Sesquiterpenoids and Flavonoids from the Aerial Parts of *Tithonia diversifolia* and Their Cytotoxic Activity

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Cytotoxicity-guided fractionation of the 80% EtOH extract of *Tithonia diversifolia* has resulted in the isolation of twelve sesquiterpenoids (1—12), including three new ones (4, 10, 12), and three known flavonoids (13—15). The structures of the new compounds were determined by analysis of their spectroscopic data. The isolated compounds showed cytotoxic activity against HL-60 leukemia cells with IC_{50} values ranging from 0.13 to 13.0 μM, when etoposide used as a positive control gave an IC_{50} value of 0.43 μM. The cancer growth inhibitory property of 9, the main cytotoxic compound in *T. diversifolia*, was examined using a disease-oriented panel composed of 39 human cancer cell lines in the Japanese Foundation for Cancer Research.

Key words *Tithonia diversifolia*; sesquiterpenoid; cytotoxicity; HL-60 leukemia cell; human cancer cell line assay

Tithonia diversifolia (HEMSL.) A. GRAY (Compositae) is native to Mexico and also grows in parts of Africa, Australia, Asia, and other countries of North America, and is commonly called Mexican sunflower or tree marigold.[1)] An extract of *T. diversifolia* has been traditionally used for the treatment of diabetes, diarrhea, menstrual pain, malaria, hematomas, hepatitis, hepatomas, and wound healing.[2—5)] Pharmacological studies of *Tithonia diversifolia* showed that it has anti-diabetic,[6,7)] anti-malarial,[8)] anti-inflammatory,[9)] analgesic,[9)] and cancer chemopreventive[10)] activity, some of which account for the folkloric claims of this medicinal plant. Several sesquiterpenoids were isolated from *T. diversifolia*.[1,10,11)] As part of our systematic survey of bioactive compounds from higher plants, we have now conducted a detailed phytochemical screening of the 80% EtOH extract of *T. diversifolia* using a cytotoxicity-guided fractionation method, which has resulted in the isolation of twelve sesquiterpenoids (**1**—**12**), including three new ones (**4**, **10**, **12**), and three known flavonoids (**13**—**15**). This paper deals with the structural identification and determination of the sesquiterpenoids and flavonoids, and with their cytotoxic activity.

Results and Discussion

The dried aerial parts of *T. diversifolia* were extracted with hot 80% EtOH. The concentrated 80% EtOH extract, which showed cytotoxic activity against HL-60 cells with an IC_{50} value of 4.10 μg/ml, was passed through a porous-polymer polystyrene resin (Diaion HP-20) column, and successively eluted with 30% MeOH, EtOH, and EtOAc. The EtOH eluate fraction exhibited cytotoxic activity against HL-60 cells (IC_{50} 1.03 μg/ml), and was repeatedly subjected to column chromatography on silica gel, octadecylsilanized (ODS) silica gel, Sephadex LH-20, and reversed phase HPLC, giving compounds **1** (8.4 mg), **2** (11.3 mg), **3** (118 mg), **4** (744 mg), **5** (112 mg), **6** (8.7 mg), **7** (67.9 mg), **8** (90.7 mg), **9** (245 mg), **10** (14.0 mg), **11** (24.2 mg), **12** (19.0 mg), **13** (18.8 mg), **14** (60.8 mg), and **15** (6.6 mg).

Compounds **1**—**3**, **5**—**9**, **11**, and **13**—**15** were identified as tirotundin (**1**),[12)] tirotundin 3-*O*-methyl ether (**2**),[13)] tagitinin A (**3**),[14)] tagitinin C (**5**),[12)] deacetylviguiestin (**6**),[15)] tagitinin F (**7**),[12)] 1β-methoxydiversifolin (**8**),[1)] 1β-methoxydiversifolin 3-*O*-methyl ether (**9**),[1)] 4β,10α-dihydroxy-3-oxo-8β-isobutyroyloxyguaia-11(13)-en-6,12-olide (**11**),[16)] luteolin (**13**),[16)] nepetin (**14**),[16)] and hispidulin (**15**),[16)] respectively.

