Acylphloroglucinol, Biyouyanagiol, Biyouyanagin B, and Related Spiro-lactones from *Hypericum chinense*

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Biyouyanagiol (**1**), an acylphloroglucinol-related compound having a unique cyclopenta-1,3-dione moiety, was isolated from a Japanese traditional medicinal plant, *Hypericum chinense*, together with three new spiro-lactone-related derivatives, biyouyanagin B (**2**), 5,6-dihydrohyperolactone D (**3**), and 4-hydroxyhyperolactone D (**4**). Their structures were established on the basis of spectroscopic evidence. In a cytotoxicity assay against human cancer cell lines including multidrug-resistant (MDR) cancer cell lines, several compounds demonstrated enhanced cytotoxicity against MDR KB cells in the presence of colchicine.

The plants of the genus *Hypericum* (Clusiaceae), occurring widely in temperate regions, have been used as traditional medicines in various parts of the world. In addition, *H. perforatum* (St. John's wort) has become a popular herbal medicine for the treatment of moderate depression. This pharmacological activity inspired many researchers to investigate the secondary metabolites from *Hypericum* plants, resulting in the isolation of a variety of bioactive compounds.[1] *H. chinense* (Biyouyanagi in Japanese) has been used for the treatment of female disorders in Japan,[2] and acylphloroglucinol derivatives, chinesins I and II, possessing antibacterial, antiviral, and thromboxane A^2 and leukotriene D_4 inhibitory activity have been characterized.[3−5] Unique spiro compounds hyperolactones A−D have also been reported.[6]

In the course of our search for bioactive plant metabolites, we previously reported on the constituents of *H. scabrum*,[7,8] *H. ascyron*,[9,10] *H. sikokumontanum*,[11,12] *H. perforatum* var. *angustifolium*,[13] and *H. pseudopetiolatum* var. *kiusianum*.[14] Biyouyanagin A (**11**), isolated from *H. chinense*, together with 37 xanthone derivatives,[2,15,16] exhibited strong selective inhibitory activity against HIV replication and lipopolysaccharide (LPS)-induced cytokine release.[2] Biyouyanagin A has become a synthetic target due to its unique structure and biological activity. Nicolaou et al. recently achieved its total synthesis and established a 24*R* absolute configuration as well as revision of the configurations of C-6 and C-7.[17,18]

Further study of the methanolic extracts of *H. chinense* has resulted in the isolation of a new acylphloroglucinol-related compound (**1**), possessing a unique cyclopenta-1,3-dione moiety, and three new spiro-lactone-related compounds (**2**−**4**), together with 10 known compounds. This paper elucidates the structures of **1**−**4** as well as the cytotoxicity of the isolated compounds and **11** against human cancer cell lines including multidrug-resistant cancer cell lines.

Results and Discussion

Dried leaves of *H. chinense* were extracted with MeOH. The MeOH extracts were partitioned between *n*-hexane and H_2O. The *n*-hexane-soluble fraction was subjected to column chromatography repeatedly to give three new (**2**−**4**) and eight known compounds. The MeOH extracts obtained from the stems of *H. chinense* were partitioned with *n*-hexane and H_2O to remove *n*-hexane-soluble compounds. The resulting aqueous suspension was then extracted with EtOAc. From the EtOAc-soluble fraction, one new (**1**) and two known compounds were isolated. Known compounds were identified as hyperolactone A (**5**),[6] hyperolactone C (**6**),[6] hyperolactone D (**7**),[6] caryophyllenol-I,[19] β-caryophyllene oxide,[20] clovandiol,[21] oxyphyllendiol B,[22] dihydrosesamin (**8**),[23] hanultarin (**9**),[24] and (−)-(2*R*,3*R*)-1,4-*O*-diferuloylsecoisolariciresinol (**10**)[24] by comparison of their physical data with reported data. Although compound **9** was elucidated as (−)-(2*R*,3*R*)-1-*O*-feruloylsecoisolariciresinol by detailed spectroscopic and chemical examination, this compound has been reported recently by Moon et al. from the seeds of *Trichosanthes kirilowii*.[24] Comparisons of the physical data of **9** with reported data showed their identity.

Biyouyanagiol (**1**), $C_{23}H_{34}O_7$, showed IR absorptions of hydroxy (3427 cm^{-1}) and carbonyl (1755 and 1726 cm^{-1}) groups. The ^1H NMR spectrum revealed, along with a prenyl and methoxy groups, the presence of two methines, four methylenes, and three tertiary methyls. The ^{13}C NMR spectrum showed the signals of 23 carbon atoms: two ketones (δ_C 218.3 and 212.9), one carboxylic carbon (δ_C 171.8), one double bond (δ_C 135.6 and 118.7), five quaternary carbons, including three with oxygen function (δ_C 86.9, 78.3, and 70.8), one methoxy group (δ_C 52.0), two methines, five methylenes, and five methyls. The $^1H-^1H$ COSY correlations of H_2-6−H-7−H-11−H_2-10−H_2-9, along with the HMBC correations of 14-Me with C-11, C-12, and C-13 and of 15-Me with C-7, C-8, and C-9, indicated the existence of a monoterpenyl moiety similar to tomoeones.[10] In contrast, the HMBC cross-peaks of 21-Me with C-2, C-3, C-4, and C-16 were suggestive of the presence of a 2-methyl-2-prenyl-1,3-dione moiety. Furthermore, the monoterpenyl and 2-methyl-2-prenyl-1,3-dione moieties were shown to be connected through C-5 from the HMBC correlations of H_2-6 with C-13, C-4, C-5, and C-1. In addition, a carbomethoxy group, shown by the HMBC correlation between the methyl protons and carboxylic carbon, and a hydroxy group were assigned at C-1, taking into account the chemical shift of C-1 (δ_C 86.9), as well as seven degrees of unsaturation of its molecular formula. Thus, the gross structure of **1** was characterized as shown in Chart 1.

