Chemical Constituents of the Bulbs of *Habranthus brachyandrus* and Their Cytotoxic Activities

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Received June 15, 2009; accepted July 15, 2009; published online August 3, 2009

The bulbs of *Habranthus brachyandrus* (Amaryllidaceae) have been extensively analyzed for their chemical constituents, resulting in the isolation of eight flavan derivatives (1—8), four of which are new naturally occurring compounds; a new hydroxybutyric acid glucoside (9); three known phenolic compounds (10—12); and six known alkaloids (13—18). The structures of the new compounds were determined on the basis of spectroscopic analysis, including two-dimensional (2D) NMR data, and chemical evidence. The isolated compounds and a few derivatives were evaluated for their cytotoxic activities against HL-60 human promyelocytic leukemia cells and HSC-2 human oral squamous cell carcinoma cells.

Key words *Habranthus brachyandrus*; Amaryllidaceae; flavan derivative; hydroxybutyric acid glucoside; HL-60 cell; HSC-2 cell

Plants of the family Amaryllidaceae are known to contain a number of alkaloids, commonly called Amaryllidaceae alkaloids, with a diversity of basic chemical structures and significant biological activities.[1] *Habranthus brachyandrus* belonging to the family Amaryllidaceae is indigenous to South America and is cultivated for ornamental purposes around the world. A literature survey concerning the second metabolites of *H. brachyandrus* showed that no systematic chemical work had been carried out on the plant and only a few alkaloids such as lycorine, lycorenine, and habranthine were reported in the 1950s to 1960s.[2,3] During our continuous screening of higher plants with anti-tumor potential, a methanolic extract of *H. brachyandrus* bulbs was found to exhibit cytotoxic activity against HL-60 human promyelocytic leukemia cells with an IC_{50} value of 11.5 μg/ml. Cytotoxicity-guided fractionation of the extract has resulted in the isolation of eight flavan derivatives (**1**—**8**), four of which are new naturally occurring compounds (**1**—**4**); a new hydroxybutyric acid glucoside (**9**); three known phenolic compounds (**10**—**12**); and six known alkaloids (**13**—**18**). The structures of the new compounds were determined on the basis of spectroscopic analysis, including two-dimensional (2D) NMR data, and chemical evidence. The isolated compounds and a few derivatives were evaluated for their cytotoxic activities against HL-60 cells and HSC-2 human oral squamous cell carcinoma cells.

Results and Discussion

The fresh bulbs of *H. brachyandrus* (2.5 kg) were extracted with hot MeOH and concentrated under reduced pressure. The MeOH extract (182 g), which showed cytotoxic activity against HL-60 cells with an IC_{50} value of 11.5 μg/ml, was passed through a porous-polymer polystyrene resin (Diaion HP-20) column. The MeOH-eluted fraction (5.0 g) and EtOH-eluted fraction (1.2 g) were cytotoxic to HL-60 cells with IC_{50} values of 0.44 μg/ml and 14.7 μg/ml, respectively. Through a series of chromatographic separations, the MeOH-eluted fraction yielded compounds **1** (5.6 mg), **2** (13.0 mg), **3** (4.2 mg), **4** (8.2 mg), **5** (12.9 mg), **6** (11.4 mg), **9** (17.4 mg), **11** (5.0 mg), **12** (2.0 mg), **13** (43.3 mg), **14** (68.6 mg), **15** (150 mg), **16** (12.0 mg), **17** (4.5 mg), and **18** (4.3 mg), and the EtOH-eluted fraction gave **7** (13.3 mg), **8** (10.7 mg), and **10** (13.3 mg). Compounds **5**—**8** and **10**—**18** were identified as (2*S*)-3′,7-dihydroxy-4′-methoxyflavan (**5**),[4] (2*S*)-4′,7-di-

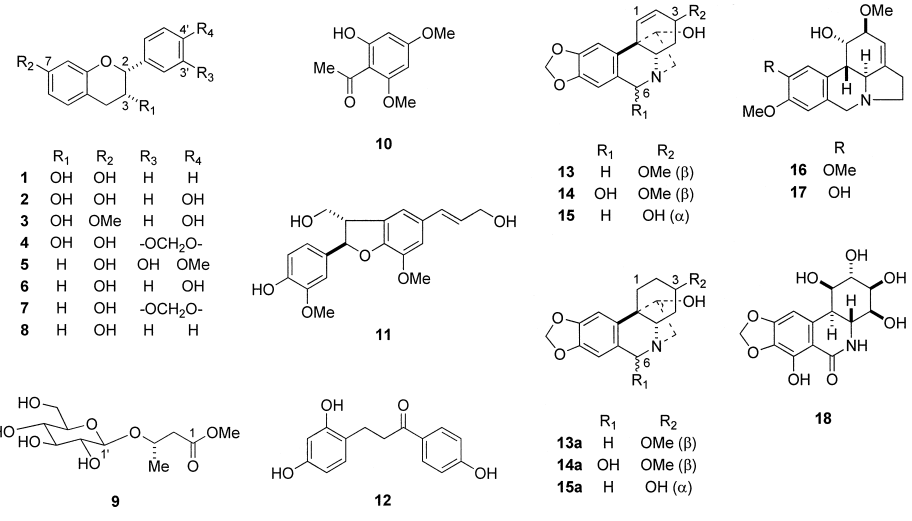

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Table 1. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) Spectral Data for Compounds **1**—**4** in Acetone-d_6

