; Martinez-Poveda et al., 2005). Therefore, cytotoxicities for tomoeones A–H (**1**–**8**) against human tumor cell lines including multidrug-resistant (MDR) cancer cell lines (KB-C2 and K562/Adr) were evaluated (Table 3). Compounds **6** and **5** demonstrated significant to relatively potent cytotoxicity against KB cells with IC_{50} values of 6.2 and 17.1 μM, respectively. They also showed relatively potent cytotoxicity against MDR cancer cell lines with IC_{50} values ranging from 17.1 to 48.0 μM. The cytotoxicity of **6** against the MDR cell lines was more toxic than doxorubicin: IC_{50} >100 μg/mL (KB-C2); 28.1 μM (K562/Adr). Overall, compounds having a 3-methylbutanoyl group at C-2 were more toxic than compounds with a 2-methylpropanoyl group at C-2. In addition, compounds **1**, **2**, **5**, and **6** were more cytotoxic than **3**, **4**, **7**, and **8**, in which all the former compounds had the axial CH_3 at C-13, and therefore, the configuration of CH_3-15 might also contribute their cytotoxicity.

Table 3
Cytotoxicity data (IC_{50}, μM) for **1**–**8** against human tumor cell lines[a] (means ± SE)[b]

Compound	Cell lines						
	KB	KB-C2	KB-C2 (+2.5 μM colchicine)	K562	K562/Adr	MCF7	COLO205
1	44.3 ± 0.8	>100	56.5 ± 1.3	>100	>100	75.7 ± 1.0	>100
2	50.9 ± 0.8	>100	94.3 ± 7.8	>100	>100	86.3 ± 3.0	>100
3	>100	>100	>100	>100	>100	>100	>100
4	>100	>100	>100	64.9 ± 3.2	94.5 ± 4.2	>100	>100
5	17.1 ± 0.8	45.8 ± 3.7	30.9 ± 1.6	>100	48.0 ± 1.6	51.1 ± 1.0	>100
6	6.2 ± 0.4	31.5 ± 1.7	28.6 ± 0.8	40.4 ± 3.1	17.1 ± 0.2	29.3 ± 0.6	46.4 ± 3.5
7	>100	>100	>100	68.4 ± 0.6	>100	>100	>100
8	>100	>100	>100	67.8 ± 4.5	87.2 ± 2.9	>100	>100
Doxorubicin	0.40 ± 0.02	>100	–	0.83 ± 0.02	28.09 ± 0.79	0.61 ± 0.04	0.92 ± 0.02

[a] Cell lines: KB, epidermoid carcinoma; KB-C2, colchicine resistant KB; K562, leukemia; K562/Adr, doxorubicin-resistant K562; MCF7, breast carcinoma; COLO205, colon carcinoma.
[b] Data are mean ± SE from three or four experiments.

3. Concluding remarks

Tomoeones A–H (**1**–**8**) have a unique spiro skeleton with geminal prenyl groups and a monoterpene moiety, and are classified as a new class of phloroglucinol derivatives. Tomoeones F (**6**) and E (**5**) demonstrated potent to moderate cytotoxicities against KB cells as well as MDR cancer cell lines (KB-C2 and K562/Adr), and whose cytotoxicities against MDR cancer cell lines were more potent than doxorubicin. Recently, some phloroglucinol derivatives, including guttiferone G, hyperforin, and aristoforin were reported as inhibitors of SIRT1 and SIRT2, and were shown to be strong inhibitors on proliferation of human umbilical vein endothelial (HUVE) cells. Though the mechanisms of action of tomoeones for showing cytotoxicity against human cancer cells, including MDR cancer cells, is not clear, inhibition of SIRTs might be one of possible mechanisms of action.

4. Experimental

4.1. General experimental procedures

NMR experiments were run on a Bruker AVANCE instrument, ^{1}H NMR: 400 MHz, ^{13}C NMR 100 MHz, using TMS as int. stand. MS was obtained on a Waters LCT Premier. CC: silica gel 60 (Merck, 63-210 μm), Sephadex LH-20 (Amersham Pharmacia Biotech AB), and Toyopearl HW-40 (TOSOH); HPLC: GPC (Shodex K-2001, 2002, $CHCl_3$; Shodex, GS-310 2G, MeOH), silica gel (Mightysil Si 60, 250 × 20 mm), ODS (Mightysil RP-18, 250 × 20 mm, Kanto Kagaku). IR spectra were recorded on a JASCO FT-IR-420 spectrometer. Optical rotations were measured with a JASCO DIP-370 digital polarimeter.