The relative configuration of **1** was elucidated by examination of the NOESY spectrum taking ^1H NMR coupling constants into consideration. The key NOE correlations are shown in Figure 1. The NOE correlation of H-11 with H-13*ax* and the coupling constants of H-7 [δ_H 1.36 (1H, ddd, J = 12.8, 12.8, 2.8 Hz)] indicated that H-7, H-11, and H-13*ax* have axial orientations. Thus,

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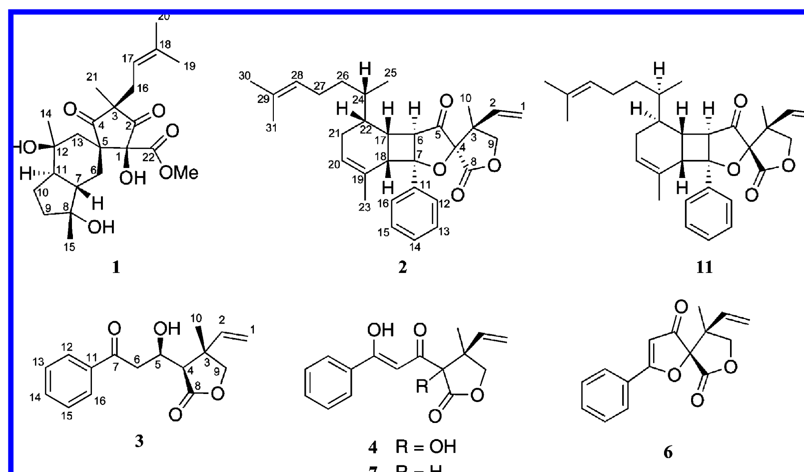
Chart 1. Structure of New Compounds (**1**−**4**), Biyouyanagin A (**11**), and Hyperlactones C (**6**) and D (**7**)



the cyclohexane ring, consisting of C-5 to C-7 and C-11 to C-13, adopts a chair conformation. An equatorial orientation of 14-Me was revealed from the NOE correlations of 14-Me with H-11, H-13*ax*, and H-13*eq*. Also, the NOE between 15-Me and H-7 indicated the *β*-orientation of 15-Me. The configurations at C-1, C-3, and C-5 of the cyclopentane ring were elucidated from the following NOE correlations: *O*-methyl protons with 15-Me, H-6*eq*, and H-7; H-17 with H-13*eq*; 21-Me with H-6*eq*. Thus, the structure of **1** was established as shown, having a structurally unique cyclopenta-1,3-dione moiety.

Biyouyanagin B (**2**) had the molecular formula $C_{31}H_{38}O_4$, identical to that of biyouyanagin A (**11**),[2] on the basis of the HRFABMS. The ^{1}H and ^{13}C NMR spectra of **2** were compared with those of **11** (Table 2), and detailed analyses of the ^{1}H−^{1}H COSY and HMBC spectra indicated that **2** had the same planar structure as **11**. However, the ^{1}H NMR resonances for H$_2$-1, H-2, 10-Me, H-17, H-18, and H-22 in **2** were different from those seen in **11**: the H$_2$-1, H-2, 10-Me, and H-17 signals of **2** were shifted downfield (+0.59 and +0.82, +0.84, +0.21, and +0.29 ppm, respectively) compared with those of **11**, while H-18 and H-22 showed upfieled shifts (−0.45 ppm in each case). In contrast, ^{13}C NMR resonances for C-2, C-6, C-7, C-10, C-17, C-21, C-24, and C-27 were slightly different from those found in **11**: the ^{13}C NMR resonances for C-6, C-7, C-10, C-17, C-24, and C-27 in **2** were shifted upfield (−3.2, −1.2, −1.3, −2.9, −1.2, and −1.1 ppm, respectively) compared with those of **11**, whereas the C-2 and C-21 signals were shifted downfield (+1.7 and +1.9 ppm, respectively). Therefore, **2** was considered to be a stereoisomer of **11**.