Compound **4** was obtained as an amorphous powder, $[\alpha]_D$ −102.0° (MeOH). The high-resolution (HR)-electrospray

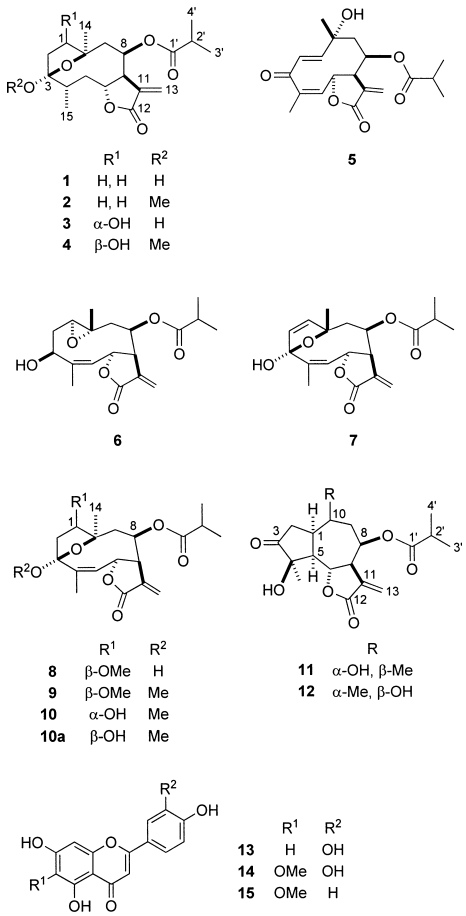

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Table 1. ^{13}C-NMR Spectral Data for Compounds **4**, **10**, and **12**

Position	**4**	**10**	**12**
1	79.7	77.2	45.8
2	41.7	45.2	39.7
3	108.8	109.7	215.0
4	46.4	138.4	77.7
5	38.2	131.2	53.8
6	82.3	75.8	77.1
7	48.2	49.6	48.1
8	70.2	71.2	65.9
9	35.1	39.7	47.4
10	81.3	87.5	71.6
11	137.7	136.2	135.4
12	169.8	169.5	170.0
13	121.7	122.7	121.2
14	24.1	20.8	32.4
15	18.3	21.6	23.1
1′	176.7	175.9	176.9
2′	34.5	34.0	34.4
3′	19.6[a)]	18.6	19.5
4′	19.6[a)]	19.0	19.2
OMe	49.2	50.1	—

a) Overlapping.