4.2. Plant material

The aerial parts of *H. ascyron* were collected in August 2005 in a mountain area more than 1500 m above sea level in Tokushima Prefecture, Japan, and separated to leaves and stems. Herbarium specimens were deposited in the botanical garden of the University of Tokushima (specimen number: UTP98010).

4.3. Extraction and isolation of compounds from the leaves of *H. ascyron*

The leaves of *H. ascyron* (4.0 kg, dried) were extracted (3 × 18 L) with hot MeOH for 4 h. The MeOH extracts were combined, then concentrated in vacuo to give a residue (1.3 kg), which was partitioned between *n*-hexane and H_2O. The *n*-hexane soluble fraction (234.1 g) was loaded on a silica gel column (12 × 40 cm) eluted with different solvents of increasing polarity *n*-hexane–EtOAc–MeOH to give 13 fractions (fr. 1–13). Fr. 7 (34.0 g) was subjected to silica gel (8 × 40 cm) CC eluted with $CHCl_3$–MeOH (100:0 to 0:100) to give six fractions (fr. 7.1–7.6). Fr. 7.2 (29.0 g) was separated on a Toyopearl column (6 × 45 cm) with $CHCl_3$–MeOH (2:1) and purified by ODS-HPLC (MeOH–H_2O, 8:2) to give **9** (25 mg) and **10** (35 mg). Fr. 7.6 (2.6 g) was applied to silica gel HPLC with *n*-hexane–EtOAc (3:1) as eluant and then purified further by ODS-HPLC (MeOH–H_2O, 9:1) to give **6** (10 mg), **5** (9 mg), **7** (10 mg), **8** (11 mg), **2** (28 mg), **1** (10 mg), **3** (40 mg), and **4** (25 mg). Fr. 8 (38.1 g) was applied to a silica gel column (8 × 40 cm) eluted with solvents of increasing polarity ($CHCl_3$–MeOH, 100:0 to 0:100) to give six fractions (fr. 8.1–8.6). Fr. 8.3 (32.2 g) was subjected to silica gel (8 × 35 cm) CC with $CHCl_3$–MeOH (100:0 to 0:100) to give three further fractions (fr. 8.3.1–8.3.3). Fr. 8.3.1 (8.4 g) was loaded on a silica gel column (5 × 30 cm) with *n*-hexane–acetone (85:15 to 0:100) and silica gel HPLC with *n*-hexane–EtOAc (3:1) and then purified by ODS-HPLC (MeOH–H_2O, 85:15) to give **11** (55 mg).

4.4. Tomoeone A (*1*)

A pale yellow amorphous powder. $[\alpha]_D$: +33.2 (*c* 2.9 MeOH); λ_{max}^{MeOH} nm (log ε): 285 (3.6), 239 (3.4); IR (KBr): ν_{MAX} cm^{-1} 3463, 2966, 1718, 1670, 1543, 1446, 1379; HRESIMS: *m*/*z* 499.3063, $[M-H]^-$ (calcd. for $C_{30}H_{43}O_6$, 499.3060); For ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) spectra, see Tables 2 and 1.

4.5. Tomoeone B (*2*)

A pale yellow amorphous powder. $[\alpha]_D$: +9.5 (*c* 1.1 MeOH); λ_{max}^{MeOH} nm (log ε): 285 (3.6), 238 (3.5); IR (KBr): ν_{MAX} cm^{-1} 3419, 2968, 1714, 1674, 1558, 1448, 1379; HRESIMS: *m*/*z* 499.3107, $[M-H]^-$ (calcd. for $C_{30}H_{43}O_6$, 499.3060); For ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) spectra, see Tables 2 and 1.

4.6. Tomoeone C (*3*)

Pale yellow gum. $[\alpha]_D$: +17.4 (*c* 4.9 MeOH); λ_{max}^{MeOH} nm (log ε): 285 (3.6), 239 (3.5); IR (KBr): ν_{MAX} cm^{-1} 3433, 2970, 1718, 1672, 1558, 1448, 1381; HRESIMS: *m*/*z* 499.3057, $[M-H]^-$ (calcd. for $C_{30}H_{43}O_6$, 499.3060); For ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) spectra, see Tables 2 and 1.