Position	**1**		**2**		**3**		**4**	
	δ_H (J)	δ_C	δ_H (J)	δ_C	δ_H (J)	δ_C	δ_H (J)	δ_C
2	5.08 (br s)	79.7	4.98 (br s)	78.7	5.00 (br s)	79.7	5.00 (br s)	79.6
3	4.27 (br s)	67.0	4.19 (br s)	66.2	4.21 (br s)	67.0	4.21 (br s)	67.1
4ax	3.13 (dd, 16.1, 4.2 Hz)	33.9	3.10 (dd, 16.8, 4.0 Hz)	32.9	3.13 (dd, 16.2, 4.2 Hz)	33.9	3.11 (dd, 16.1, 4.2 Hz)	33.9
eq	2.73 (dd, 16.1, 3.3 Hz)		2.71 (dd, 16.8, 3.2 Hz)		2.74 (dd, 16.2, 3.2 Hz)		2.71 (dd, 16.1, 3.2 Hz)	
5	6.88 (d, 8.1 Hz)	131.4	6.86 (d, 8.2 Hz)	130.5	6.94 (d, 8.4 Hz)	131.4	6.86 (d, 8.2 Hz)	131.5
6	6.39 (dd, 8.1, 2.5 Hz)	109.2	6.37 (dd, 8.2, 2.4 Hz)	108.2	6.45 (dd, 8.4, 2.5 Hz)	108.0	6.38 (dd, 8.2, 2.4 Hz)	109.2
7		157.5		156.6		160.1		157.6
8	6.36 (d, 2.5 Hz)	103.6	6.33 (d, 2.4 Hz)	102.7	6.39 (d, 2.5 Hz)	102.0	6.33 (d, 2.4 Hz)	103.7
9		156.2		155.4		156.4		156.2
10		111.6		110.7		112.8		111.5
11							5.97 (s)	101.8
1′		140.6		130.4		131.2		134.6
2′	7.52 (dd, 7.4, 1.3 Hz)	127.8	7.34 (d, 8.5 Hz)	128.2	7.34 (d, 8.5 Hz)	129.1	7.08 (d, 1.4 Hz)	108.6
3′	7.35 (dd, 7.4, 7.4 Hz)	128.6	6.81 (d, 8.5 Hz)	114.5	6.81 (d, 8.5 Hz)	115.5		148.2
4′	7.28 (m)	128.1		156.7		157.8		147.8
5′	7.35 (dd, 7.4, 7.4 Hz)	128.6	6.81 (d, 8.5 Hz)	114.5	6.81 (d, 8.5 Hz)	115.5	6.81 (d, 8.4 Hz)	108.3
6′	7.52 (dd, 7.4, 1.3 Hz)	127.8	7.34 (d, 8.5 Hz)	128.2	7.34 (d, 8.5 Hz)	129.1	6.97 (dd, 8.4, 1.4 Hz)	121.0
3-OH	3.75 (br s)		3.67 (d, 5.4 Hz)		3.78 (br s)		3.82 (br s)	
7-OH	8.10 (s)		8.25[a] (s)				8.19 (br s)	
4′-OH			8.08[a] (s)		8.47 (br s)			
7-OMe					3.73 (s)	55.4		

a) Interchangeable.

hydroxyflavan (**6**),[5] (2*S*)-7-hydroxy-3′,4′-methylenedioxyflavan (**7**),[6] (2*S*)-7-hydroxyflavan (**8**),[7] 2-hydroxy-4,6-dimethoxyacetophenone (**10**),[8] (±)-dehydrodiconiferyl alcohol (**11**),[9] 2,4,4′-trihydroxydihydrochalcone (**12**),[10] haemanthamine (**13**),[11] haemanthidine (**14**),[12] (+)-bulbispermine (**15**),[13] galanthine (**16**),[11] 10-*O*-demethylgalanthine (**17**)[11] and pancratistatine (**18**),[14] respectively, by comparison of their physical and spectroscopic data with literature values. Although these compounds were previously obtained from other plant sources, this is the first report on their isolation from *H. brachyandrus*.

Compound **1** was isolated as an amorphous solid and showed an accurate $[M+H]^+$ ion at m/z 243.1008 in the high-resolution electrospray ionization mass spectrum (HR-ESI-MS), corresponding to the empirical molecular formula $C_{15}H_{14}O_3$. The IR spectrum of **1** suggests the presence of hydroxy groups (3118 cm^{-1}) and aromatic rings (1625, 1509, 1457 cm^{-1}) in the molecule. The ^{1}H-NMR spectrum of **1** in acetone-d_6 showed signals for a monosubstituted aromatic ring at δ 7.52 (2H, dd, J=7.4, 1.3 Hz, H-2′ and H-6′), 7.35 (2H, dd, J=7.4, 7.4 Hz, H-3′ and H-5′), and 7.28 (1H, m, H-4′); a 1,2,4-trisubstituted aromatic ring at δ 6.88 (1H, d, J=8.1 Hz, H-5), 6.39 (1H, dd, J=8.1, 2.5 Hz, H-6), and 6.36 (1H, d, J=2.5 Hz, H-8); two oxymethine protons at δ 5.08 (1H, br s, H-2) and 4.27 (1H, br s, H-3); and a pair of deshielded methylene protons at δ 3.13 (1H, dd, J=16.1, 4.2 Hz, H-4ax) and 2.73 (1H, dd, J=16.1, 3.3 Hz, H-4eq) (Table 1). In addition, two exchangeable proton signals at δ 8.10 and 3.75 (each 1H, br s), which disappeared on the addition of D_2O, indicates the presence of two free hydroxy groups in **1**. These spectral data suggest that **1** is a flavan derivative with two hydroxy groups. In the ^{1}H-detected multiple-quantum coherence (HMQC) spectrum of **1**, the H-6 and H-8 aromatic protons were associated with the corresponding one-bond coupled carbons at δ 109.2 (C-6) and 103.6 (C-8), with which the proton of a hydroxy group at δ 8.10 showed