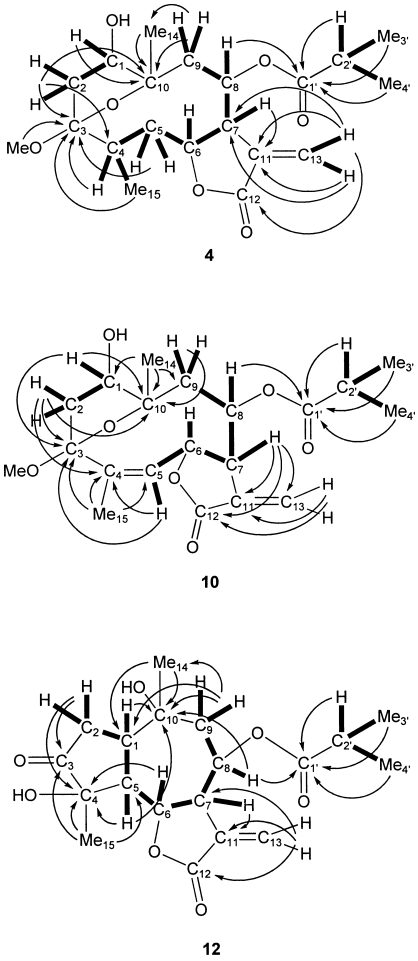

Fig. 1. HMBC Correlations of Compounds **4**, **10**, and **12**

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

ionization (ESI)-MS showed an accurate $[M+H]^+$ peak at *m*/*z* 383.2058 in accordance with the empirical molecular formula $C_{20}H_{30}O_7$. The IR spectrum of **4** suggested the presence of a hydroxy (3446 cm^{-1}), an ester (1735 cm^{-1}), and an α-methylene γ-lactone (1760, 1659 cm^{-1}) group in the molecule. The UV spectrum exhibited absorption maxima at 216 nm. The ^{1}H-NMR spectrum of **4** showed signals for a tertiary and a secondary methyl group [δ 1.37 (3H, s), 0.99 (d, J=7.0 Hz)], a methoxy group [δ 3.10 (3H, s)], an isobutyroyloxy group [δ 1.01, 0.99 (each 3H, d, J=7.0 Hz), 2.37 (1H, m)], an exomethylene group [δ 6.19, 5.47 (each 1H, d, J=3.2 Hz)], and three methine protons geminally bearing an oxygen atom [δ 5.54 (1H, ddd, J=11.4, 5.4, 2.6 Hz), 4.46 (1H, ddd, J=10.6, 6.9, 1.1 Hz), 4.06 (1H, dd, J=9.5, 8.6 Hz)]. In the ^{13}C-NMR spectrum of **4** (Table 1), signals for an acetalic carbon and a quaternary carbon with an oxygen atom were observed at δ 108.8 and 81.3, respectively. These NMR spectral properties of **4** were similar to those of **2**.[13)] However, the molecular formula of **4** was higher by one oxygen atom than that of **2** and an oxymethine proton signal could be observed at δ 4.06, indicating that **4** has a free hydroxy group. The locus and configuration of the hydroxy group was assigned by the following spectral analysis. The oxymethine proton at δ 4.06 was shown to have spin-coupling correlations with a pair of methylene protons at δ 2.49 and 1.81 in the ^{1}H–^{1}H shift correlation spectroscopy (COSY) spectrum of **4**. The methylene protons (H_2-2), which were associated with the one-bond coupled carbon at δ 41.7 (C-2) by the ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) spectrum, showed long-range correlations with the carbons at δ 108.8 (C-3), 46.4 (C-4), and 81.3 (C-10) in the ^{1}H-detected heteronuclear multiple-bond connectivities (HMBC) spectrum (Fig. 1). Consequently, the presence of the C-1 hydroxy group is evident. The stereostructure of **4**, including the configuration of the C-1 hydroxy group, was determined from the phase-sensitive NOE correlation spectroscopy (NOESY) spectrum (Fig. 2). NOEs between the H-1 and H-2α/Me-14/methoxy group allowed the configuration of the C-1 hydroxy group to be assigned as α, and those between the H-4 and H-2β/H-5β/H-6, H-6 and H-9β, H-7 and H-5α/H-13b, and between Me-14 and H-8/H-9α showed that the configurations of the other asymmetric carbons of **4** were the same as those of **2**. The structure of **4** was elucidated as 1α-hydroxytirotundin 3-*O*-methyl ether.

Compound **10** was isolated as an amorphous solid with a molecular formula of $C_{20}H_{28}O_7$, as determined by the data of HR-ESI-MS, which showed an $[M+H]^+$ peak at *m*/*z* 381.1947 (Calcd for $C_{20}H_{28}O_7$ 381.1913). The ^{1}H-NMR spectrum of **10** exhibited signals due to two tertiary methyl groups [δ 1.70 (t-like, J=1.8 Hz), 1.44 (s)], a methoxy group [δ 3.11 (s)], an isobutyroyloxy group [δ 1.00, 0.97 (each 3H, d, J=7.2 Hz), 2.34 (1H, m)], an exomethylene group [δ 6.20, 5.55 (each 1H, d, J=2.6 Hz)], an olefinic proton [δ 5.66 (m)] and three methine protons geminally bearing an oxygen atom [δ 5.59 (1H, ddd, J=11.2, 5.0, 4.6 Hz), 5.20 (m), 3.77 (dd, J=11.1, 3.9 Hz)]. The existence of an acetalic carbon and a quaternary carbon with an oxygen atom were shown by the ^{13}C-NMR signals at δ 109.7 and 87.5, respectively. These NMR data were closely related to those of 1β-hydroxydiversifolin 3-*O*-methyl ether (**10a**),[17)] and the plane structure of **10** was confirmed to be the same as that of **10a** by analysis of its ^{1}H–^{1}H COSY, HMQC, and HMBC spectra. The H-1 pro-

