# Bulbinelonesides A−E, Phenylanthraquinone Glycosides from the Roots of *Bulbinella floribunda*

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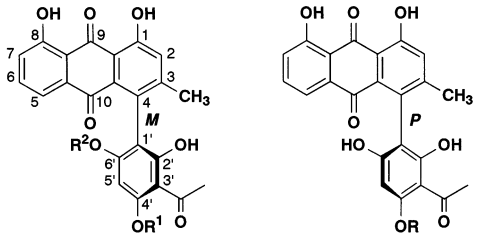
The roots of *Bulbinella floribunda* have been analyzed for the phenolic constituents, resulting in the isolation of five new phenylanthraquinone glycosides, named bulbinelonesides A−E (**1**−**5**), along with two known phenylanthraquinones, (+)-*M*-knipholone (**6**) and (+)-*M*-isoknipholone (**7**). The structures of the new compounds were determined on the basis of extensive spectroscopic analysis, including 2D NMR, and the results of enzymatic hydrolysis. Although the new compounds **3**−**5**, whose absolute stereochemistry of the unsymmetric biaryl moiety was determined to be *P* by the CD spectrum, did not show apparent cytotoxicity against cultured HSC-2 tumor cells and HPC normal cells, the new compounds **1** and **2**, as well as the known compounds **6** and **7**, whose biaryl bond was assigned as *M*, exhibited a tumor-specific cytotoxicity against HSC-2 cells comparable to or slightly weaker than etoposide, used as a positive control.

Several plants belonging to the subfamily Asphodeloideae (Liliaceae) such as *Aloe*, *Asphdeline*, *Bulbine*, *Bulbinella*, and *Kniphofia* species have been shown to produce a variety of anthraquinones.[1,2] Phenylanthraquinones are a new class of anthraquinone derivatives composed of an anthraquinone moiety and an acetylphloroglucinol part, and the first phenylanthraquinone, knipholone (**6**), was isolated from *Kniphofia foliosa* in 1984.[3] Subsequent phytochemical studies have resulted in the isolation of knipholone and its derivatives exclusively from *Kniphofia* and *Bulbine* species,[1,2,4−10] with the exception of the isolation of knipholone from *Senna didymobotrya* (Leguminosae).[11] All of the naturally occurring knipholone derivatives reported up to the present have an optically active biaryl moiety and the absolute configuration has been determined to be *M* by quantum chemical CD calculations[12] and atropeenantioselective total synthesis.[13,14]

The genus *Bulbinella*, composed of about 12 species, is taxonomically related to *Bulbine* and *Kniphofia*,[15] and a literature survey concerning the secondary metabolites of *Bulbinella* species showed that they produce several anthraquinone derivatives, including phenylanthraquinones, by conventional TLC and HPLC analysis,[2] but no systematic phytochemical examination has been carried out on this species. *Bulbinella floribunda* (Ait.) T. Durand et Schinz (Liliaceae) is native to the western area of Cape Province and commercially obtainable from the market in Japan. The present investigation aimed at the phenolic constituents of the roots of *B. floribunda* has resulted in the isolation of five new phenylanthraquinone glycosides, named bulbinelonesides A−E (**1**−**5**). The cytotoxic activities of the isolated compounds against cultured tumor and normal cells are also described.

The fresh roots of *B. floribunda* (2.8 kg) were extracted with hot MeOH. After removal of solvent, the MeOH extract was subjected to column chromatography over silica gel and octadecylsilanized (ODS) silica gel, as well as Sephadex LH-20, giving compounds **1**−**7**. Compounds **6** and **7** were identified as (+)-*M*-knipholone and (+)-*M*-isoknipholone, respectively, by comparison of their $[\alpha]_D$, CD, and $^1$H and $^{13}$C NMR data with those of the reported values in the literature.[3,5,14]




| | $R^1$ | $R^2$ |
|---|---|---|
| **1** | Me | Glc'' |
| **2** | H | Xyl'' |
| **6** | Me | H |
| **7** | H | Me |

| | R |
|---|---|
| **3** | Xyl'' |
| **4** | Glc'' |
| **5** | Glc'''-(1→3)-Xyl'' |