The relative configuration of biyouyanagin B was elucidated by NOESY analyses. The ^{1}H NMR spectrum of **2** in CDCl$_3$ showed overlapping of H-6 and H-17 [δ_H 3.30 × 2 (each 1H, m)], whereas these resonances separated when the ^{1}H NMR spectrum was recorded in C$_6$D$_6$ [δ_H 3.21 (1H, d, J = 7.6 Hz, H-6), 3.47 (1H, m, H-17)]. The NOE correlation of H-6 with phenyl protons indicated that they were located on the same side. The *trans*-fusion of the cyclobutane ring was revealed by the NOE correlations of H-21*ax* with H-6 and phenyl protons. The α-orientation of the C-22 side chain as well as the *S** configuration at C-24 were established from the following NOE correlations: H-6 with H-24; H-17 with H-26; 25-Me with H$_2$-21 (Figure 2).

Although no other important NOESY correlations that might provide information on the stereostructure of **2** were observed, the ^{1}H NMR resonances for H-1, H$_2$-2, and 10-Me in biyouyanagins A (**11**) and B (**2**) were significantly different; those of **11** were shifted upfield as compared with those of hyperolactone C (**6**),[6] while the signals for H-1, H$_2$-2, and 10-Me of **2** were quite similar to those of **6**. The upfield shift found in **11** could be explained by an anisotropic effect of the aromatic ring in the hyperolactone moiety, as the NOESY correlations were observed between 10-Me and the aromatic proton in **11**. Since such a NOESY correlation was not observed in **6**, these functional groups are in closer proximity than those in **6**, probably owing to the strain in the cyclobutane ring. In contrast, the resonances of H-1, H$_2$-2, and 10-Me found in **2**, being quite similar to those of **6**, suggested that these protons are not affected anisotropically by the aromatic ring in the hyperolactone moiety. This observation strongly suggested that the aromatic ring and the 10-Me and vinyl substituent at C-4 in **2** are located on opposite faces. This was consistent with the absence of an NOE between the aromatic proton and Me-10, H$_2$-1, or H-2 in **2**. The configuration of C-3 in **2** was considered to be the same as that of **11**, allowing for its possible biogenetic origin.[2] Thus, biyouyanagins A (**11**) and B (**2**) are biogenetically produced by the [2+2]-cycloaddition of a bisabolene-type sesquiterpene, *ent*-zingiberene, and hyperolactone C (**6**), and zingiberene and the C-4

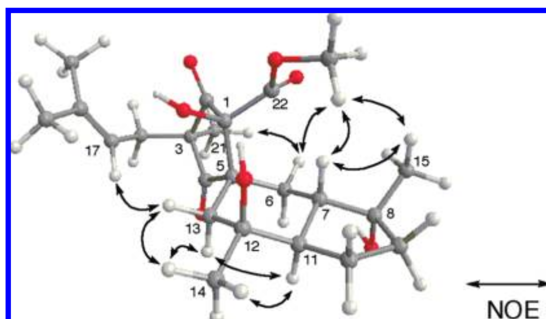


Figure 1. NOE correlations in the NOESY spectrum of **1**.

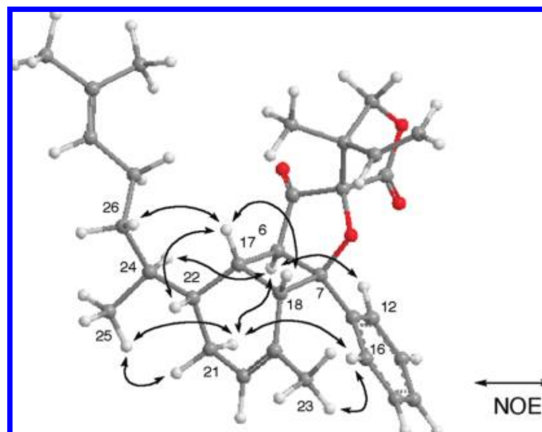


Figure 2. NOE correlations in the NOESY spectrum of **2**.

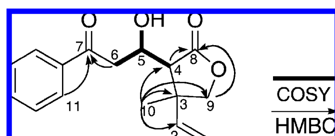


Figure 3. COSY and HMBC correlations for **3**.

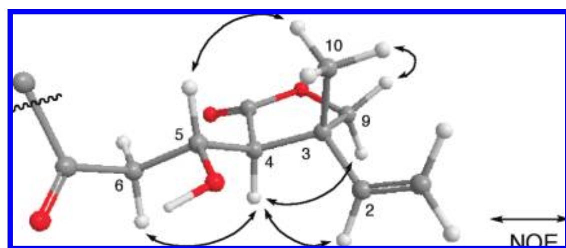


Figure 4. NOE correlations in the NOESY spectrum of **3**.

epimer of **6**, respectively. The latter two compounds derived from hyperolactone D (**7**)[6] possess the same C-3 configuration. Accordingly, the structure of **2** was concluded to be the C-4, C-22, and C-24-isomer of **11** as shown.