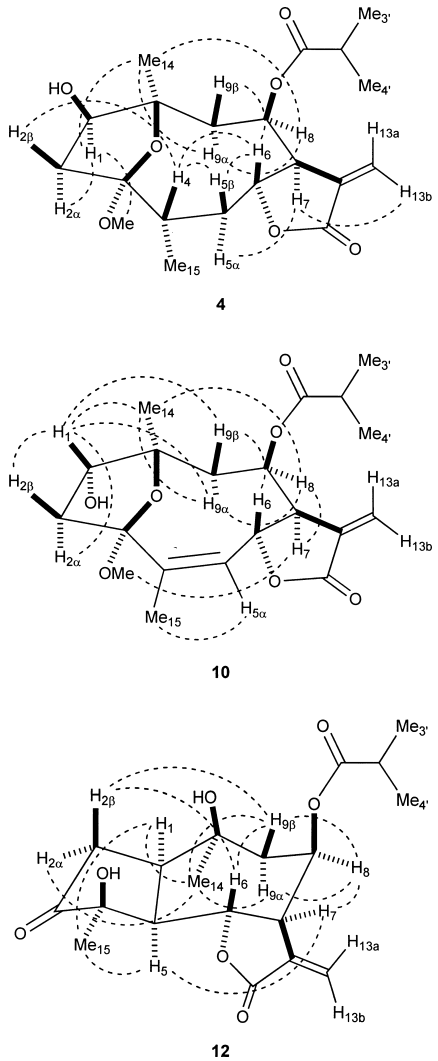

Fig. 2. NOE Correlations of Compounds **4**, **10**, and **12**

ton signal of **10** was observed at δ 3.77 (dd, J=11.1, 3.9 Hz)], whereas that of **10a** appeared at δ 4.37 (dd, J=10.0, 6.0 Hz)]. These findings suggest that **10** is a stereoisomer of **10a** with regard to the C-1 hydroxy group. The stereostructure of **10**, including the configuration of the C-1 hydroxy group, was determined by the NOESY spectrum (Fig. 2), which showed NOE correlations between H-1 and H-2α/H-2β/H-9α/H-9β/Me-14, H-6 and H-9β, and H-8 and H-7/H-9α/Me-14. Based on the above evidence, the structure of **10** was determined to be 1α-hydroxydiversifolin 3-*O*-methyl ether.

Compound **12** was obtained as an amorphous solid. Its molecular formula was determined to be $C_{19}H_{26}O_7$ by an $[M+H]^+$ peak in the HR-ESI-MS at *m/z* 367.1764 (Calcd for $C_{19}H_{27}O_7$ 367.1757). The ^{1}H-NMR spectrum of **12** displayed signals for two tertiary methyl groups [δ 1.44, 1.23 (each 3H, s)], an isobutyroyloxy group [δ 1.02 (3H×2, d, J=7.0 Hz), 2.40 (1H, m)], an exomethylene group [δ 6.17, 5.36 (each 1H, d, J=3.4 Hz)], and two methine protons geminally bearing an oxygen atom [δ 5.54 (1H, m), 4.71 (1H, dd, J=9.7, 9.7 Hz)]. The presence of a carbonyl, an ester carbonyl, and a γ-lactone carbonyl group in **12** was shown by the ^{13}C-NMR signals at δ 215.0, 176.9, and 170.0, respectively. The molecular formula of **12** was the same of that of **11**, and the ^{13}C-NMR spectral data of **12** showed close similarity to those of **11**, except for the signals due to the C-10 and its neighboring carbons. When the ^{13}C-NMR spectrum of **12** was compared with that of **11**, the signal due to C-10 was shifted upfield by 2.1 ppm, whereas that due to C-9 moved downfield by 7.3 ppm. Furthermore, a downfield shift at H-6 (+0.36 ppm), and upfield shifts at H-1 (−0.68 ppm) and H-5 (−0.44 ppm) could be recognized on comparison of the ^{1}H-NMR spectrum of **12** with that of **11**. In the NOESY spectrum of **12** (Fig. 2), NOE correlations between Me-14 and H-1/H-2α/H-2β/H-9α/H-9β were observed. The above data indicate that **12** is an epimer of **11** with regard to the C-10 hydroxy group. The structure of **12** was determined to be 4β,10β-dihydroxy-3-oxo-8β-isobutyroyloxyguaia-11(13)-en-6,12-olide.