Glc: β-D-glucopyranosyl
Xyl: β-D-xylopyranosyl

Bulbineloneside A (**1**) was obtained as an amorphous red powder, giving rise to UV−vis absorptions at 256, 287, and 433 nm. The molecular formula of **1** was determined to be $C_{30}H_{28}O_{13}$ on the basis of the positive-ion HRFABMS (*m*/*z* 597.1627 $[M + H]^+$) data. The IR spectrum showed a broad absorption band for free hydroxyl groups at 3332 cm$^{-1}$, as well as absorptions due to unchelated and chelated carbonyl groups at 1678 and 1601 cm$^{-1}$, respectively. As in the case of knipholone (**6**), the $^1$H NMR spectrum of **1** measured in DMSO-$d_6$ displayed three aromatic proton signals as an ABC system at $\delta$ 7.72 (dd, $J$ = 8.2, 7.4 Hz, H-6), 7.47 (dd, $J$ = 7.4, 0.7 Hz, H-5), and 7.31 (dd, $J$ = 8.2, 0.7 Hz, H-7), two singlet aromatic proton signals at $\delta$ 7.31 (H-2) and 6.57 (H-5′), and three three-proton singlet signals at $\delta$ 3.98, 2.64, and 2.07, which were assigned to a methoxy group attached at C-4′, an aryl acetyl at C-3′, and an aryl methyl at C-3, respectively. Furthermore, three chelated protons of the hydroxyl groups at $\delta$ 13.75, 12.44, and 11.86 and an anomeric proton of a hexopyranose at $\delta$ 4.90 (d, $J$ = 7.9 Hz) were observed. The anomeric proton was shown to be associated with the six carbon signals at $\delta$ 100.7 (CH), 72.7 (CH), 76.9 (CH), 69.9 (CH), 77.6 (CH), and 60.8 ($CH_2$), which were consistent with the presence of a $\beta$-D-glucopy-

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ranosyl unit in **1**.[16,17] Enzymatic hydrolysis of **1** with β-D-glucosidase in acetate buffer gave (+)-*M*-knipholone (**6**) and D-glucose. The linkage position of the β-D-glucopyranosyl group to the aglycon was confirmed by the following HMBC correlations. The anomeric proton signal at δ 4.90 showed a $^3J_{C,H}$ correlation with the oxygenated carbon signal at δ 160.6, which exhibited a long-range correlation with the H-5′ proton resonance at δ 6.57 and was assigned to C-6′ of the aglycon. Thus, the structure of **1** was defined as (+)-*M*-knipholone 6′-*O*-β-D-glucopyranoside.

Bulbineloneside B (**2**) was obtained as an amorphous red powder with a molecular formula $C_{28}H_{24}O_{12}$, as determined by the positive-ion HRFABMS (*m*/*z* 553.1331 $[M + H]^+$). The spectral data of **2** were essentially analogous with those of **1** and suggestive of a phenylanthraquinone glycoside of the same type. However, slight differences were recognized in the signals from both the aglycon and sugar moieties. The signal for the methoxy group linked to C-4′ of the aglycon, which was observed in the $^1$H NMR spectrum of **1** at δ 3.98, could not be detected in that of **2**. Instead, the signal for one more chelated proton of a hydroxyl group was observed at δ 10.35 in addition to the δ 13.96, 12.43, and 11.83 resonances. On the other hand, the anomeric proton signal at δ 5.53 (d, *J* = 7.5 Hz) was shown to be associated with the five sugar carbon signals at δ 101.4 (CH), 72.9 (CH), 76.5 (CH), 69.2 (CH), and 65.9 ($CH_2$), which were consistent with the presence of a β-D-xylopyranosyl unit in **2**.[16,17] Enzymatic hydroysis of **2** with naringinase liberated a genuine aglycon (**2a**), identified as (+)-*M*-4′-demethylknipholone by the $[\alpha]_D$, CD, and $^1$H and $^{13}$C NMR data,[7,14] and D-xylose. Long-range correlations from the signals due to the anomeric proton of the xylosyl moiety at δ 5.53 and the H-5′ proton at δ 6.31 to the carbon signal at δ 159.8 in the HMBC spectrum indicated the location of the xylosyl at either C-4′ or C-6′. The possibility of the xylosyl linkage to C-4′ was ruled out by the fact that the chelated proton signal at δ 10.35 showed long-range correlations with C-3′ (δ 104.8), C-4′ (δ 161.3), and C-5′ (δ 93.7) and was unambiguously assigned to C-4′-OH. Thus, the structure of **2** was shown to be (+)-*M*-4′-demethylknipholone 6′-*O*-β-D-xylopyranoside.

