Cytotoxic Constituents of the Fruit Body of *Daedalea dickisii*

Kazuko Yoshikawa,* Kazuaki Kouso, Jyoji Takahashi, Akiko Matsuda, Mina Okazoe, Akemi Umeyama, and Shigenobu Arihara

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-Cho, Tokushima 770-8514, Japan

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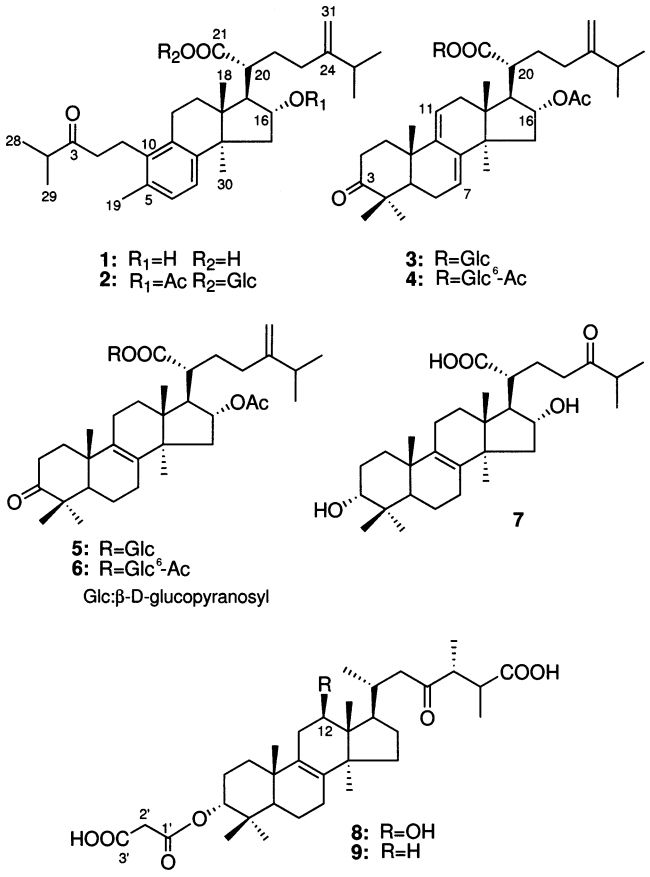
Three new lanostane triterpenoids (**1**, **7**, **8**) and five new lanostane triterpene glucosides (**2**−**6**) have been isolated from the fruit bodies of *Daedalea dickinsii*. Their structures were established primarily by NMR experiments, and their biological activity against HL-60 and HCT-15 cell lines was investigated. Compounds **3**−**6** induced internucleosomal DNA fragmentation characteristic of apoptotic cell death in the HL-60 cell line.

In the course of our program aimed at the discovery of biologically active compounds from fungi,[1] we have initiated the chemical study of *Daedalea dickinsii* (Berk. ex Cke.) Yasuda (Polyporaceae), which grows on dead trees in broad-leaved forests throughout Japan.[2,3] An earlier study of this fungus resulted in the isolation of lanostane triterpenes[4−7] and was also reported to have collagenase inhibitory and antibiotic activities.[8,9] The fruit bodies of *D. dickinsii* were extracted with 70% ethanol, and the EtOH extract after concentration was dissolved in ethyl acetate. The EtOAc-soluble portion showed moderate cytotoxic activity (IC_{50} 39.5 μg/mL) against human promyelocytic leukemia (HL-60) cell line. Fractionation of the EtOAc-soluble portion led to the isolation and characterization of three new lanostane triterpenes, which we designated as daedaleanic acids A (**1**), B (**7**), and C (**8**), and five new lanostanoid glucosides designated as daedaleasides A (**2**), B (**3**), C (**4**), D (**5**), and E (**6**), along with one known compound, carboxyacetylquercinic acid (**9**),[6] which was isolated from this fungi by Inoue et al. We describe here the isolation and structure elucidation of **1**−**8**, primarily by extensive NMR experiments. The cytotoxic activities of **1**−**9** against HL-60 and human colon tumor (HCT-15) cell lines are also described.