Table 2. Cytotoxic Activity of Compounds **1**—**15** and Etoposide against HL-60 Cells

Compound	IC_{50} (μM)
1	7.1
2	2.1
3	10.5
4	13.0
5	1.1
6	2.6
7	3.0
8	0.13
9	1.5
10	6.6
11	5.7
12	6.4
13	8.7
14	12.3
15	8.0
Etoposide	0.43

The isolated compounds (**1**—**15**) were evaluated for their cytotoxic activity against HL-60 cells. The cells were continuously treated with each sample for 72 h and the cell growth was measured by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) reduction assay procedure.[18] Compounds **1**—**15** showed IC_{50} values ranging from 0.13 to 13.0 μM, when etoposide used as a positive control gave an IC_{50} value of 0.43 μM (Table 2). The cancer growth inhibitory property of **9**, the main cytotoxic compound in *T. diversifolia*, was examined using a disease-oriented panel composed of 39 human cancer cell lines (HCC panel) at the Japanese Foundation for Cancer Research.[19] The breast BSY-1 ($\log GI_{50}$ −5.51), CNS [SF 539 ($\log GI_{50}$ −5.50), SNB-78 ($\log GI_{50}$ −5.48)], lung [DMS273 ($\log GI_{50}$ −5.41), DMS114 ($\log GI_{50}$ −5.41)], ovary [OVCAR-3 ($\log GI_{50}$ −5.48), OVCAR-5 ($\log GI_{50}$ −5.53), OVCAR-8 ($\log GI_{50}$ −5.45), SK-OV-3 ($\log GI_{50}$ −5.40)], stomach [MKN1 ($\log GI_{50}$ −5.62), MKN28 ($\log GI_{50}$ −5.42), MKN74 ($\log GI_{50}$ −5.48)], prostate [DU-145 ($\log GI_{50}$ −5.38), PC-3 ($\log GI_{50}$ −5.49)] cell lines (Table 3) were relatively sensitive to **9**, and the average logarithm of the GI_{50} (MG-MID) across all cell lines tested was −5.17. The delta and range values of **9** were 0.45 and 0.97, respectively. The pattern of differential cytotoxicity of **9** was evaluated by the COMPARE program and was shown not to be correlated with those of any other compounds listed in our database.

Table 3. Cytotoxicity of Compound **9** against a Panel Composed of 39 Human Cancer Cell Lines

Origin of cancer	Cell line	log GI_{50} (M)[a]
Breast	HBC-4	−4.74
	BSY-1	−5.51
	HBC-5	−5.25
	MCF-7	−4.85
	MDA-MB-231	−5.23
Central nervous system	U251	−4.78
	SF-268	−5.23
	SF-295	−4.97
	SF-539	−5.50
	SNB-75	−5.00
	SNB-78	−5.48
Colon	HCC2998	−4.98
	KM-12	−4.87
	HY-29	−5.31
	HCT-15	−5.10
	HCT-116	−5.09
Lung	NCI-H23	−5.00
	NCI-H226	−5.11
	NCI-H522	−5.23
	NCI-H460	−5.16
	A549	−4.78
	DMS273	−5.41
	DMS114	−5.41
Melanoma	LOX-IMVI	−4.75
Ovary	OVCAR-3	−5.48
	OVCAR-4	−5.25
	OVCAR-5	−5.53
	OVCAR-8	−5.45
	SK-OV-3	−5.40
Kidney	RXF-631L	−4.65
	ACHN	−5.10
Stomach	St-4	−4.86
	MKN1	−5.62
	MKN7	−4.96
	MKN28	−5.42
	MKN45	−4.88
	MKN74	−5.48
Prostate	DU-145	−5.38
	PC-3	−5.49
MG-MID[b]		−5.17
Delta[c]		0.45
Range[d]		0.94