Bulbineloneside C (**3**) analyzed for $C_{28}H_{24}O_{12}$ by the positive-ion HRESIMS (*m*/*z* 553.1307 $[M + H]^+$). Comparison of the $^1$H and $^{13}$C NMR spectra of **3** with those of **2** showed their considerable structural similarity and suggested that **3** differed from **2** in the linkage position of the xylosyl moiety to the aglycon. Enzymatic hydrolysis of **3** with naringinase gave 4′-demethylknipholone (**3a**) and D-xylose. The $[\alpha]_D$ value of **3a**, which was opposite that of **2a**, and the CD spectrum confirmed the configuration of the biaryl moiety as *P*.[7,14] The HMBC spectrum indicated that the xylosyl group was linked to either C-4′ or C-6′, and NOE correlations from the xylose H-2″ proton at δ 3.41 (m) to the acetyl methyl protons at δ 2.69, as well as from the anomeric proton at δ 4.95 (d, *J* = 7.6 Hz, H-1″) to the H-5′ proton at δ 6.35 (s), excluded the possibility of the xylosyl linkage to C-6′ of the aglycon, thus resulting in the conclusion that it was attached at C-4′ of the aglycon. Thus, the structure of **3** was established as (−)-*P*-4′-demethylknipholone 4′-*O*-β-D-xylopyranoside.

Bulbineloneside D (**4**) was deduced as $C_{29}H_{26}O_{13}$ from its positive-ion HRESIMS (*m*/*z* 605.1314 $[M + Na]^+$). Analysis of the CD and $^1$H and $^{13}$C NMR spectra of **4** and comparison with those of **3** implied that the aglycon of **4** was identical with that of **3**, but differed from **3** in terms of the monosaccharide constituent. Instead of the signals for a xylosyl moiety, six signals assignable to a β-D-glucopyranosyl residue were observed at δ 100.8 (CH), 73.2 (CH), 76.8 (CH), 69.8 (CH), 77.3 (CH), and 60.0 ($CH_2$) in the $^{13}$C NMR spectrum.[16,17] Hydrolysis of **4** with naringinase gave **3a** and D-glucose. The glycoside linkage of the glucosyl group to C-4′ of the phenyl moiety was ascertained by comparison with the $^{13}$C NMR spectrum of **4** with that of **3** and interpretation of the HMBC and NOESY spectra. The structure of **4** was assigned as (−)-*P*-4′-demethylknipholone 4′-*O*-β-D-glucopyranoside.

Bulbineloneside E (**5**) gave an $[M + H]^+$ ion at *m*/*z* 715.1893 in the HRESIMS, appropriate for a molecular formula $C_{34}H_{34}O_{17}$. The $^1$H NMR spectrum contained signals for two anomeric protons at δ 5.09 (d, *J* = 7.6 Hz) and 4.38 (d, *J* = 7.8 Hz), as well as signals for the phenylanthraquinone moiety. Enzymatic hydrolysis of **5** with naringinase furnished a partial hydrolysate, identified as **3**, and **3a**, along with D-glucose and D-xylose. On comparison of the entire $^{13}$C NMR spectrum of **5** with that of **3**, a set of six additional signals corresponding to a terminal β-D-glucopyranosyl moiety appeared at δ 104.0 (CH), 73.7 (CH), 76.0 (CH), 70.3 (CH), 76.9 (CH), and 61.2 ($CH_2$),[16,17] and the signal due to C-3 of the xylosyl moiety was displaced downfield by 11.0 ppm and was observed at δ 87.5, suggesting that the C-3 hydroxyl group of the xylosyl moiety was the position at which the additional D-glucose was linked. This was confirmed by a long-range correlation from the anomeric proton of the glucosyl unit at δ 4.38 to the xylose C-3 carbon at δ 87.5. Thus, the structure of **5** was elucidated as (−)-*P*-4′-demethylknipholone 4′-*O*-β-D-glucopyranosyl-(1→3)-β-D-xylopyranoside.

Bulbinelonesides A−E (**1**−**5**) are new phenylanthraquinone glycosides, and this is the first report on the isolation of the phenylanthraquinone derivatives from a *Bulbinella* plant. It is also notable that bulbinelonesides C−E (**3**−**5**) are the first naturally occurring phenylanthraquinones with the *P* configuration around the optically active biaryl moiety.

Since a representative plant anthraquinone, emodine, has been reported to be highly active in suppressing the proliferation of several tumor cell lines,[18] we evaluated cytotoxic activities of the isolated phenylanthraquinones against HSC-2 human squamous cell carcinoma cells and normal human pulp cells (HPC).[19−21] Although **3**−**5**, with the *P* biaryl bond and the sugar moiety at C-4′, did not show apparent cytotoxicity against HSC-2 tumor cells and HPC normal cells, **1**, **2**, **6**, and **7** with the *M* biaryl bond and the sugar moiety at C-6′ exhibited a tumor-specific cytotoxicity against HSC-2 cells comparable to or slightly weaker than etoposide, used as a positive control (Table 2).