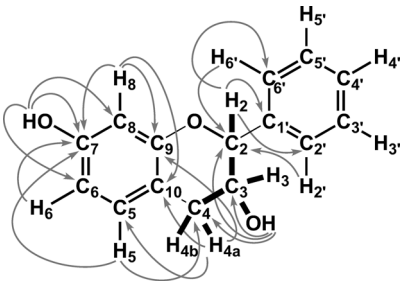

Fig. 1. HMBC Correlations of **1**

Bold lines indicate the ^{1}H–^{1}H spin-couplings traced by the ^{1}H–^{1}H COSY spectrum and arrows indicate ^{1}H/^{13}C long-range couplings observed in the HMBC spectrum.

long-range correlations in the ^{1}H-detected heteronuclear multiple-bond connectivity (HMBC) spectrum. Thus the locus of one hydroxy group at C-7 is evident. Another hydroxy group was determined to be attached to C-3 because proton spin-coupling systems consistent with an –O–$C_{(2)}$H–$C_{(3)}$H(OH)–$C_{(4)}H_2$– unit were traced by the ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY) spectrum of **1**. On the basis of the above data, the plane structure of **1** was shown to be 3,7-dihydroxyflavan. Detailed HMBC correlations supporting the proposed structure are depicted in Fig. 1. The following spectroscopic analysis made the absolute configurations at C-2 and C-3 assignable. The small coupling constants of H-2 and H-3, and H-3 and H-4ax in the ^{1}H-NMR spectrum of **1**, and NOE correlations between H-2 and H-3/H-4ax, and between H-3 and H_2-4 in the NOE correlation spectroscopy (NOESY) spectrum were indicative of the 2,3-*cis* relative configuration with the 2-phenyl group in a *pseudo*-equatorial orientation and the 3-hydroxy group in a *pseudo*-axial orientation, that is, the (2*S*,3*S*) or (2*R*,3*R*) configurations. In the circular dichroism (CD) spectrum of **1**, a negative Cotton effect was observed at 281.6 nm, which provided evidence for the *P*-helicity of the ring-C part,[15] thus

possessing the (2*R*,3*R*) configurations. Accordingly, the structure of **1** was determined to be (2*R*,3*R*)-3,7-dihydroxyflavan.

Compound **2** was shown to have a molecular formula $C_{15}H_{14}O_4$ on the basis of the HR-ESI-MS data (*m/z* 259.0988 $[M+H]^+$). The ^{1}H- and ^{13}C-NMR spectral features of **2** were similar to those of **1**; however, the deduced molecular formula of **2** was higher than that of **1** by one oxygen atom, and three exchangeable proton signals were observed at δ 8.25 (1H, br s), 8.08 (1H, br s) and 3.67 (1H, d, J=5.4 Hz), suggesting that **2** has one more hydroxy group in addition to C-3 and C-7. The ^{1}H-NMR spectrum of **2** showed signals of an A_2B_2-type proton spin-coupling system at δ 7.34 (2H, d, J=8.5 Hz, H-2′ and H-6′) and 6.81 (2H, d, J=8.5 Hz, H-3′ and H-5′) assignable to a 1,4-disubstituted aromatic ring, as well as signals for a 1,2,4-trisubstituted aromatic ring at δ 6.86 (1H, d, J=8.2 Hz, H-5), 6.37 (1H, dd, J=8.2, 2.4 Hz, H-6), and 6.33 (1H, d, J=2.4 Hz, H-8); two oxymethine protons at δ 4.98 (1H, br s, H-2) and 4.19 (1H, br s H-3); and a pair of deshielded methylene protons at δ 3.10 (1H, dd, J=16.8, 4.0 Hz, H-4ax) and 2.71 (1H, dd, J=16.8, 3.2 Hz, H-4eq). In the HMBC spectrum of **2**, long-range correlations were observed between H-2 (δ 4.98) and C-2′/C-6′ (δ 128.2), indicating a linkage of the 1,4-disubstituted aromatic ring to C-2. A negative Cotton effect at 274.1 nm in the CD spectrum of **2** in conjunction with the proton coupling constants between H-2 and H-3, and between H-3 and H-4ax, and NOE correlations among the –O–$C_{(2)}$H–$C_{(3)}$H(OH)–$C_{(4)}H_2$– unit allowed the absolute configurations of C-2 and C-3 to be assigned as (2*R*,3*R*). The structure of **2** was formulated as (2*R*,3*R*)-3,4′,7-trihydroxyflavan.

Compound **3** had a molecular formula $C_{16}H_{16}O_4$ on the basis of the HR-ESI-MS data (*m/z* 273.1100 $[M+H]^+$). Comparison of the ^{1}H- and ^{13}C-NMR spectra of **3** with those of **2** showed their considerable structural similarity. The ^{1}H-NMR spectrum of **3** exhibited signals assignable to a 1,4-disubstituted aromatic ring, a 1,2,4-trisubstituted aromatic ring, and an –O–$C_{(2)}$H–$C_{(3)}$H(OH)–$C_{(4)}H_2$– unit, as observed for **2**. Compound **3** was only different from **2** in the lack of one of the three free hydroxy groups and in the presence of a methoxy group [δ_H 3.73 (3H, s)/δ_C 55.4 (–OMe)]. In the HMBC spectrum of **3**, the methoxy proton signal at δ 3.73 showed long-range correlations with the carbon signals at δ 160.1 (C-7), indicating methylation of the C-7 hydroxy group. The absolute configurations at C-2 and C-3 of **3** were confirmed to be the same as those of **2** by analysis of the ^{1}H-NMR, NOESY, and CD spectra. The structure of **3** was established as (2*R*,3*R*)-3,4′-dihydroxy-7-methoxyflavan.