a) Log concentration of compound for inhibition of cell grouth at 50% compared to control. *b*) Mean value of log GI_{50} over all cell lines tested. *c*) The difference in log GI_{50} value of the most sensitive cell and MG-MID value. *d*) The difference in log GI_{50} value of the most sensitive cell and the least sensitive cell.

Experimental

Optical rotations were measured by using a JASCO DIP-360 automatic digital polarimeter. IR spectra were recorded on a JASCO FT-IR 620 spectrophotometer and UV spectra on a JASCO V-520 spectrophotometer. NMR spectra were recorded on a Bruker DRX-500 spectrometer (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR) using standard Bruker pulse programs. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as an internal standard. MS were recorded on a Micromass LCT mass spectrometer (Manchester, U.K.). Silica gel (Fuji-Silysia Chemical, Aichi, Japan), ODS silica gel (Nacalai Tesque, Kyoto, Japan), Sephadex LH-20 (Pharmacia, Uppsala, Sweden), and Diaion HP-20 (Mitsubishi-Chemical, Tokyo, Japan) were used for column chromatography. TLC was 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 the spots were visualized by spraying the plates with 10% H_2SO_4 solution, followed by heating. HPLC was performed by using an LC system comprised of a CCPM pump (Tosoh, Tokyo, Japan), a CCP PX-8010 controller (Tosoh), an RI-8010 detector (Tosoh), and a Rheodyne injection port with a 2.0 ml sample loop. A Capcell Pak C_{18} UG80 or UG120 column (10 mm i.d.×200 mm, 5 μm, Shiseido, Tokyo, Japan) was employed for preparative HPLC. The following materials and reagents were used for cell culture and assay of cytotoxic activity: 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), Bio-Whittaker (Walkersville, MD, U.S.A.); RPMI 1640 medium, etoposide and MTT, Sigma (St. Louis, MO, U.S.A.); penicillin G and streptomycin sulfate, Meiji-Seika (Tokyo, Japan). All other chemicals used were of biochemical reagent grade.

Plant Material The aerial parts of *T. diversifolia* were provided by Hiro International Co., Ltd. (Tokyo, Japan) in April 2003 and were identified by Dr. Yutaka Sashida, emeritus professor of the Tokyo University of Pharmacy and Life Sciences. A voucher specimen has been deposited in our laboratory (voucher No. 03-4-05-TD, Laboratory of Medicinal Pharmacognosy).