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured using a JASCO DIP-360 (Tokyo, Japan) automatic digital polarimeter. IR spectra were recorded on a JASCO FT-IR 620 spectrophotometer, UV spectra on a JASCO V-520 spectrophotometer, and CD spectra on a JASCO J-720 instrument. NMR spectra were recorded on a Bruker DRX-500 spectrometer (500 MHz for $^1$H NMR, Karlsruhe, Germany) using standard Bruker pulse programs. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as internal standard. MS were recorded on a Finnigan MAT TSQ-700 (San Jose, CA) mass spectrometer, using a dithiothreitol and dithioerythritol (3:1) matrix, or a Micromass LCT (Manchester, UK) mass spectrometer. Silica gel (Fuji-Silysia Chemical, Aichi, Japan), ODS silica gel (Nacalai Tesque, Kyoto, Japan), and Sephadex LH-20 (Pharmacia, Uppsala, Sweden) were used for column chromatography. TLC was

**Table 1.** $^{13}C$ NMR Spectral Data for **1**−**5** in DMSO-$d_6$

| C | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 | 161.3 | 161.3 | 161.4 | 161.4 | 161.3 |
| 2 | 124.7 | 124.5 | 124.6 | 124.6 | 124.5 |
| 3 | 151.4 | 151.0 | 151.1 | 151.1 | 151.0 |
| 4 | 127.6 | 128.1 | 128.1 | 128.1 | 128.0 |
| 4a | 131.0 | 131.3 | 131.3 | 131.3 | 131.2 |
| 5 | 119.2 | 119.1 | 119.1 | 119.1 | 119.1 |
| 6 | 137.3 | 137.3 | 137.4 | 137.4 | 137.3 |
| 7 | 123.5 | 123.5 | 123.5 | 123.5 | 123.5 |
| 8 | 160.7 | 160.7 | 160.8 | 160.8 | 160.7 |
| 8a | 115.4 | 115.4 | 115.5 | 115.5 | 115.5 |
| 9 | 192.0 | 192.0 | 192.0 | 192.0 | 191.9 |
| 9a | 114.4 | 114.6 | 114.6 | 114.7 | 114.6 |
| 10 | 181.8 | 181.9 | 181.9 | 182.0 | 181.9 |
| 10a | 134.1 | 134.2 | 134.2 | 134.2 | 134.1 |
| Me-3 | 20.5 | 20.3 | 20.4 | 20.4 | 20.3 |
| 1′ | 109.1 | 108.3 | 108.3 | 108.3 | 108.4 |
| 2′ | 161.9 | 162.5 | 162.5 | 162.5 | 162.5 |
| 3′ | 106.0 | 104.8 | 104.9 | 104.8 | 104.7 |
| 4′ | 162.1 | 161.3 | 159.8 | 160.0 | 159.5 |
| 5′ | 90.9 | 93.7 | 93.9 | 93.9 | 94.0 |
| 6′ | 160.6 | 159.8 | 161.4 | 161.4 | 161.3 |
| MeCO-3′ | 203.4 | 203.1 | 203.1 | 203.1 | 202.9 |
| MeCO-3′ | 33.0 | 33.1 | 33.1 | 33.1 | 33.0 |
| OMe-4′ | 55/0 | | | | |
| 1″ | 100.7 | 101.4 | 101.3 | 100.8 | 100.7 |
| 2″ | 72.7 | 72.9 | 73.0 | 73.2 | 71.3 |
| 3″ | 76.9 | 76.5 | 76.5 | 76.8 | 87.5 |
| 4″ | 69.9 | 69.2 | 69.3 | 69.8 | 68.1 |
| 5″ | 77.6 | 65.9 | 66.0 | 77.3 | 65.2 |
| 6″ | 60.8 | | | 60.0 | |
| 1‴ | | | | | 104.0 |
| 2‴ | | | | | 73.7 |
| 3‴ | | | | | 76.0 |
| 4‴ | | | | | 70.3 |
| 5‴ | | | | | 76.9 |
| 6‴ | | | | | 61.2 |

**Table 2.** Cytotoxic Activities of **1**−**7** and Etoposide

| | $IC_{50}$ ($\mu$g/mL) | |
|---|---|---|
| compound | HSC-2 | HPC |
| **1** | 36 | >120 |
| **2** | 56 | >120 |
| **3** | >120 | >120 |
| **4** | >120 | >120 |
| **5** | >120 | >120 |
| **6** | 33 | >120 |
| **7** | 25 | 57 |
| etoposide | 24 | >120 |

[a] Key to cell lines: HSC-2 (human squamous cell carcinoma); HPC (normal human pulp cells).