Compound **4** exhibited a molecular formula $C_{16}H_{14}O_5$ on the basis of its HR-ESI-MS data (*m/z* 287.0922 $[M+H]^+$). The ^{1}H-NMR spectrum of **4** showed signals for two 1,2,4-trisubstituted aromatic rings at δ 7.08 (1H, d, J=1.4 Hz, H-2′), 6.97 (1H, dd, J=8.4, 1.4 Hz, H-6′), and 6.81 (1H, d, J=8.4 Hz, H-5′), and δ 6.86 (1H, d, J=8.2 Hz, H-5), 6.38 (1H, dd, J=8.2, 2.4 Hz, H-6), and 6.33 (1H, d, J=2.4 Hz, H-8); two oxymethine protons at δ 5.00 (1H, br s, H-2) and 4.21 (1H, br s H-3); a pair of deshielded methylene protons at δ 3.11 (1H, dd, J=16.1, 4.2 Hz, H-4ax) and 2.71 (1H, dd, J=16.1, 3.2 Hz, H-4eq)]; a methylenedioxy group at δ 5.97 (2H, s); and two exchangeable protons at δ 8.19 and 3.82 (each 1H, br s). These ^{1}H-NMR data and comparison with those of **2** suggest that **4** is a 3,7-dihydroxyflavan derivative with a methylenedioxy group at the ring B portion. This was confirmed by long-range correlations between H-2′ (δ 7.08) and δ 148.2/δ 147.8, H-5′ (δ 6.81) and δ 148.2/δ 147.8, and between H-6′ (δ 6.97) and δ 147.8, which allowed the carbon signals at δ 148.2 and δ 147.8 to be assigned to C-3′ and C-4′, respectively, and between the methylenedioxy protons at δ 5.97 and C-3′/C-4′. The ^{1}H-NMR, NOESY, and CD spectral data were consistent with the 2*R* and 3*R* configurations. The structure of **4** was determined to be (2*R*,3*R*)-3,7-dihydroxy-3′,4′-methylenedioxyflavan.

Compound **9** was deduced to be $C_{11}H_{20}O_8$ from its HR-ESI-MS data (*m/z* 303.1057 $[M+Na]^+$). The IR spectrum of **9** was indicative of a glycoside (3417 cm^{-1}) and showed an absorption band for an ester carbonyl group (1714 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra of **9** showed signals for a CH_3–CH(O–)–CH_2– group at δ_H 1.39 (3H, d, J=6.3 Hz)/δ_C 22.1, δ_H 4.56 (1H, m)/δ_C 72.0, and δ_H 2.90 (1H, dd, J=15.5, 6.3 Hz) and 2.57 (1H, dd, J=15.5, 6.3 Hz)/δ_C 41.9; a β-glucopyranosyl moiety at δ_H 4.95 (1H, d, J=7.7 Hz, H-1′)/δ_C 104.1 (C-1′), 75.1 (C-2′), 78.4 (C-3′), 71.5 (C-4′), 78.4 (C-5′), and 62.7 (C-6′); an ester carbonyl group at δ_C 171.9; and a methoxy group at δ_H 3.60 (3H, s)/δ_C 51.4 (OMe). In the HMBC spectrum of **9**, the ester carbonyl carbon showed $^2J_{C,H}$ correlations with the methylene protons at δ 2.90 and 2.57, and $^3J_{C,H}$ correlations with the methoxy protons and oxymethine proton at δ 4.56. A long-range correlation was also observed between the anomeric proton of the β-glucosyl moiety and the oxymethine carbon at δ 72.0. Thus **9** was shown to be methyl 3-[(β-glucopyranosyl)oxy]butyrate. The absolute configuration at C-3, as well as that of the glucosyl unit in **9**, was determined by comparison of its physical and spectral data with synthetic compounds. Methyl (*R*)-3-hydroxybutyrate and methyl (*S*)-3-hydroxybutyrate were independently treated with *o*-nitrophenyl-β-D-glucopyranoside in the presence of β-glucosidase from almond at room temperature for 1 h to give methyl (*R*)-3-[(β-D-glucopyranosyl)-oxy]butyrate (**9a**) and methyl (*S*)-3-[(β-D-glucopyranosyl)-oxy]butyrate (**9b**), respectively. The physical and spectral data of **9** were in complete agreement with those of **9b**. The full structure of **9** was characterized as methyl (*S*)-3-[(β-D-glucopyranosyl)oxy]butyrate.

The isolated compounds and the 1,2-hydrogenerated derivatives (**13a**—**15a**) of **13**—**15** were evaluated for their cytotoxic activity against HL-60 cells (Table 2). Compounds **13**, **14**, and **18** were also subjected to a cytotoxic screening test using HSC-2 cells. Compound **18** (pancratistatine), as expected from previous data, **13** (haemanthamine), and **14** (haemanthidine) showed potent cytotoxic activities against both HL-60 cells and HSC-2 cells and are considered to contribute mainly to the cytotoxicity of the MeOH-eluted fraction. In the crinum-type alkaloids (**13**—**15**), the introduction of a hydroxy group at C-6 did not affect the cytotoxic activity, whereas displacement of the C-3β methoxy group by the C-3α hydroxy group reduced the activity. It is notable that hydrogenation of the double bond between C-1 and C-2 in **13**—**15** resulted in a decrease in the activity. The two flavan and acetophenone derivatives (**7** and **8**, and **10**) exhibited moderate cytotoxic activity against HL-60 cells and seem to contribute partially to the cytotoxicity of the EtOH-eluted fraction. In the flavan derivatives (**1**—**8**), those with a hy-

Table 2. Cytotoxic Activities of Compounds **1**—**18**, **13a**—**15a**, Etoposide, and Cisplatin against HL-60 Cells and HSC-2 Cells

Compound	IC_{50} (μM)[a]	
	HL-60 cells	HSC-2 cells
1	>55.0	—
2	>55.0	—
3	>55.0	—
4	>55.0	—
5	42.6±2.08	—
6	—[b]	—
7	19.0±1.30	—
8	27.9±1.91	—
9	>55.0	—
10	>55.0	—
11	13.8±0.37	—
12	23.3±0.44	—
13	2.0±0.05	33.2±3.19
13a	23.3±0.92	—
14	2.0±0.09	13.3±0.14
14a	22.5±1.23	—
15	17.8±0.17	—
15a	45.9±4.60	—
16	>55.0	—
17	>55.0	—
18	0.16±0.003	1.1±0.14
Etoposide	0.34±0.003	17.2±1.48
Cisplatin	1.2±0.09	15.6±0.04

a) Data represent the mean value±S.E.M. of three experiments performed in triplicate. *b*) Not measured.

droxy group at C-3 did not show apparent cytotoxicty at a sample concentration of 55 μM.