Extraction and Isolation The plant material (dry weight, 3.0 kg) was extracted with hot 80% EtOH (45 l). The 80% EtOH extract was concentrated under reduced pressure, and the viscous concentrate (320 g) was passed through a Diaion HP-20 column, successively eluted with 30% MeOH, EtOH, and EtOAc. The EtOH and EtOAc eluate fractions exhibited cytotoxic activity against HL-60 cells (IC_{50} 1.03 μg/ml and 1.35, respectively), while the 30% MeOH fraction did not show apparent cytotoxic activity (IC_{50} >20 μg/ml). Column chromatography of the EtOH eluate portion (90 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), and finally with MeOH alone, gave seven fractions (I—VII). Fraction II was subjected to ODS silica gel column chromatography eluted with MeOH–H_2O (1 : 1) to collect four subfractions (IIa—IId). Fraction IIa was further separated by a silica gel column eluted with $CHCl_3$–MeOH (49 : 1) to give four fractions (IIa-1—4). Fraction IIa-1 was subjected to an ODS silica gel column eluted with MeOH–H_2O (11 : 9) and MeCN–H_2O (1 : 2) and a silica gel column with $CHCl_3$–acetone (19 : 1; 4 : 1) to afford **3** (118 mg), **4** (744 mg), and **12** (19.0 mg). Fraction IIa-2 was subjected to an ODS silica gel column eluted with MeCN–H_2O (2 : 3) and MeOH–H_2O (11 : 9), a silica gel column with $CHCl_3$–acetone (99 : 1), and finally to preparative HPLC using MeCN–H_2O (3 : 2) to furnish **5** (112 mg), **8** (90.7 mg), and **9** (245 mg). Fraction IIa-3 was purified by a silica gel column eluted with $CHCl_3$–acetone (19 : 1) and an ODS silica gel column with MeOH–H_2O (3 : 2) and MeCN–H_2O (1 : 2) to give **6** (8.7 mg) and **10** (14.0 mg). Compound **7** (67.9 mg) was isolated from fraction IIa-4 by subjecting it to a silica gel column eluted with $CHCl_3$–acetone (19 : 1) and an ODS silica gel column with MeCN–H_2O (3 : 1; 5 : 8). Fraction IIb was subjected to column chromatography on silica gel eluted with $CHCl_3$–acetone (19 : 1; 4 : 1) and ODS silica gel with MeOH–H_2O (3 : 2) to afford **15** (6.6 mg). Fraction IIc was chromatographed on silica gel eluted with $CHCl_3$–acetone (19 : 1; 4 : 1; 2 : 1) and an ODS silica gel with MeCN–H_2O (4 : 1; 3 : 1; 2 : 1; 1 : 1) to give **1** (8.4 mg) and **2** (11.3 mg). Fraction III was chromatographed on ODS silica gel eluted with MeCN–H_2O (3 : 2; 1 : 1; 2 : 3), silica gel with $CHCl_3$–acetone (4 : 1), and Sephadex LH-20 with MeOH to yield **11** (24.2 mg) and **14** (60.8 mg). Fraction IV was subjected to ODS silica gel column chromatography eluted with MeCN–H_2O (2 : 1; 1 : 1; 1 : 2; 1 : 3) and Sephadex LH-20 column chromatography with MeOH to give **13** (18.8 mg).

Compound **4**: Amorphous solid, $[\alpha]_D^{26}$ −102.0° (c=0.10, MeOH). HR-ESI-MS m/z: 383.2058 $[M+H]^+$ (Calcd for $C_{20}H_{31}O_7$: 383.2070). UV (MeOH) λ_{max} (log ε): 216 (3.81). IR (film) cm^{-1}: 3446 (OH), 2971 (CH), 1760 and 1735 (C=O), 1659. ^{1}H-NMR ($CDCl_3$) δ: 6.19 (1H, d, J=3.2 Hz, H-13a), 5.54 (1H, ddd, J=11.4, 5.3, 2.6 Hz, H-8), 5.47 (1H, d, J=3.2 Hz, H-13b), 4.46 (1H, ddd, J=10.6, 6.9, 1.1 Hz, H-6), 4.06 (1H, dd, J=9.5, 8.6 Hz, H-1), 3.92 (1H, m, H-7), 3.10 (3H, s, OMe), 2.49 (1H, dd, J=14.5, 9.5 Hz, H-2β), 2.37 (1H, m, H-2′), 2.06 (1H, m, H-5β), 1.99 (1H, m, H-4), 1.97 (1H, dd, J=14.5, 5.4 Hz, H-9α), 1.81 (1H, dd, J=14.5, 8.6 Hz, H-2α), 1.72 (1H, br d, J=13.3 Hz, H-5α), 1.63 (1H, dd, J=14.5, 11.4 Hz, H-9β), 1.37 (3H, s, Me-14), 1.01 and 0.99 (each 3H, d, J=7.0 Hz, Me-3′ and Me-4′), 0.99 (3H, d, J=7.0 Hz, Me-15). ^{13}C-NMR: see Table 1.