carried out on precoated Kieselgel 60 $F_{254}$ (0.25 mm thick, Merck, Darmstadt, Germany) and RP-18 $F_{254}S$ (0.25 mm thick, Merck) plates, and spots were visualized by spraying the plates with 10% $H_2SO_4$ solution, followed by heating. HPLC was performed using a system comprised of a Tosoh CCPM pump (Tokyo, Japan), a Tosoh CCP PX-8010 controller, a Tosoh RI-8010 detector, a Shodex OR-2 detector (Showa-Denko, Tokyo, Japan), and a Rheodyne injection port. The following reagents were obtained from the indicated companies: Dulbecco's modified Eagle medium (DMEM) (Gibco, Grand Island, NY); fetal bovine serum (FBS) (JRH Biosciences, Lenexa, KS); penicillin and streptomycin sulfate (Meiji-Seika, Tokyo, Japan); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) (Sigma, St. Louis, MO).

**Plant Material.** *Bulbinella floribunda* was purchased from a nursery in Heiwaen, Japan, in October 1998, and was identified by one of the authors (Y.S.). A voucher specimen has been deposited in our laboratory (voucher No. 98-4-BF, Laboratory of Medicinal Plant Science).

**Extraction and Isolation.** Fresh roots of *B. floribunda* (2.8 kg) were extracted with hot MeOH. Following removal of MeOH, the residue was subjected to column chromatogarphy on silica gel, eluting with a stepwise gradient mixture of $CHCl_3$−MeOH (19:1; 9:1; 4:1; 2:1) and finally with MeOH to give five fractions (I−V). Fraction I was chromatographed on silica gel eluting with $CHCl_3$−AcOEt (19:1) and hexane−$Me_2$-CO (2:1) to give **6** (2.85 g) and **7** (90.6 mg). Fraction II was subjected to a silica gel column eluting with $CHCl_3$−AcOEt (19:1; 9:1; 4:1) to collect an additional four fractions (IIa−IId). Fraction IIc was subjected to silica gel column chromatography eluting with $CHCl_3$−MeOH (12:1; 10:1; 9:1) and AcOEt−MeOH (12:1), and Sephadex LH-20 column chromatography with MeOH, to yield **1** (90.8 mg), **2** (272 mg), and **3** (870 mg). Fraction III was chromatographed on silica gel eluting with $CHCl_3$−MeOH−$H_2O$ (40:10:1; 30:10:1), on ODS silica gel with MeOH−$H_2O$ (20:1) and MeCN−$H_2O$ (5:8), and on Sephadex LH-20 with MeOH to give **4** (118 mg) and **5** (52.2 mg).

**Bulbineloneside A (1):** red powder; $[\alpha]^{26}_D$ +238.1° (*c* 0.10, MeOH); UV (EtOH) $\lambda_{max}$ 433 (log $\epsilon$ 4.00), 287 (log $\epsilon$ 4.40), 256 (log $\epsilon$ 4.39) nm; UV (EtOH + 3% NaOMe) $\lambda_{max}$ 512, 288 nm; CD (EtOH) $\Delta_{\epsilon 294}$ −1.83, $\Delta_{\epsilon 277}$ +19.80, $\Delta_{\epsilon 251}$ −2.45, $\Delta_{\epsilon 239}$ −11.39, $\Delta_{\epsilon 219}$ −26.22, $\Delta_{\epsilon 207}$ −33.12; FT-IR (film) $\nu_{max}$ 3332 (OH), 2920 (C−H), 1678 and 1601 (C=O), 1446, 1365, 1273, 1200, 1041 (C−O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 13.75 (1H, s, OH-2′), 12.44 (1H, s, OH-1), 11.86 (1H, s, OH-8), 7.72 (1H, dd, *J* = 8.2, 7.4 Hz, H-6), 7.47 (1H, dd, *J* = 7.4, 0.7 Hz, H-5), 7.31 (1H, s, H-2), 7.31 (1H, dd, *J* = 8.2, 0.7 Hz, H-7), 6.57 (1H, s, H-5′), 4.90 (1H, d, *J* = 7.9 Hz, H-1″), 3.98 (3H, s, OMe-4′), 3.72 (1H, m, H-6″a), 3.39 (1H, ddd, *J* = 9.0, 5.2, 2.2 Hz, H-5″), 3.31 (1H, m, H-6″b), 3.19 (1H, m, H-3″), 2.98 (1H, m, H-4″), 2.90 (1H, m, H-2″), 2.64 (3H, s, Ac-3′), 2.07 (3H, s, Me-3); HRFABMS (positive-ion mode) *m*/*z* 597.1627 $[M + H]^+$ (calcd for $C_{30}H_{29}O_{13}$, 597.1608).