Experimental

Optical rotations were measured using a JASCO P-1030 (Tokyo, Japan) automatic digital polarimeter. IR, UV, and CD spectra were recorded on a JASCO FT-IR 620, a JASCO V-520 or a JASCO V-630, and a JASCO J-720 spectrophotometer, respectively. NMR spectra were recorded on a Bruker DPX-400 spectrometer (400 MHz for ^{1}H-NMR, Karlsruhe, Germany) or a Bruker DRX-500 spectrometer (500 MHz for ^{1}H-NMR) using standard Bruker pulse programs. Chemical shifts are given as the δ-value with reference to tetramethylsilane (TMS) as an internal standard. ESI-MS data were obtained on a Waters-Micromass LCT mass spectrometer (Manchester, U.K.). Diaion HP-20 (Mitsubishi-Chemical, Tokyo, Japan), silica gel (Fuji-Silysia Chemical, Aichi, Japan), aminopropyl-bonded (NH) silica gel (Fuji-Silysia Chemical), and octadecylsilanized (ODS) silica gel (Nacalai Tesque, Kyoto, Japan) were used for column chromatography. TLC was carried out on Silica gel 60 F_{254} (0.25 mm, Merck, Darmstadt, Germany), NH_2 F_{254S} (0.25 mm thick, Merck), and RP-18 F_{254S} (0.25 mm thick, Merck) plates, and spots were visualized by spraying with 10% H_2SO_4 followed by heating. The following materials and reagents were used for cell culture assay: microplate reader, Spectra Classic, Tecan (Salzburg, Austria); 96-well flat-bottom plate, Iwaki Glass (Chiba, Japan); HL-60 cells, Human Science Research Resources Bank (JCRB 0085, Osaka, Japan); fetal bovine serum (FBS), BioWhittaker (Walkersville, MD, U.S.A.); RPMI-1640 medium, etoposide, cisplatin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT), and 0.25% Tripsin-EDTA solution, Sigma (St. Louis, MO, U.S.A.); and Dulbecco's modified Eagle's medium (DMEM), penicillin G, and streptomycin sulfate, Gibco (Grand Island, NY, U.S.A). All other chemicals used were of biochemical reagent grade.

Plant Material The bulbs of *H. brachyandrus* were purchased from a garden center in Heiwaen, Japan, in September 2005. The bulbs were cultivated and the flowered plants were identified by one of the authors (Y.M.). A voucher specimen has been deposited in our laboratory (voucher No. 05-9-01-HB, Laboratory of Medicinal Pharmacognosy).

Extraction and Isolation The bulbs of *H. brachyandrus* (fresh weight, 2.5 kg) were extracted with hot MeOH (11 l). The MeOH extract was concentrated under reduced pressure, and the viscous concentrate (182 g) was passed through a Diaion HP-20 column, successively eluted with 30% MeOH, 50% MeOH, MeOH, EtOH, and EtOAc. The MeOH- and EtOH-eluate fractions exhibited cytotoxic activity against HL-60 cells (IC_{50} 0.44 μg/ml and 14.7 μg/ml, respectively). Column chromatography of the MeOH-eluate portion (5.0 g) on silica gel and elution with a stepwise gradient mixture of $CHCl_3$–MeOH–H_2O (19 : 1 : 0; 9 : 1 : 0; 40 : 10 : 1; 20 : 10 : 1; 7 : 4 : 1), and finally with MeOH alone, gave 10 fractions (I—X). Fraction II was chromatographed on ODS silica gel eluted with MeOH–H_2O (2 : 3; 3 : 1) to collect four subfractions (IIa—IId). Fraction IIa was further separated on a silica gel column eluted with hexane–EtOAc (3 : 1) to afford **1** (5.6 mg). Fraction IIb was subjected to column chromatography on silica gel eluted with hexane–Me_2CO (3 : 1) and hexane–EtOAc (2 : 1) to give **4** (8.2 mg). Fraction IIc was subjected to silica gel column chromatography eluted with hexane–EtOAc (5 : 2; 3 : 1; 1 : 3) to furnish **3** (4.2 mg). Fraction IId was subjected to a silica gel column eluted with hexane–EtOAc (3 : 1) and hexane–$CHCl_3$–MeOH (23 : 19 : 1) to yield **5** (12.9 mg). Fraction III was chromatographed on ODS silica gel eluted with MeOH–H_2O (2 : 5; 2 : 3; 3 : 2) to collect two subfractions (IIIa and IIIb). Fraction IIIa was subjected to a silica gel column eluted with hexane–EtOAc (1 : 2; 1 : 4) and an NH silica gel column eluted with hexane–EtOAc (1 : 8; 1 : 19) to afford **16** (12.0 mg). Fraction IIIb was subjected to column chromatography on silica gel eluted with hexane–Me_2CO (3 : 1; 5 : 2) and hexane–$CHCl_3$–MeOH (10 : 19 : 1) to give **6** (11.4 mg). Fraction IV was chromatographed on ODS silica gel eluted with MeOH–H_2O (2 : 5; 2 : 3) to collect three subfractions (IVa—IVc). Fraction IVa was further separated on a silica gel column eluted with hexane–EtOAc (1 : 2) to afford **2** (13.0 mg). Fraction IVb was subjected to column chromatography on silica gel eluted with hexane–EtOAc (1 : 2) to give **11** (5.0 mg). Fraction IVc was subjected to silica gel column chromatography eluted with hexane–EtOAc (1 : 2) and $CHCl_3$–MeOH (25 : 1) to yield **12** (2.0 mg). Fraction V was chromatographed on NH silica gel eluted with $CHCl_3$–MeOH (20 : 1; 10 : 1; 5 : 1; 2 : 1) to give **13** (43.3 mg), **14** (68.6 mg), and **17** with few impurities, which was purified on an NH silica gel column eluted with hexane–$CHCl_3$–MeOH (2 : 20 : 1) to yield **17** (4.5 mg). Fraction VIII was chromatographed on NH silica gel eluted with $CHCl_3$–MeOH (20 : 1) to afford **15** (150 mg) and a mixture of **9** and **18**. The mixture was separated on a silica gel column eluted with EtOAc–MeOH (9 : 1) to furnish **9** (17.4 mg) and **18** (4.3 mg). Column chromatography of the EtOH eluate portion (1.2 g) on NH silica gel and elution with hexane–EtOAc (1 : 1) gave eight fractions (XI—XVIII). Fraction XII was separated on a silica gel column eluted with hexane–EtOAc (19 : 1; 9 : 1; 4 : 1) to give **10** (13.3 mg). Fraction IX was subjected to column chromatography on silica gel eluted with hexane–EtOAc (9 : 1; 4 : 1) and ODS silica gel eluted with MeOH–H_2O (2 : 1) to furnish **8** (10.7 mg). Fraction XVI was subjected to silica gel column chromatography eluted with hexane–EtOAc (17 : 3) to yield **7** (13.3 mg).