The HREIMS of compound **3** gave a molecular ion peak at m/z 274.1208 ($[M]^+$, calcd 274.1205), indicating the molecular formula $C_{16}H_{18}O_4$. It showed IR absorptions of hydroxy (3480 cm^{-1}), five-membered lactone (1763 cm^{-1}), and ketone (1680 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra revealed the signals of phenyl, vinyl, oxygenated methine, oxygenated methylene, methylene attached to a carbonyl carbon, methine, methyl, hydroxy, ketone and lactone carbonyl groups, and a quaternary carbon atom. These data suggested the structure of **4** was similar to that of a polyketide derivative, hyperolactone D (**7**), isolated previously from this plant.[6] The ^1H and ^{13}C NMR spectra of **7** gave duplicated signals, which was attributable to the existence of an equilibrium mixture of keto−enol tautomers, whereas such signals were not observed in the NMR spectra of **3**. The planar structure of **3** was elucidated by the $^1H-^1H$ COSY and HMBC data analyses as shown in Figure 3.

The NOE correlations of H-4 with H-6α, H-9α, and H-2 indicated that these protons were located at the α-face, while the β-orientations of H-5 and 10-Me were revealed by the correlations of 10-Me with H-5 and H-9β (Figure 4). In addition, these NOE observations also suggested the relative configuration of C-5 to be *R**. From these data, the relative configuration of **3** was elucidated as shown and was designated as 5,6-dihydrohyperolactone D.

Compound **4** had the molecular formula $C_{16}H_{16}O_5$ on the basis of HREIMS. The ^1H and ^{13}C NMR spectra were similar to those of **3** except for the signals due to C-4 to C-7. Instead of the signals due to ketone, oxygenated methine, methine, and methylene groups seen in **3**, the presence of an α,β-unsaturated ketone [δ_H 15.59 (1H, s) and 6.63 (1H, s); δ_C 195.6, 181.7, and 93.3] and an oxygenated quaternary carbon (δ_C 84.0) was obsesrved. The long-range correlations of aromatic protons with C-7 and an olefinic proton with C-5, C-7, and C-11 were observed in the HMBC spectrum. The configuration of C-4 still remains to be determined. Thus, the structure of **4**, designated as 4-hydroxyhyperolactone D, was elucidated as shown.

Compounds **1**, **4**−**10**, and biyouyanagin A (**11**) were evaluated for their cytotoxicity against human tumor cell lines including multidrug-resistant (MDR) cancer cell lines, and their IC_{50} values are shown in Table 3. Biyouyanagin A (**11**) displayed moderate cytotoxicity against all the tested cell lines, with IC_{50} values ranging from 16.6 to 38.8 μg/mL. Its cytotoxicity against KB-C2 was enhanced in the presence of colchicine; colchicine had no effect on the growth of KB-C2 cells at this concentration level. A similar trend against MDR cancer cell lines was also observed in **4**, **5**, **6**, and **7**, suggesting that the spiro-lactone moiety could play an important role in this activity. Since these compounds are more sensitive to K562/Adr MDR cancer cells and their cytotoxicity against KB-C2 was enhanced by colchicine, they might have some effect on the P-glycoprotein (P-gp) function of KB-C2 cells. Biyouyanagiol (**1**) also showed moderate cytotoxicity against KB-C2 MDR cancer cells in the presence of colchicine, although it was not cytotoxic against all the tested cell lines. This compound also might have some effect on P-gp function. Compound **10**, (−)-(2*R*,3*R*)-1,4-*O*-diferuloylsecoisolariciresinol, possessing two feruloyl groups, showed potent cytotoxicity against COLO205 cells with an IC_{50} value of 5.2 μg/mL. The cytotoxicities of **9** against all cell lines were lower than those of **10**, suggesting that the feruloyl group might contribute to cytotoxicity.

Table 1. NMR Spectroscopic Data (acetone-d_6) of Compound **1**

position	δ_C	δ_H (J in Hz)	HMBC
1	86.9		
2	212.9		
3	55.1		
4	218.3		
5	60.9		
6	30.4	1.87 (1H, dd, 14.4, 12.8)	1, 4, 5, 7, 8, 11
		1.72 (1H, m)	
7	45.1	1.36 (1H, ddd, 12.8, 12.8, 2.8)	9, 11, 12
8	78.3		
9	40.3	1.71 (2H, m)	
10	21.3	1.69 (2H, m)	
11	51.5	1.82 (1H, m)	
12	70.8		
13	42.7	2.26 (1H, dd, 14.4, 2.0)	1, 4, 5, 6, 11,
		1.45 (1H, d, 14.4)	12, 14
14	28.5	1.25 (3H, s)	11, 12, 13
15	26.3	1.19 (3H, s)	7, 8, 9
16	36.4	2.42 (1H, dd, 14.0, 8.4)	2, 3, 4, 17, 18, 21
		2.33 (1H, dd, 14.0, 6.8)	
17	118.7	5.01 (1H, ddq, 8.4, 6.8, 1.2)	3, 16, 19, 20
18	135.6		
19	17.4	1.57 (3H, s)	17, 18, 19
20	25.6	1.67 (3H, s)	17, 18, 20
21	21.0	1.22 (3H, s)	2, 3, 4, 16
22	171.8		
OMe	52.0	3.74 (3H, s)	22