**Enzymatic Hydrolysis of 1.** Compound **1** (11.1 mg) was dissolved in AcOH−AcONa buffer (pH 5.0, 5 mL) with $\beta$-D-glucosidase (Sigma, EC 3.2.1.21) (40 mg) and incubated at room temperature for 24 h. The crude reaction mixture was chromatographed on silica gel eluting with $CHCl_3$−MeOH (19:1; 1:1) to yield **6** (1.0 mg) and D-glucose. D-Glucose was identified by direct TLC comparison with an authentic sample: $R_f$ 0.40 (*n*-BuOH−$Me_2CO$−$H_2O$, 4:5:1).

**Bulbineloneside B (2):** red powder; $[\alpha]^{26}_D$ +209.5° (*c* 0.11, MeOH); UV (EtOH) $\lambda_{max}$ 432 (log $\epsilon$ 4.03), 287 (log $\epsilon$ 4.40), 256 (log $\epsilon$ 4.42) nm; UV (EtOH + 3% NaOMe) $\lambda_{max}$ 501, 333, 289, 252 nm; CD (EtOH) $\Delta_{\epsilon 295}$ −2.59, $\Delta_{\epsilon 279}$ +15.60, $\Delta_{\epsilon 255}$ −2.21, $\Delta_{\epsilon 240}$ −10.89, $\Delta_{\epsilon 219}$ −23.32, $\Delta_{\epsilon 209}$ −20.40; IR (film) $\nu_{max}$ 3383 (OH), 2918 (C−H), 1676 and 1616 (C=O), 1427, 1369, 1281, 1203, 1076 (C−O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 13.96 (1H, s, OH-2′), 12.43 (1H, s, OH-1), 11.83 (1H, s, OH-8), 10.35 (1H, s, OH-4′), 7.72 (1H, dd, *J* = 8.3, 7.5 Hz, H-6), 7.49 (1H, dd, *J* = 7.5, 1.0 Hz, H-5), 7.36 (1H, s, H-2), 7.31 (1H, dd, *J* = 8.3, 1.0 Hz, H-7), 6.31 (1H, s, H-5′), 5.53 (1H, d, *J* = 7.5 Hz, H-1″), 4.27 (1H, dd, *J* = 10.6, 5.1 Hz, H-5″a), 4.16 (1H, m, H-3″), 4.15 (1H, m, H-4″), 4.08 (1H, m, H-3″), 3.67 (1H, dd, *J* = 10.6, 10.6 Hz, H-5″b), 2.70 (3H, s, Ac-3′), 2.07 (3H, s, Me-3); HRFABMS (positive-ion mode) *m*/*z* 553.1331 $[M + H]^+$ (calcd for $C_{28}H_{25}O_{12}$, 553.1346).

**Enzymatic Hydrolysis of 2.** Compound **2** (30.7 mg) was subjected to enzymatic hydrolysis with naringinase (Sigma, EC 232-962-4) (36.7 mg) in AcOH−AcONa buffer (pH 5.0, 10 mL) at room temperature for 20 h. The reaction mixture was passed through a combination of a Sep-Pak $C_{18}$ cartridge (Waters, Milford, MA) and a Toyopak IC-SP M cartridge (Tosoh) eluting with 20% MeOH followed by MeOH. The MeOH eluted fraction was purified by silica gel chromatography eluting with $CHCl_3$−MeOH (19:1) to yield **2a** (3.4 mg). The 20% MeOH eluted fraction was analyzed by HPLC under the following conditions: column, Kaseisorb $NH_2$-60-5 (4.6 mm i.d. × 250 mm, 5 $\mu$m, Tokyo-Kasei, Tokyo, Japan); solvent, MeCN−$H_2O$ (3:1); flow rate, 0.8 mL/min; detection, RI and optical rotation (OR). Identification of D-xylose was carried out by comparison of its retention time and OR with those of an authentic sample; $t_R$(min) 9.20 (positive optical rotation).