Compound **1**: Amorphous solid, $[\alpha]_D^{25}$ −60.4° (c=0.34, MeOH). HR-ESI-MS (positive mode) m/z: 243.1008 $[M+H]^+$ (Calcd for $C_{15}H_{15}O_3$: 243.1021). IR (film) ν_{max} cm^{-1}: 3118 (OH), 2925 and 2855 (CH), 1625, 1509 and 1457 (aromatic rings). UV (MeOH) λ_{max} nm (log ε): 283.0 (3.13). CD (c=0.0002, MeOH) λ_{max} nm ($\Delta\varepsilon$): 255.0 (+0.14), 281.6 (−0.17). ^{1}H-NMR (acetone-d_6) and ^{13}C-NMR (acetone-d_6): see Table 1. ^{1}H-NMR (acetone-d_6+D_2O) δ: 7.51 (2H, dd, J=7.7, 1.4 Hz, H-2′, H-6′), 7.34 (2H, dd, J=7.7, 7.3 Hz, H-3′, H-5′), 7.28 (1H, m, H-4′), 6.86 (1H, d, J=8.2 Hz, H-5), 6.38 (1H, dd, J=8.2, 2.4 Hz, H-6), 6.36 (1H, d, J=2.4 Hz, H-8), 5.07 (1H, br s, H-2), 4.26 (1H, br s, H-3), 3.12 (1H, dd, J=16.2, 4.3 Hz, H-4ax), 2.71 (1H, dd, J=16.2, 3.4 Hz, H-4eq).

Compound **2**: Amorphous solid, $[\alpha]_D^{25}$ −75.1° (c=0.13, MeOH). HR-ESI-MS (positive mode) m/z: 259.0988 $[M+H]^+$ (Calcd for $C_{15}H_{15}O_4$: 259.0970). IR (film) ν_{max} cm^{-1}: 3371 (OH), 2931 and 2847 (CH), 1621, 1513 and 1463 (aromatic rings). UV (MeOH) λ_{max} nm (log ε): 282.0 (3.57). CD (c=0.0002, MeOH) λ_{max} nm ($\Delta\varepsilon$): 236.6 (+0.99), 274.1 (−0.86). ^{1}H-NMR (acetone-d_6) and ^{13}C-NMR (acetone-d_6): see Table 1. ^{1}H-NMR (acetone-d_6+D_2O) δ: 7.32 (2H, d, J=8.5 Hz, H-2′, H-6′), 6.84 (1H, d, J=8.2 Hz, H-5), 6.79 (2H, d, J=8.5 Hz, H-3′, H-5′), 6.36 (1H, dd, J=8.2, 2.5 Hz, H-6), 6.32 (1H, d, J=2.5 Hz, H-8), 4.96 (1H, br s, H-2), 4.18 (1H, br s, H-3), 3.08 (1H, dd, J=16.1, 4.3 Hz, H-4ax), 2.69 (1H, dd, J=16.1, 3.5 Hz, H-4eq).

Compound **3**: Amorphous solid, $[\alpha]_D^{25}$ −56.8° (c=0.21, MeOH). HR-ESI-MS (positive mode) m/z: 273.1100 $[M+H]^+$ (Calcd for $C_{16}H_{17}O_4$: 273.1127). IR (film) ν_{max} cm^{-1}: 3303 (OH), 2922 and 2853 (CH), 1618, 1503 and 1448 (aromatic rings). UV (MeOH) λ_{max} nm (log ε): 282.0 (3.38). CD (c=0.0002, MeOH) λ_{max} nm ($\Delta\varepsilon$): 238.3 (+0.28), 277.1 (−0.54). ^{1}H-NMR (acetone-d_6) and ^{13}C-NMR (acetone-d_6): see Table 1. ^{1}H-NMR (acetone-d_6+D_2O) δ: 7.34 (2H, d, J=8.5 Hz, H-2′, H-6′), 6.94 (1H, d, J=8.3 Hz, H-5), 6.81 (2H, d, J=8.5 Hz, H-3′ and H-5′), 6.45 (1H, dd, J=8.3, 2.6 Hz, H-6), 6.39 (1H, d, J=2.6 Hz, H-8), 5.00 (1H, br s, H-2), 4.21 (1H, br s, H-3), 3.73 (3H, s, OMe), 3.13 (1H, dd, J=16.2, 4.2 Hz, H-4ax), 2.74 (1H, dd,

J=16.2, 3.3 Hz, H-4eq).