Results and Discussion

Daedaleanic acid A (**1**) gave a molecular ion peak at *m/z* 482.3399 $[M]^+$ (HREIMS), corresponding to a molecular formula of $C_{31}H_{46}O_4$ and requiring nine unsaturation equivalents. The IR spectrum of **1** showed absorptions due to hydroxy and carbonyl groups at 3400, 1710, 1070, and 1040 cm^{-1}. The ^1H NMR spectrum of **1** exhibited three singlet methyls at δ 1.05, 1.59, and 2.25, one oxygenated methine at δ 4.62 (dd, J = 8.0, 4.9 Hz), two coupled broad singlet protons at δ 4.85 and 4.99 characteristic of 24-methylenelanostane, and two aromatic protons at δ 6.96 (d, J = 7.7 Hz) and 7.07 (d, J = 7.7 Hz), which were mutually coupled. Further, two pairs of equivalent secondary methyl signals at δ 0.99 (6H, s) and 1.02 (6H, s) indicated the presence of two isopropyl groups. The 31 carbon signals observed in the ^{13}C NMR spectrum were sorted into seven methyl, seven methylene, five methine (one of which had an oxygen substituent, δ 76.6), two sp^3 quaternary carbons, a carboxylic acid and a ketone (δ 178.7 and 213.3), and four double bonds by DEPT experiment. The planar structure of **1** was constructed using the COSY and HMBC data. Namely, analysis of the COSY spectrum led to the partial structures depicted by the bold lines in Figure 1, which were connected on the basis of the long-range correlations observed in the HMBC spectrum. The C and D rings and the side chain, except for the A and B rings, in the lanostane skeleton were determined to have the hydroxy group at C-16, a carboxylic acid at C-21, and an exomethylene at C-24. Most important, the HMBC correlations from the two secondary methyls at δ 1.02 (6H, s, H_3-28/29) to a ketone at δ 213.3 (C-3) and from the remaining singlet methyl at δ 2.25 (H_3-19) to three aromatic carbons, C-5 [δ 133.1 (s)], C-6 [δ 128.1 (d)], and C-10 [δ 138.0 (s)], could construct the A and B rings, suggesting the cleavage of the A ring and the transference of the methyl group (C-19) to C-5 and aromatization (C-5−C-10) of the B ring. The relative configuration of **1** was established by ROESY experiment. Significant NOE correlations between H_3-18 (δ 1.05) and H-16 (δ 4.62), and

* To whom correspondence should be addressed. Tel: +81-88-622-9611. Fax: +81-88-655-3051. E-mail: yosikawa@ph.bunri-u.ac.jp.

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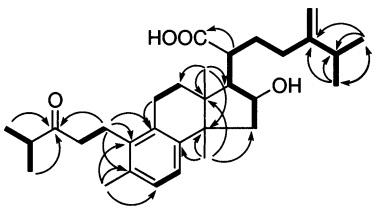

Figure 1. COSY (bold line) and selected HMBC (arrow line) of **1**.

H-20 [δ 2.94 (m)], and between H_3-30 (δ 1.59) and H-17 [δ 2.96 (m)] indicated α-orientations of OH-16 and H-17 and *R*-configuration at C-20. From the analysis of all of these data and from biosynthetic considerations, the structure of daedaleanic acid A was assigned as 16α-hydroxy-24-methylene-3-oxo-19(10 → 5)*abeo*-4,5-secolanosta-5,7,9-trien-21-oic acid.