**Bulbineloneside C (3):** red powder; $[\alpha]^{26}_D$ −95.2° (*c* 0.10, MeOH); UV (EtOH) $\lambda_{max}$ 432 (log $\epsilon$ 3.83), 288 (log $\epsilon$ 4.21), 255 (log $\epsilon$ 4.23) nm; UV (EtOH + 3% NaOMe) $\lambda_{max}$ 501, 334, 289,

253 nm; CD (EtOH) $\Delta_{\epsilon 294}$ +4.39, $\Delta_{\epsilon 279}$ −8.85, $\Delta_{\epsilon 252}$ +6.67, $\Delta_{\epsilon 244}$ +11.27, $\Delta_{\epsilon 237}$ +11.02, $\Delta_{\epsilon 233}$ +9.72, $\Delta_{\epsilon 219}$ +23.44, $\Delta_{\epsilon 212}$ +21.86, $\Delta_{\epsilon 208}$ +26.15; IR (film) $\nu_{max}$ 3371 (OH), 2924 (C−H), 1665 and 1601 (C═O), 1427, 1365, 1277, 1203, 1072 (C−O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 13.94 (1H, s, OH-2′), 12.43 (1H, s, OH-1), 11.84 (1H, s, OH-8), 7.74 (1H, dd, $J$ = 8.3, 7.6 Hz, H-6), 7.49 (1H, dd, $J$ = 7.6, 0.9 Hz, H-5), 7.36 (1H, s, H-2), 7.32 (1H, dd, $J$ = 8.3, 0.9 Hz, H-7), 6.35 (1H, s, H-5′), 4.95 (1H, d, $J$ = 7.6 Hz, H-1″), 3.89 (1H, dd, $J$ = 11.2, 5.2 Hz, H-5″a), 3.46 (1H, m, H-4″), 3.41 (1H, m, H-2″), 3.31 (1H, m, H-3″), 3.30 (1H, dd, $J$ = 11.2, 10.7 Hz, H-5″b), 2.69 (3H, s, Ac-3′), 2.06 (3H, s, Me-3); HRESIMS $m/z$ 553.1307 $[M + H]^+$ (calcd for $C_{28}H_{25}O_{12}$, 553.1346).

**Bulbineloneside D (4):** red powder; $[\alpha]^{26}_D$ −47.6° ($c$ 0.11, MeOH); UV (EtOH) $\lambda_{max}$ 432 (log $\epsilon$ 4.01), 289 (log $\epsilon$ 4.39), 255 (log $\epsilon$ 4.41) nm; UV (EtOH + 3% NaOMe) $\lambda_{max}$ 501, 334, 289, 254 nm; CD (EtOH) $\Delta_{\epsilon 293}$ +2.31, $\Delta_{\epsilon 281}$ −4.92, $\Delta_{\epsilon 252}$ +5.04, $\Delta_{\epsilon 243}$ +8.35, $\Delta_{\epsilon 237}$ +6.72, $\Delta_{\epsilon 217}$ +13.91, $\Delta_{\epsilon 208}$ +16.85; IR (film) $\nu_{max}$ 3379 (OH), 2924 (C−H), 1676 and 1616 (C═O), 1427, 1369, 1280, 1203, 1080 (C−O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 13.96 (1H, s, OH-2′), 7.74 (1H, dd, $J$ = 8.3, 7.4 Hz, H-6), 7.48 (1H, dd, $J$ = 7.4, 0.7 Hz, H-5), 7.36 (1H, s, H-2), 7.32 (1H, dd, $J$ = 8.3, 0.7 Hz, H-7), 6.34 (1H, s, H-5′), 4.97 (1H, d, $J$ = 7.7 Hz, H-1″), 3.78 (1H, dd, $J$ = 11.9, 1.5 Hz, H-6″a), 3.62 (1H, dd, $J$ = 11.9, 3.8 Hz, H-6″b), 3.40 (1H, m, H-2″), 3.40 (1H, m, H-3″), 3.32 (1H, m, H-4″), 2.70 (3H, s, Ac-3′), 2.05 (3H, s, Me-3); HRESIMS $m/z$ 605.1314 $[M + Na]^+$ (calcd for $C_{29}H_{26}O_{13}Na$, 605.1271).

**Enzymatic Hydrolysis of 3 and 4.** Compounds **3** (50.1 mg) and **4** (7.4 mg) were separately subjected to enzymatic hydrolysis with naringinase as described for **2** to give an aglycon **3a** (2.5 mg) and a sugar fraction, and an aglycon **3a** (0.6 mg) and a sugar fraction, respectively. HPLC analysis of each sugar fraction under the same conditions as in the case of that of **2** showed the presence of D-xylose in **3** and D-glucose in **4**; $t_R$(min) 9.22 (positive optical rotation, D-xylose), 12.80 (positive optical rotation, D-glucose).

**(−)-*P*-4′-Demethylknipholone (3a):** red powder; $[\alpha]^{26}_D$ −80.0° ($c$ 0.02, MeOH); CD (EtOH) $\Delta_{\epsilon 295}$ +0.74, $\Delta_{\epsilon 279}$ −1.38, $\Delta_{\epsilon 241}$ +1.92, $\Delta_{\epsilon 227}$ −0.31, $\Delta_{\epsilon 213}$ +0.99; HREIMS $m/z$ 420.0855 (calcd for $C_{23}H_{16}O_8$, 420.0843).