Compound **4**: Amorphous solid, $[\alpha]_D^{25}$ −59.8° (c=0.14, MeOH). HR-ESI-MS (positive mode) m/z: 287.0922 $[M+H]^+$ (Calcd for $C_{16}H_{15}O_5$: 287.0919). IR (film) ν_{max} cm^{-1}: 3212 (OH), 2916 and 2853 (CH), 1618, 1598 and 1489 (aromatic rings). UV (MeOH) λ_{max} nm (log ε): 284.0 (3.66). CD (c=0.0002, MeOH) λ_{max} nm ($\Delta\varepsilon$): 248.5 (+0.27), 289.9 (−1.03). ^{1}H-NMR (acetone-d_6) and ^{13}C-NMR (acetone-d_6): see Table 1. ^{1}H-NMR (acetone-d_6+D_2O) δ: 7.08 (1H, d, J=1.6 Hz, H-2′), 6.96 (1H, dd, J=8.5, 1.6 Hz, H-6′), 6.86 (1H, d, J=8.2 Hz, H-5), 6.80 (1H, d, J=8.5 Hz, H-5′), 6.37 (1H, dd, J=8.2, 2.4 Hz, H-6), 6.33 (1H, d, J=2.4 Hz, H-8), 5.97 (2H, s, –OCH_2O–), 4.99 (1H, br s, H-2), 4.20 (1H, br s, H-3), 3.10 (1H, dd, J=16.1, 4.2 Hz, H-4ax), 2.71 (1H, dd, J=16.1, 3.3 Hz, H-4eq).

Compound **9**: Amorphous solid, $[\alpha]_D^{25}$ −16.7° (c=0.10, MeOH). HR-ESI-MS (positive mode) m/z: 303.1057 $[M+Na]^+$ (Calcd for $C_{11}H_{20}O_8Na$: 303.1056). IR (film) ν_{max} cm^{-1}: 3417 (OH), 2925 (CH), 1714 (C=O). ^{1}H-NMR (pyridine-d_5) δ: 4.95 (1H, d, J=7.7 Hz, H-1′), 4.56 (1H, m, H-3), 4.51 (1H, dd, J=11.7, 2.4 Hz, Ha-6′), 4.36 (1H, dd, J=11.7, 5.2 Hz, Hb-6′), 4.22 (1H, dd, J=9.0, 9.0 Hz, H-4′), 4.20 (1H, dd, J=9.0, 9.0 Hz, H-3′), 3.95 (1H, dd, J=9.0, 7.7 Hz, H-2′), 3.91 (1H, ddd, J=9.0, 5.2, 2.4 Hz, H-5′), 3.60 (3H, s, OMe), 2.90 (1H, dd, J=15.5, 6.3 Hz, Ha-2), 2.57 (1H, dd, J=15.5, 6.3 Hz, Hb-2), 1.39 (3H, d, J=6.3 Hz, Me-4).

Preparation of 9a and 9b Methyl (*R*)-3-hydroxybutylate (Wako Pure Chemical Industries, Osaka, Japan; 2.5 g) was dissolved in acetone (2.0 ml), to which was added *o*-nitrophenyl β-D-glucopyranoside (130 mg), β-glucosidase (Sigma, 232-589-7; 100 mg), and an acetate buffer (pH 5.0, 3.2 ml), and the solution was allowed to stand at room temperature for 1 h. The reaction mixture was purified on a silica gel column eluted with EtOAc–MeOH (9-1) to give **9a** (6.5 mg). Following this procedure using methyl (*S*)-3-hydroxybutylate, **9b** (4.8 mg) was prepared.

Catalytic Hydrogenation of 13—15 A mixture of **13** (10.2 mg) and 10% Pd–C (11.5 mg) in EtOH (4.2 ml) was stirred under an H_2 atmosphere at ambient temperature for 7 h. The reaction mixture, after the removal of Pd–C by filtration, was subjected to preparative TLC using $CHCl_3$–MeOH–H_2O (40 : 10 : 1) to yield **13a** (4.8 mg).[16] Following this procedure, **14** (10.2 mg) and **15** (40.1 mg) were converted to **14a** (3.3 mg)[17] and **15a** (34.0 mg), respectively.

Compound **13a**: Amorphous powder. $[\alpha]_D^{25}$ +41.6° (c=0.10, $CHCl_3$). HR-ESI-MS m/z: 304.1550 $[M+H]^+$ (Calcd for $C_{17}H_{22}NO_4$: 304.1549). ^{1}H-NMR (chloroform-d) δ: 6.72 (1H, s, H-10), 6.41 (1H, s, H-7), 5.88 (2H, d, J=2.4 Hz, –OCH_2O–), 4.32 (1H, d, J=16.8 Hz, Ha-6), 4.13 (1H, m, H-11), 3.70 (1H, m, H-3), 3.68 (1H, d, J=16.8 Hz, Hb-6), 3.32—3.27 (3H, m, H_2-12 and H-4a), 3.29 (3H, s, OMe), 2.34 (1H, m, Ha-1), 2.11 (1H, m, Ha-4), 2.08 (1H, m, Ha-2), 2.03 (1H, m, Hb-1), 1.97 (1H, m, Hb-2), 1.82 (1H, m, Hb-4). ^{13}C-NMR (chloroform-d) δ: 146.8 (C-9), 146.1 (C-8), 138.8 (C-10a), 121.9 (C-6a), 106.1 (C-7), 103.7 (C-10), 100.8 (–OCH_2O–), 80.2 (C-11), 75.3 (C-3), 63.1 (C-12), 62.8 (C-4a), 60.4 (C-6), 55.7 (OMe), 46.2 (C-10b), 29.8 (C-4), 26.8 (C-1 or C-2), 22.7 (C-1 or C-2).