Daedaleaside A (**2**) gave a $[M + Na]^+$ peak at *m/z* 709.4070 in its HRFABMS, which matched a formula of $C_{39}H_{58}O_{10}$. The IR spectrum of **2** showed absorptions for many hydroxy (3395 cm^{-1}) groups and acetoxy (1730 and 1235 cm^{-1}) function, suggesting the presence of a sugar moiety in **2**. The ^1H NMR spectrum of **2** exhibited one acetyl methyl at δ 2.16, one downfield-shifted oxymethine proton at δ 5.52 (dd, J = 8.0, 6.3 Hz), and one anomeric proton as a doublet (J = 8.4 Hz) at δ 6.45. Analysis of the COSY spectrum, starting from the anomer proton, disclosed the presence of a β-glucopyranosyl unit. Acid hydrolysis of **2** with 5% H_2SO_4 afforded D-glucose, which was confirmed by specific rotation using chiral detection by HPLC analysis.[10] Comparison of the ^1H NMR and ^{13}C NMR data of **2** and **1** revealed that **2** differed from **1** in the acylation shift at H-16 (δ 4. 62 → δ 5.52) and in the glycosylation shift[10] at C-21 (δ 178.7 → δ 175.2). Moreover, HMBC long-range correlations were observed between H-16 and the carbonyl carbon of the acetyl group at δ 170.4, and H-1 (δ 6.45) of glucose and C-21 (δ 175.2). The 16-*O*-Ac was confirmed as α-oriented from NOE between H-16 (δ 5.52) and H_3-18 (δ 1.01) in the ROESY experiment. The relative stereochemistry, including H-17 and H-20 of **2**, was also established on the basis of ROESY analysis described as **1**. Thus, the structure of daedaleaside A was assigned as 16α-acetoxy-24-methylene-3-oxo-19(10 → 5)*abeo*-4,5-secolanosta-5,7,9-trien-21-oic acid 21-*O*-β-D-glucopyranoside.

Daedaleaside B (**3**) had a quasi-molecular ion peak at *m/z* 709.3911 $[M + Na]^+$ in the HRFABMS and matched a molecular formula of $C_{39}H_{58}O_{10}$ requiring 11 unsaturations. The UV spectrum of **3** showed an absorption band at 230 nm (log ϵ 3.98), 237 (4.03), and 244 (3.88), indicating the presence of a heteroannular diene system. The ^1H NMR spectrum showed signals for two olefin protons at δ 5.49 (2H, d, J = 6.0 Hz), one acetyl methyl at δ 2.11, the characteristic exomethylene at δ 4.86 (brs) and 4.91 (brs), an oxygenated methine at δ 5.45 (dd, J = 8.2, 6.3 Hz), and one anomeric proton at δ 6.47 (d, J = 8.0 Hz). The ^{13}C NMR spectrum of **3** showed signals due to a conjugated diene group at δ 118.0 (d), 120.9 (d), 141.9 (s), 144.3 (s), an ester carbonyl at δ 174.9, a ketone at δ 215.0, and the β-glucopyranosyl moiety. Analysis of 2D NMR (COSY, HMQC, HMBC, and ROESY) data revealed the prosapogenin of **3**, 16α-acetoxy-24-methylene-3-oxolanosta-7,9(11)-dien-21-oic acid (16-*O*-acetylpolyporenic acid C).[11] The glucose moiety was determined to be at C-21 by observation of the glycosylation shift (−3.8 ppm) and HMBC correlation. Therefore, the structure of daedaleaside B was 16α-acetoxy-24-methylene-3-oxolanosta-7,9(11)-dien-21-oic acid 21-*O*-β-D-glucopyranoside.

The ^1H NMR spectrum for daedaleaside C (**4**) showed the presence of two acetyl methyls at δ 1.97 and 2.11. This was further supported by the mass spectral data, the molecular formula of $C_{41}H_{60}O_{11}$ from a $[M + Na]^+$ peak at *m/z* 751.4009 in HRFABMS, which indicated a difference of 42 amu (C_2H_2O) between **4** and **3**. The ^{13}C NMR spectrum of **4** was similar to that obtained for **3**, except for the downfield shift for C-6 of glucose in **4**: the H_2-6 signals of glucose in the ^1H NMR spectrum were shifted by +0.43 and +0.36 ppm due to the acylation shift on comparison with those of **3**. Thus, daedaleaside C was determined to be 16α-acetoxy-24-methylene-3-oxolanosta-7,9(11)-dien-21-oic acid 21-*O*-6-*O*-acetyl-β-D-glucopyranoside.