**Bulbineloneside E (5):** orange powder; $[\alpha]^{26}_D$ −104.8° ($c$ 0.11, MeOH); UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 432 (log $\epsilon$ 4.01), 288 (log $\epsilon$ 4.38), 255 (log $\epsilon$ 4.41); UV (EtOH + 3% NaOMe) $\lambda_{max}$ 501, 337, 334, 289, 254 nm; CD (EtOH) $\Delta_{\epsilon 293}$ +3.58, $\Delta_{\epsilon 280}$ −12.35, $\Delta_{\epsilon 255}$ +8.42, $\Delta_{\epsilon 242}$ +15.20, $\Delta_{\epsilon 239}$ +13.69, $\Delta_{\epsilon 218}$ +28.58, $\Delta_{\epsilon 207}$ +30.55, $\Delta_{\epsilon 202}$ −16.84; IR (film) $\nu_{max}$ 3390 (OH), 2920 (C−H), 1668 and 1604 (C═O), 1427, 1369, 1273, 1207, 1076 (C−O), 1034 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 13.94 (1H, s, OH-2′), 7.74 (1H, dd, $J$ = 8.5, 7.6 Hz, H-6), 7.48 (1H, dd, $J$ = 7.6, 0.8 Hz, H-5), 7.36 (1H, s, H-2), 7.33 (1H, dd, $J$ = 8.5, 0.8 Hz, H-7), 6.30 (1H, s, H-5′), 5.09 (1H, d, $J$ = 7.6 Hz, H-1″), 4.38 (1H, d, $J$ = 7.8 Hz, H-1‴), 3.99 (1H, dd, $J$ = 11.5, 5.3 Hz, H-5″a), 3.74 (1H, m, H-6‴a), 3.67 (1H, m, H-2″), 3.62 (1H, m, H-4″), 3.53 (1H, m, H-3″), 3.42 (1H, dd, $J$ = 11.5, 9.2 HZ, H-5″b), 3.41 (1H, m, H-6‴b), 3.24 (1H, m, H-3‴), 3.12 (1H, m, H-2‴), 3.07 (1H, m, H-4‴), 2.71 (3H, s, Ac-3′), 2.06 (3H, s, Me-3); HRESIMS $m/z$ 715.1893 $[M + H]^+$ (calcd for $C_{34}H_{35}O_{17}$, 715.1874).

**Enzymatic Hydrolysis of 5.** Compound **5** (8.7 mg) was subjected to enzymatic hydrolysis with naringinase as described for **2** to give an aglycon (**3a**, 1.9 mg), a deglucosyl compound (**3**, 4.1 mg), and a sugar fraction. HPLC analysis of the sugar fraction under the same conditions as in the case of **4** showed the presence of D-xylose and D-glucose.

**Cell Culture.** HSC-2 human squamous cell carcinoma cells were provided through the courtesy of Prof. M. Nagumo, Showa University, Tokyo, Japan. Normal human pulp cells (HPC) were prepared from the explants of pulp of first premolars extracted for orthodontics purposes, after obtaining approval by the Institutional Review Board, Meikai University School of Dentistry. HPC were used between the fifth and 10th passages. The cells were cultured in DMEM supplemented with 10% heat-inactivated FBS, 100 U/mL penicillin, and 100 $\mu$g/mL streptomycin sulfate in a humidified 5% $CO_2$ atmosphere.

**Assay for Cytotoxic Activity.** Cells were trypsinized and inoculated at $6 \times 10^3$ to $1.2 \times 10^4$ per each 96-microwell plate (Falcon, flat bottom, treated polystyrene, Becton Dickinson, San Jose, CA) and incubated for 24 h. After washing once with phosphate-buffered saline (PBS, 0.01 M phosphate buffer, 0.15 M NaCl, pH 7.4) supplemented with 100 U/mL penicillin and 100 $\mu$g/mL streptomycin sulfate, they were treated for 24 h without or with test compounds. They were washed once with PBS and incubated for 4 h with 0.2 mg/mL MTT in DMEM supplemented with 10% FBS. After the medium was removed, the cells were lysed with 0.1 mL of DMSO and the relative viable cell number was determined by measuring the absorbance at 540 nm of the cell lysate, using Labsystems Multiskan (Biochromatic, Helsinki, Finland) connected to a Star/DOT Matrix printer JL-10. The $LD_{50}$ value, which reduces the viable cell number by 50%, was determined from the dose−response curve.

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