Compound **10**: Amorphous solid, $[\alpha]_D^{26}$ −110.0° (c=0.10, MeOH). HR-ESI-MS m/z: 381.1947 $[M+H]^+$ (Calcd for $C_{20}H_{29}O_7$: 381.1913). UV (MeOH) λ_{max} (log ε): 213 (3.88). IR (film) cm^{-1}: 3446 (OH), 2971 (CH), 1760 and 1735 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 6.20 (1H, d, J=2.6 Hz, H-13a), 5.66 (1H, m, H-5), 5.59 (1H, ddd, J=11.2, 5.0, 4.6 Hz, H-8), 5.55 (1H, d, J=2.6 Hz, H-13b), 5.20 (1H, m, H-6), 4.16 (1H, m, H-7), 3.77 (1H, dd, J=11.1, 3.9 Hz, H-1), 3.11 (3H, s, OMe), 2.91 (1H, br d, J=11.3 Hz, H-2α), 2.34 (1H, m, H-2′), 2.31 (1H, m, H-2β), 1.82 (1H, dd, J=14.1, 5.0 Hz, H-9α), 1.70 (3H, t-like, J=1.8 Hz, Me-15), 1.66 (1H, dd, J=14.1, 11.2 Hz, H-9β), 1.44 (3H, s, Me-14), 1.00 and 0.97 (each 3H, d, J=7.2 Hz, Me-3′ and Me-4′). ^{13}C-NMR: see Table 1.

Compound **12**: Amorphous solid, $[\alpha]_D^{26}$ +44.6° (c=0.10, MeOH). HR-ESI-MS m/z: 367.1764 $[M+H]^+$ (Calcd for $C_{19}H_{27}O_7$: 367.1757). UV

(MeOH) λ_{max} (log ε): 211 (2.68). IR (film) cm^{-1}: 3450 (OH), 2971 (CH), 1743 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 6.17 (1H, d, J=3.4 Hz, H-13a), 5.54 (1H, m, H-8), 5.36 (1H, d, J=3.4 Hz, H-13b), 4.71 (1H, dd, J=9.7, 9.7 Hz, H-6), 3.71 (1H, m, H-7), 2.57 (1H, dd, J=16.4, 9.2 Hz, H-9α), 2.46 (1H, m, H-2α), 2.40 (1H, m, H-2′), 2.26 (1H, m, H-2β), 2.23 (1H, m, H-1), 2.20 (1H, m, H-5), 1.56 (1H, dd, J=16.4, 4.8 Hz, H-9β), 1.44 (3H, s, Me-14), 1.23 (3H, s, Me-15), 1.02 (3H×2, d, J=7.0 Hz, Me-3′ and Me-4′). ^{13}C-NMR: see Table 1.

HL-60 Cell Culture Assay HL-60 cells were maintained in the RPMI 1640 medium containing 10% FBS supplemented with L-glutamine, 100 units/ml penicillin G, and 100 μg/ml streptomycin sulfate. The leukemia cells were washed and resuspended in the above medium to 3×10^4 cells/ml, and 196 μl of this cell suspension was placed in each well of a 96-well flat-bottom 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 0.1 or 0.01—20 μM; 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 by an MTT assay procedure. After termination of the cell culture, 10 μl of 5 mg/ml MTT 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 formazan. An aliquot of 150 μl of the supernatant was removed from every well, and 175 μl of dimethyl sulfoxide (DMSO) 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}) was calculated from a dose–response curve.

Human Cancer Cell Line Panel Assay The system to evaluate samples for the cell growth inhibition profile was developed according to the method of the National Cancer Institute,[20] with modification. The cells were plated at proper density in 96-well plates in RPMI 1640 medium with 5% FBS and allowed to attach overnight. The cells were exposed to **9** for 48 h. Then, the cell growth was determined according to the sulforhodamine B assay.[21] The mean graph was drawn on the basis of a calculation using a set of GI_{50}.[21] The COMPARE computer algorithm was used to analyze the correlation between the mean graphs of two drugs.[22]

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