Conclusions

As part of our search for bioactive plant metabolites of *Hypericum* plants, we undertook an additional investigation of *H. chinense* that resulted in the isolation of four new (**1**−**4**) and 10 known compounds. Biyouyanagiol (**1**), a new acylphloroglucinol-related compound, possesses a spiro-skeleton with a monoterpene moiety, as well as a unique cyclopenta-1,3-dione moiety. Recently, our group and Wu et al. reported tomoeones[10] and hyperielliptone HB,[25] a new class of phloroglucinol derivatives fused with a monoterpene moiety by a spiro-linkage. This type of compound was considered to be derived from chinesins, a phloroglucinol derivative with a monoterpenyl moiety.[10] Biyouyanagiol is presumed to be derived from spirophloroglucinols, such as tomoeones and hyperielliptone HB, as shown in Scheme 1. In contrast, 4-hydroxyhyperolactone D (**4**) was considered to be a biogenetic precursor of hyperolactone C (**3**).

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a 1720 infrared Fourier transform spectrometer (Perkin-Elmer). NMR spectra were run on a Bruker AVANCE instrument (^1H NMR 400 MHz, ^{13}C NMR 100 MHz) using TMS as an internal standard. MS was obtained on a JEOL JMSD-300 instrument. Chromatography column: silica gel 60 (Merck, 63-210 μm), Sephadex LH-20 (Amersham Pharmacia), and Toyopearl HW-40C (Tosoh). HPLC: GPC (Shodex H-2001, 2002, $CHCl_3$; Asahipak, GS-310 2G, MeOH), silica gel (YMC-Pack SIL-06 SH-043-5-06, 250 × 20 mm), ODS (YMC-R-ODS-5, Yamamura).

Table 2. NMR Spectroscopic Data ($CDCl_3$) of Compounds **2** and **11**

position	**2** δ_C	**2** δ_H (*J* in Hz)	**2** HMBC	**11** δ_C	**11** δ_H (*J* in Hz)
1	119.1	5.39 (1H, d, 10.8) 5.44 (1H, d, 16.8)	3	118.4	4.80 (1H, d, 11.2) 4.62 (1H, d, 17.6)
2	136.2	6.08 (1H, dd, 17.6, 10.8)	3, 9, 10	134.5	5.24 (1H, dd, 17.6, 11.2)
3	48.7			49.0	
4	92.6			93.1	
5	209.3			209.6	
6	48.7	3.30 (1H, m)	5, 17, 22	51.9	3.16 (1H, dd, 6.0, 1.2)
7	88.5			89.7	
8	170.8			171.6	
9	73.9	4.73 (1H, d, 8.8) 3.98 (1H, d, 8.8)	2, 3, 4, 8, 10	73.7	4.71 (1H, d, 8.8) 3.98 (1H, d, 8.8)
10	18.8	1.52 (3H, s)	2, 3, 4, 9	20.1	1.31 (3H, s)
11	138.6			139.6	
12	126.0	7.28 (1H, m)	7	125.9	7.37−7.26 (1H, m)
13	127.0	7.28 (1H, m)		127.7	7.37−7.26 (1H, m)
14	127.3	7.28 (1H, m)		127.8	7.37−7.26 (1H, m)
15	127.0	7.28 (1H, m)		127.7	7.37−7.26 (1H, m)
16	126.0	7.28 (1H, m)		125.9	7.37−7.26 (1H, m)
17	33.0	3.30 (1H, m)	5, 22, 24	35.9	3.01 (1H, ddd, 8.4, 6.6, 6.6)
18	51.1	3.04 (1H, brd, 6.4)	6, 7, 17, 19, 23	50.3	3.49 (1H, d, 8.4)
19	131.7			131.4	
20	124.7	5.58 (1H, brd, 6.4)	18, 21, 22, 23	123.9	5.46 (1H, m)
21	25.4	2.29 (1H, m) 1.82 (1H, m)		23.5	2.09 (1H, m) 1.99 (1H, m)
22	37.8	1.28 (1H, m)		38.8	1.73 (1H, m)
23	22.1	0.93 (3H, s)	18, 19, 20	21.7	1.02 (3H, d, 1.2)
24	33.9	1.47 (1H, m)		35.1	1.46 (1H, m)
25	16.5	0.97 (3H, d, 6.8)	22, 24, 26	16.8	0.83 (3H, d, 6.4)
26	34.3	1.47 (1H, m) 1.08 (1H, m)		35.0	1.45 (1H, m) 1.20 (1H, m)
27	24.8	1.98 (2H, m)		25.9	2.02 (1H, m) 1.94 (1H, m)
28	124.6	5.12 (1H, t, 6.4)	30, 31	124.6	5.11 (1H, brt, 5.6)
29	131.1			131.4	
30	25.6	1.71 (3H, s)	28, 29, 31	25.7	1.70 (3H, d, 1.2)
31	17.6	1.65 (3H, s)	28, 29, 30	17.7	1.61 (3H, s)

Table 3. Cytotoxicity Data (IC_{50},[h] μg/mL) for **1** and **4**−**11** against Human Tumor Cell Lines