4.7. Tomoeone D (*4*)

Pale yellow gum. $[\alpha]_D$: +10.4 (*c* 3.0 MeOH); λ_{max}^{MeOH} nm (log ε): 284 (3.5), 237 (3.5); IR (KBr): ν_{MAX} cm^{-1} 3431, 2970, 1716, 1672, 1558, 1448, 1381; HRESIMS: *m*/*z* 523.3057, $[M+Na]^+$ (calcd. for $C_{30}H_{44}O_6Na$, 523.3036); For ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) spectra, see Tables 2 and 1.

4.8. Tomoeone E (*5*)

A pale yellow amorphous powder. $[\alpha]_D$: +40.3 (*c* 1.0 MeOH); λ_{max}^{MeOH} nm (log ε): 285 (3.5), 239 (3.4); IR (KBr): ν_{MAX} cm^{-1} 3409, 2960, 1718, 1674, 1558, 1448, 1385; HRESIMS: *m*/*z* 537.3165, $[M+Na]^+$ (calcd. for $C_{31}H_{46}O_6Na$, 537.3192)For ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) spectra, see Tables 2 and 1.

4.9. Tomoeone F (*6*)

A pale yellow amorphous powder. $[\alpha]_D$: +1.8 (*c* 1.8 MeOH); λ_{max}^{MeOH} nm (log ε): 285 (3.6), 239 (3.6); IR (KBr): ν_{MAX} cm^{-1} 3390, 2964, 1714, 1674, 1556, 1448, 1379; HRESIMS: *m*/*z* 537.3169, $[M+Na]^+$ (calcd. for $C_{31}H_{46}O_6Na$, 537.3192); For ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) spectra, see Tables 2 and 1.

4.10. Tomoeone G (*7*)

Pale yellow gum. $[\alpha]_D$: +25.4 (*c* 1.7 MeOH); λ_{max}^{MeOH} nm (log ε): 283 (3.7), 237 (3.6); IR (KBr): ν_{MAX} cm^{-1} 3429, 2964, 1718, 1674, 1556, 1450, 1380; HRESIMS: *m*/*z* 513.3235, $[M-H]^-$ (calcd. for $C_{31}H_{45}O_6$, 513.3216); For ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) spectra, see Tables 2 and 1.

4.11. Tomoeone H (*8*)

Pale yellow gum. $[\alpha]_D$: +5.2 (*c* 2.7 MeOH); λ_{max}^{MeOH} nm (log ε): 283 (3.5), 238 (3.5); IR (KBr): ν_{MAX} cm^{-1} 3432, 2964, 1714, 1672, 1558, 1448, 1381; HRESIMS: *m*/*z* 513.3235, $[M-H]^-$ (calcd. for $C_{31}H_{45}O_6$, 513.3216); For ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) spectra, see Tables 2 and 1.

4.12. Cell lines and cell culture

KB (human epidermoid carcinoma of the nasopharynx), MCF7 (breast carcinoma), COLO205 (colon carcinoma), K562 (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 5 μM colchicine. MCF7, COLO205, and K562 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 °C in a humidified atmosphere with 5% CO_2–95% air.

4.13. In vitro cytotoxicity assay

Cells were seeded at each density (1×10^5 cells/well for K562 and K562/Adr, 5×10^4 cells/well for KB and KB-C2, or 5×10^4 cells/well for MCF7 and COLO205) in 96-well plate and pre-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 concentration (0.4, 2, 10, 50, and 100 μg/mL) of test compound were added and further incubated for 48 h. Cell viability was determined by 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay (Mosmann, 1983). IC_{50} values (concentration in μg/mL required to inhibit cell viability by 50%) were calculated using the concentration–inhibition curve. Cytotoxic activities are shown as mean ± SE from four experiments.

References

Aramaki, Y., Chiba, K., Tada, M., 1995. Absolute stereochemistry of chinesin I and II. J. Chem. Soc. Perkin Trans. I, 683–688.

Arisawa, M., Fujita, A., Hayashi, T., Morita, N., Kikuchi, T., Tezuka, Y., 1990. Studies on cytotoxic constituents in pericarps of *Mallotus japonicus*. IV. Chem. Pharm. Bull. 38, 698–700.

Decosterd, L.A., Stoeckli-Evans, H., Chapuis, J.C., Sordat, B., Hostettmann, K., 1989. New cell growth-inhibitory cyclohexadienone derivatives from *Hypericum calycinum* L.. Helv. Chim. Acta 72, 1833–1845.