Compound **14a**: Amorphous powder. $[\alpha]_D^{25}$ +18.4° (c=0.10, $CHCl_3$). HR-ESI-MS m/z: 320.1493 $[M+H]^+$ (Calcd for $C_{17}H_{22}NO_5$: 320.1498). ^{1}H-NMR (chloroform-d) δ: 6.90/6.75 (s, H-7), 6.68/6.66 (s, H-10), 5.91/5.89 (m, –OCH_2O–), 5.75/5.04 (s, H-6), 4.15/3.28 (m, H-12*exo*), 4.06 (m, H-11), 3.81/3.58 (m, H-4a), 3.71 (m, H-3), 3.29/3.27 (s, OMe), 3.26/2.00 (m, H-12*endo*), 2.23/2.10 (each 1H, m, H-4β), 2.02/1.86 (m, H-4α), 2.34—1.95 (m, H_2-1 and H_2-2). ^{13}C-NMR (chloroform-d) δ: 147.9 (C-9), 146.6/146.3 (C-8), 139.8/138.1 (C-10a), 127.0/126.4 (C-6a), 108.6/107.5 (C-7), 103.2/103.1 (C-10), 101.1/101.0 (–OCH_2O–), 88.0/85.6 (C-6), 80.9/80.7 (C-11), 75.1/74.9 (C-3), 62.2/56.4 (C-4a), 57.6/51.6 (C-12), 55.9/55.6 (OMe), 46.7/46.3 (C-10b), 29.7/29.2/29.1/27.1/26.4/22.6 (C-1, C-2 and C-4).

Compound **15a**: Amorphous powder. $[\alpha]_D^{25}$ +52.5° (c=0.10, MeOH). HR-ESI-MS m/z: 290.1410 $[M+H]^+$ (Calcd for $C_{16}H_{20}NO_4$: 290.1392). UV λ_{max} (MeOH) nm: 296.5 (log ε=3.54). IR ν_{max} (film) cm^{-1}: 3234 (OH), 2934 and 2828 (CH), 1623 and 1482 (aromatic ring), 1035 (CN). ^{1}H-NMR (chloroform-d) δ: 6.81 (1H, s, H-10), 6.58 (1H, s, H-7), 5.93 (2H, dd, J=3.6, 1.1 Hz, –OCH_2O–), 4.50 (1H, d, J=16.2 Hz, H-6b), 4.14 (1H, br dd, J=7.1, 3.2 Hz, H-11), 3.98 (1H, d, J=16.2 Hz, H-6α), 3.69 (1H, m, H-3), 3.59 (1H, dd, J=13.8, 7.1 Hz, H-12*endo*), 3.44 (1H, dd, J=13.8, 3.2 Hz, H-12*exo*), 3.38 (1H, dd, J=12.8, 5.1 Hz, H-4a), 2.72 (1H, ddd, J=14.0, 4.9, 1.8 Hz, H-1α), 2.15 (1H, m, H-2α), 2.10 (1H, m, H-4β), 1.96 (1H, ddd, J=12.8, 12.1, 11.4 Hz, H-4α), 1.94 (1H, m, H-2β), 1.87 (1H, ddd, J=14.0, 13.7, 5.5 Hz, H-1β). ^{13}C-NMR (chloroform-d) δ: 146.9 (C-8), 146.2 (C-9), 137.0 (C-10a), 121.7 (C-6a), 105.3 (C-7), 102.9 (C-10), 100.6 (–OCH_2O–), 79.9 (C-11), 67.5 (C-3), 66.8 (C-4a), 61.2 (C-12), 58.4 (C-6), 45.5 (C-10b), 34.0 (C-4), 31.3 (C-2), 24.9 (C-1).

Cell Culture Assays HL-60 cells were maintained in RPMI-1640 medium containing 10% FBS supplemented with L-glutamine, 100 units/ml of penicillin G, and 100 μg/ml of streptomycin sulfate. The leukemia cells were washed and resuspended in the above medium to 4×10^4 cells/ml, and 196 μl of this cell suspension was placed in each well of a 96-well flat-bottomed plate. The cells were incubated in 5% CO_2/air for 24 h at 37 °C. After incubation, 4 μl of EtOH–H_2O (1 : 1) solution containing the sample was added to give the final concentrations of 0.052—20.0 μg/ml; 4 μl of EtOH–H_2O (1 : 1) was added into control wells. The cells were further incubated for 72 h in the presence of each agent, and then cell growth was evaluated using an MTT assay procedure. After termination of the cell culture, 10 μl of MTT 5 mg/ml in phosphate-buffered saline (PBS) was added to every well and the plate was further incubated in 5% CO_2/air for 4 h at 37 °C. The plate was then centrifuged at 1500 rpm for 5 min to precipitate cells and MTT formazan. An aliquot of 150 μl of the supernatant was removed from every well, and 175 μl of dimethyl sulfoxide was added to dissolve the formazan crystals. The plate was mixed on a microshaker for 10 min, and then read on a microplate reader at 550 nm. The concentration giving 50% inhibition (IC_{50} value) was calculated from the dose-response curve.

HSC-2 cells were maintained in DMEM containing 10% FBS supplemented with L-glutamine, D-glucose 4.5 g/ml, penicillin G 100 units/ml, and streptomycin sulfate 100 μg/ml. The cells were washed and resuspended in the above medium to 1×10^5 cells/ml, and 100 μl of this cell suspension was placed in each well of a 96-well flat-bottomed plate. The cells were incubated in 5% CO_2/air for 24 h at 37 °C. After removal of the medium, 196 μl of fresh medium and 4 μl of EtOH–H_2O (1 : 1) solution containing the sample were added to give the final concentrations of 0.16—55.0 μM; 196 μl of fresh medium and 4 μl of EtOH–H_2O (1 : 1) were added into control wells. The cells were further incubated for 24 h in the presence of each agent, and then cell growth was evaluated in the same way as for HL-60 cells.

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