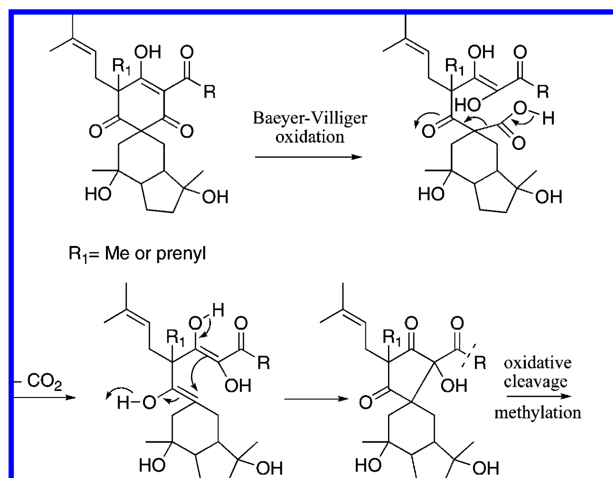
compound	KB[a]	KB-C2[b]	KB-C2 (+Col.)[c]	MCF-7[d]	K562[e]	K562/Adr[f]	COLO205[g]
1	>100	>100	47.8 ± 4.0	>100	>100	>100	>100
4	>100	>100	53.1 ± 6.5	62.1 ± 3.7	>100	33.7 ± 2.0	79.0 ± 2.6
5	>100	>100	70.8 ± 5.2	>100	>100	47.1 ± 2.0	>100
6	>100	>100	51.8 ± 3.7	77.7 ± 2.4	>100	32.8 ± 1.4	58.5 ± 4.3
7	61.0 ± 1.4	22.3 ± 0.6	24.6 ± 0.9	52.9 ± 1.9	47.8 ± 1.5	20.0 ± 0.7	37.5 ± 3.4
8	>100	>100	53.1 ± 6.5	62.1 ± 3.7	>100	33.7 ± 2.0	79.0 ± 2.7
9	33.7 ± 1.0	20.7 ± 0.8	20.9 ± 0.6	18.9 ± 0.7	35.2 ± 0.8	33.1 ± 0.6	16.8 ± 0.6
10	14.1 ± 0.3	9.4 ± 0.1	4.5 ± 0.2	5.9 ± 0.4	17.3 ± 0.9	19.9 ± 0.4	5.2 ± 1.9
11	38.8 ± 1.3	36.2 ± 2.1	16.8 ± 0.8	27.1 ± 1.1	31.1 ± 0.4	16.6 ± 0.9	26.9 ± 0.8
daunorubicin	0.22 ± 0.01	>100		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.

Plant Material. The aerial parts of *H. chinense* were collected in October 2002 in Tokushima Prefecture, Japan, and separated into leaves and stems. Herbarium specimens were deposited in the botanical garden of the University of Tokushima (specimen number UTP98008).

Extraction and Isolation of Compounds from the Leaves of *H. chinense.* The leaves of *H. chinense* (1.48 kg, dried) were crushed and extracted (3 × 18 L) with MeOH at 60 °C for 4 h. The MeOH extracts were concentrated *in vacuo* to give a residue (633 g), which was partitioned between *n*-hexane and H_2O. The *n*-hexane-soluble fraction (92.6 g) was subjected to silica gel CC eluted with solvents of increasing polarity (*n*-hexane−EtOAc−MeOH) to give 12 fractions (fr. 1−12). Fr. 1 (4.4 g) was loaded on a silica gel CC column eluted with solvents of increasing polarity (*n*-hexane−acetone) to give eight fractions (fr. 1.1−1.8). Fr. 1.3 was subjected to a Toyopearl HW-40C column ($CHCl_3$−MeOH, 2:1) to give three fractions (fr. 1.3.1−1.3.3). Fr. 1.3.2 was subjected to silica gel CC (*n*-hexane−EtOAc, 9:1) to give β-caryophyllene oxide (7 mg). Fr. 1.3.3 was purified by silica gel HPLC (*n*-hexane−EtOAc, 9:1) to give caryophyllenol-I (1 mg). Fr. 1.6 was loaded on a Sephadex LH-20 column with MeOH to give two fractions (fr. 1.6.1 and 1.6.2). Fr. 1.6.2 was purified by GPC (MeOH) and preparative TLC ($CHCl_3$−MeOH, 9:1) to give oxyphyllendiol B (1 mg). Fr. 2 (13.2 g) was subjected to silica gel CC eluted with solvents of increasing polarity (*n*-hexane−$CHCl_3$−MeOH) to give eight fractions (fr. 2.1−2.8). Fr. 2.6 was separated by a Toyopearl HW-40C column ($CHCl_3$−MeOH, 1:1) and purified by GPC ($CHCl_3$) and silica gel HPLC (*n*-hexane−EtOAc, 95:5) to give **2** (2 mg). Fr. 4 (19.9 g) was loaded on a silica gel column (*n*-hexane−acetone, 4:1 to 0:1) to give **5** (8.8 g) and **6** (972 mg), together with five fractions (fr. 4.1−4.5). Fr. 4.2 was purified by GPC ($CHCl_3$) and silica gel HPLC (*n*-hexane−acetone, 3:1) to give **7** (770 mg). Fr. 7 was applied to a Sephadex LH-20 column with MeOH to give four fractions (fr. 7.1−7.4). Fr. 7.2 was purified by GPC with MeOH and silica gel HPLC (*n*-hexane−acetone, 3:1) to give **3** (2 mg). Fr. 8 was loaded on a Toyopearl HW-40C column ($CHCl_3$−MeOH, 1:1) to give seven fractions (fr. 8.1−8.7). Fr. 8.4 was subjected to silica gel CC with $CHCl_3$ to give two fractions (fr. 8.4.1 and 8.4.2). Fr. 8.4.1 was separated by silica gel HPLC ($CHCl_3$−MeOH, 98:2) to give two fractions (fr. 8.4.1.1 and 8.4.1.2). Fr. 8.4.1.1 was purified by preparative TLC ($CHCl_3$−MeOH, 98:2) to give **4** (2 mg).