Ishii, R., Horie, M., Saito, K., Arisawa, M., Kitanaka, S., 2003. Inhibition of lipopolysaccharide-induced pro-inflammatory cytokine expression via suppression of nuclear factor-κB activation by *Mallotus japonicus* phloroglucinol derivatives. Biochim. Biophys. Acta 1620, 108–118.

Ito, H., Muranaka, T., Mori, K., Jin, Z.X., Tokuda, H., Nishino, H., Yoshida, T., 2000. Ichthyotoxic phloroglucinol derivatives from *Dryopteris fragrans* and their anti-tumor promoting activity. Chem. Pharm. Bull. 48, 1190–1195.

Jiang Su New Medical College, 1977. Dictionary of Chinese Crude Drugs. Scientific Technological Publishers, Shanghai, p. 1002.

Komissarenko, N.F., Levashova, V.P., 1992. Flavonoids of *Hypericum ascyron*. Khim. Prirod. Soedinenii 5, 580–581.

Martinez-Poveda, B., Quesada, A.R., Medina, M.A., 2005. Hyperforin, a bio-active compound of St. John's Wort, is a new inhibitor of angiogenesis targeting several key steps of the process. Int. J. Cancer 117, 775–780.

Matsuhisa, M., Shikishima, Y., Takaishi, Y., Honda, G., Ito, M., Takeda, Y., Shibata, H., Higuti, T., Kodzhimatov, O.K., Ashumetov, O., 2002. Benzoylphloroglucinol derivatives from *Hypericum scabrum*. J. Nat. Prod. 65, 290–294.

Medina, M.A., Martinez-Poveda, B., Amores-Sanchez, M.I., Quesada, A.R., 2006. Hyperforin: more than an antidepressant bioactive compound? Life Sci. 79, 105–111.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Method 65, 55–63.

Nagai, M., Tada, M., 1987. Antimicrobial compounds, chinesin I and II from flowers of *Hypericum chinense* L.. Chem. Lett., 1337–1340.

Park, H.J., Kwon, S.H., Yun, S.Y., Lee, K.T., 2000. Isolation of steroids and flavonoids from the herbs of *Hypericum ascyron* L.. Saengyak Hakhoechi 31, 39–44.

Pinarosa, A., 2005. A survey on the *Hypericum* genus: secondary metabolites and bioactivity. Stud. Nat. Prod. Chem. 30, 603–634.

Tada, M., Chiba, K., Yamada, H., Maruyama, H., 1991. Phloroglucinol derivatives as competitive inhibitors of against thromboxane A_2 and leukotriene D_4 from *Hypericum erectum*. Phytochemistry 30, 2559–2562.

Tanaka, N., Takaishi, Y., Shikishima, Y., Nakanishi, Y., Bastow, K., Lee, K.H., Honda, G., Ito, M., Takeda, Y., Kodzhimatov, O.K., Ashurmetov, O., 2004. Prenylated benzophenones and xanthones from *Hypericum scabrum*. J. Nat. Prod. 67, 1870–1875.

Tanaka, N., Okasaka, M., Ishimaru, Y., Takaishi, Y., Sato, M., Okamoto, M., Oshikawa, T., Ahmed, S.U., Consentino, L.M., Lee, K.H., 2005. Biyouyanagin A, an anti-HIV agent from *Hypericum chinense* L. var. *salicifolium*. Org. Lett. 7, 2997–2999.

Verotta, L., Lovaglio, E., Sterner, O., Appendino, G., Bombardelli, E., 2004. Modulation of chemoselectivity by protein additives. Remarkable effects in the oxidation hyperforin. J. Org. Chem. 69, 7869–7874.

Wang, Z.Q., Wang, X.R., 1980. Studies on the active principles of Hong Han Lian (*Hypericum ascyron* L.). Yaoxue Xuebao 6, 365–367.

Zhao, F., Watanabe, Y., Nozawa, H., Daikonnya, A., Kondo, K., Kitanaka, S., 2005. Prenylflavonoids and phloroglucinol derivatives from hops (*Humulus lupulus*). J. Nat. Prod. 68, 43–49.

Zhang, L., Zhao, C., Wu, D., Mai, Y., 1998. Extraction of flavones from *Hypericum ascyron* L.. Shanxi Daxue Xuebao, Ziran Kexueban 21, 401–402.