Scheme 1. Possible Biogenetic Pathway of **1**



Compound **8** (14 mg) was isolated from fr. 8.4.1.2 by GPC with $CHCl_3$. Fr. 9 was subjected to a Toyopearl HW-40C column ($CHCl_3$−MeOH, 2:1) to give six fractions (fr. 9.1−9.6). Fr. 9.5 was subjected to silica gel CC eluted with solvents of increasing polarity ($CHCl_3$−MeOH) to give seven fractions (fr. 9.5.1−9.5.7). Fr. 9.5.5 was purified by GPC ($CHCl_3$) to give clovandiol (4 mg).

Extraction and Isolation of Compounds from the Stems of *H. chinense*. The stems of *H. chinense* (4.54 kg, dried) were crushed and extracted (3 × 18 L) with MeOH at 60 °C for 4 h. The MeOH extracts were concentrated *in vacuo* to give a residue (548 g). This residue was partitioned with *n*-hexane and H_2O to remove *n*-hexane-soluble compounds, and the aqueous suspension was then extracted with EtOAc. The EtOAc-soluble fraction (96.8 g) was subjected to a silica gel column eluted with solvents of increasing polarity (*n*-hexane−EtOAc−MeOH) to give 13 fractions (fr. 1−13). Fr. 9 was dissolved in MeOH to give a MeOH-soluble fraction and residue. The MeOH-soluble fraction of fr. 9 was applied to a Sephadex LH-20 column with MeOH to give five fractions (fr. 9.1−9.5). Fr. 9.3 was separated by silica gel CC eluted with solvents of increasing polarity with $CHCl_3$−acetone to give five fractions (fr. 9.3.1−9.3.5). Fr. 9.3.1 was applied to GPC ($CHCl_3$) and ODS HPLC (MeOH−H_2O, 9:1) and purified by silica gel HPLC (*n*-hexane−EtOAc, 1:2) to give **1** (4 mg). Fr. 9.4 was separated by silica gel CC eluted with solvents of increasing polarity ($CHCl_3$−MeOH) to give **10** (242 mg) and eight fractions (fr. 9.4.1−9.4.9). Fr. 9.4.4 was loaded on a Sephadex LH-20 column with MeOH to give two fractions (fr. 9.4.4.1 and 9.4.4.2). Fr. 9.4.4.1 was separated by GPC with MeOH to give **9** (24 mg).

Biyouyanagiol (1): amorphous powder; $[\alpha]^{22}_{D}$ +10.5 (*c* 0.5, $CHCl_3$); IR (KBr) ν_{max} 3427, 2966, 2933, 1755, 1726, 1457, 1377, 1265, and 1032 cm^{-1}; ^1H and ^{13}C NMR data (acetone-d_6) see Table 1; HREIMS *m/z* 422.2307, $[M]^+$ (calcd for $C_{23}H_{34}O_7$, 422.2305).

Biyouyanagin B (2): amorphous powder; $[\alpha]^{22}_{D}$ −5.9 (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 2929, 2856, 1795, 1741, 1448, 1091, and 1009 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$), see Table 2; ^1H NMR (400 MHz, C_6D_6) δ 7.35 (2H, d, *J* = 7.2 Hz, H-12 and H-16), 7.25 (2H, t, *J* = 7.2 Hz, H-13 and H-15), 7.20 (1H, m, H-14, overlapped with the signal of C_6D_5H), 6.07 (1H, dd, *J* = 17.6, 10.8 Hz, H-2), 5.40 (1H, m, H-28), 5.39 (1H, m, H-20), 5.24 (1H, d, *J* = 10.8 Hz, H-1a), 5.16 (1H, d, *J* = 17.6 Hz, H-1b), 4.62 (1H, d, *J* = 8.8 Hz, H-9a), 3.48 (1H, d, *J* = 8.8 Hz, H-1b), 3.32 (1H, m, H-17), 3.18 (1H, d, *J* = 7.6 Hz, H-6), 3.03 (1H, brd, *J* = 7.6 Hz, H-18), 2.15 (2H, m, H_2-27), 1.96 (1H, dt, *J* = 17.6, 5.2 Hz, H-21β), 1.83, 1.74 (each 3H, s, Me-30 and 31), 1.60 (1H, m, H-26a), 1.47 (1H, m, H-21α), 1.41 (1H, m, H-24), 1.30 (3H, s, Me-10), 1.16 (1H, m, H-26b), 1.11 (1H, m, H-22), 1.02 (3H, s, Me-23), 0.92 (3H, d, *J* = 6.8 Hz, Me-25); HRFABMS *m/z* 475.2853, $[M + H]^+$ (calcd for $C_{31}H_{39}O_4$, 475.2848).

5,6-Dihydrohyperolactone D (3): amorphous powder; $[\alpha]^{22}_{D}$ +8.9 (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3480, 2923, 2852, 1763, 1680, 1598, 1450, 1136, and 1020 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.99 (2H, d, *J* = 7.8 Hz, H-12, 16), 7.61 (1H, t, *J* = 7.6 Hz, H-14), 7.49 (2H, dd, *J* = 7.8, 7.6 Hz, H-13, 15), 6.12 (1H, dd, *J* = 17.8, 10.4 Hz, H-2), 5.19 (1H, d, *J* = 10.4, H-1a), 5.18 (1H, d, *J* = 17.8 Hz, H-1b), 4.47 (1H, dddd, *J* = 10.4, 10.0, 4.4, 2.8 Hz, H-5), 4.08 (1H, d, *J* = 8.8 Hz, H-9a), 4.01 (1H, dd, *J* = 18.0, 2.8 Hz, H-6a), 3.94 (1H, d, *J* = 8.8 Hz, H-9b), 3.60 (1H, d, *J* = 4.4 Hz, OH-5), 3.30 (1H, dd, *J* = 18.0, 10.4 Hz, H-6b), 2.82 (1H, d, *J* = 10.0 Hz, H-4), and 1.44 (3H, s, Me-10); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.2 (C-7), 175.7 (C-8), 141,8 (C-2), 136.5 (C-11), 133.7 (C-14), 128.7 × 2 (C-13 and C-15), 128.1 × 2 (C-12 and C-16), 114.4 (C-1), 75.9 (C-9), 65.9 (C-5), 53.4 (C-4), 46.2 (C-3), 42.7 (C-6), and 18.1 (C-10); HREIMS *m/z* 274.1208, $[M]^+$ (calcd for $C_{16}H_{18}O_4$, 274.1205).

4-Hydroxyhyperolactone D (4): amorphous powder; $[\alpha]^{22}_{D}$ −118.0 (*c* 0.3, $CHCl_3$); IR (KBr) ν_{max} 3466, 2924, 2852, 1773, 1698, 1604, 1570, 1453, 1347, 1268, 1160, 1120, and 1014 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 15.59 (1H, s, OH-7), 7.91 (2H, d, *J* = 7.6 Hz, H-12, 16), 7.58 (1H, t, *J* = 7.6 Hz, H-14), 7.48 (2H, t, *J* = 7.6 Hz, H-13, 15), 6.63 (1H, s, H-6), 5.82 (1H, dd, *J* = 17.4, 11.2 Hz, H-12), 5.23 (1H, d, *J* = 11.2 Hz, H-1a), 5.20 (1H, d, *J* = 17.4 Hz, H-1b), 4.70 (1H, d, *J* = 8.8 Hz, H-9a), 4.13 (1H, d, *J* = 8.8 Hz, H-9b), 3.45 (1H, s, OH-4), and 1.35 (3H, s, Me-10); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.6 (C-5), 181.7 (C-7), 175.4 (C-8), 135.7 (C-2), 133.3 (C-11), 133.1 (C-14), 128.8 × 2 (C-13 and C-15), 127.3 × 2 (C-12 and C-16), 117.6 (C-1), 93.3 (C-6), 84.0 (C-4), 75.2 (C-9), 49.8 (C-3), and 19.8 (C-10); HREIMS *m/z* 288.0998, $[M]^+$ (calcd for $C_{16}H_{16}O_5$, 288.0998).

Bioassay. Cell Lines and Cell Culture. Human epidermoid carcinoma cells (KB) were cultured in Dulbecco's modified Eagles medium (DMEM) with 10% fetal bovine serum (FBS). Multidrug-resistant KB cells (KB-C2) were maintained in DMEM medium in the presence of 10% FBS and 5 μM colchicines. MCF-7 (breast carcinoma), COLO205 (colon carcinoma), and K-562 (leukemia) 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 doxoruibicin. All cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2−95% air.

***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 a 96-well plate and preincubated 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 preincubated culture medium, 100 μL of medium containing various concentrations of test compound was added and further incubated for 48 h. Cell viability was determined by a 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay.[26] IC_{50} values (concentration in μg/mL required to inhibit cell viability by 50%) were calculated using the concentration−inhibition curve.

Note Added after ASAP Publication: Several typographical errors were corrected related to author names in the references. The corrected version was posted on July 22, 2009.

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