Table 1. ^{13}C NMR Spectroscopic Data (δ) for **1**−**8** (in pyridine-d_5, 150 MHz)

C no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	23.8	23.8	36.5	36.5	35.9	36.0	30.7	31.3
2	39.1	39.1	34.8	34.8	34.6	34.6	26.8	23.5
3	213.3	213.3	215.0	215.1	216.1	216.1	75.0	79.0
4	40.9	40.9	47.4	47.4	47.3	47.3	38.1	37.0
5	133.1	133.2	50.8	50.8	51.2	51.2	44.6	45.7
6	128.1	128.3	23.8	23.8	19.5	19.4	18.5	18.2
7	123.2	123.3	120.9	121.1	26.3	26.3	26.6	25.9
8	145.8	144.8	141.9	142.0	134.1	134.0	134.5	134.0
9	133.3	133.4	144.3	144.3	134.6	134.7	135.0	136.5
10	138.0	138.2	37.4	37.4	37.0	37.1	37.4	37.0
11	23.4	23.4	118.0	118.0	21.3	21.0	20.9	34.8
12	29. 7	29.3	35.8	35.8	29.1	29.1	29.7	71.8
13	45.4	45.0	44.5	44.5	45.8	45.7	46.2	52.3
14	49.4	49.0	48.9	48.4	48.4	48.4	48.8	49.7
15	45.1	42.2	41.4	41.3	40.7	40.7	43.6	31.6
16	76.6	79.3	78.9	78.9	78.9	78.6	76.4	25.9
17	57.3	53.8	53.8	53.7	53.7	53.7	56.9	50.7
18	18.0	17.7	17.5	17.4	17.6	17.4	17.8	10.8
19	19.7	19.7	21.9	22.3	18.5	18.5	19.3	19.0
20	48.5	46.8	46.9	46.8	47.0	46.9	47.8	30.2
21	178.7	175.2	174.9	175.1	175.1	175.0	178.6	22.9
22	31.5	31.1	31.0	30.9	31.2	30.9	26.8	48.5
23	33.1	31.8	31.8	31.5	31.9	31.6	38.6	213.4
24	156.0	155.7	155.7	155.9	155.8	155.9	213.8	49.7
25	34.1	34.4	34.4	34.5	34.5	34.6	40.9	41.9
26	21.9	22.0	22.0	21.9	22.0	22.0	18.3	178.4
27	22.0	22.0	22.0	21.9	22.0	22.0	18.4	14.8
28	18.2	18.3	25.5	25.7	26.3	26.4	29.0	27.9
29	18.3	18.4	22.3	21.9	21.3	21.3	22.5	21.9
30	29.2	28.7	25.8	25.5	24.9	24.9	25.3	24.1
31	107.0	106.9	106.8	107.0	106.8	107.0		13.6

Daedaleaside D (**5**) had the molecular formula of $C_{41}H_{60}O_{11}$ (HRFABMS), which differed from **3** by containing two additional hydrogen atoms. The ^{13}C NMR spectrum for **5** was also similar to that of **3**, except for the absence of a heteroannular diene system and the appearance of a tetrasubstituted double bond. The carbon signals due to the rings B and C of **5** were in good agreement with those of 16α-acetoxy-24-methylene-3-oxolanosta-8-en-21-oic acid.[11] Thus, the structure of daedaleaside D was assigned as 16α-acetoxy-24-methylene-3-oxolanosta-8-en-21-oic acid 21-*O*-β-D-glucopyranoside.

Daedaleaside E (**6**) had the molecular formula of $C_{41}H_{62}O_{11}$ by the HRFABMS, 42 amu (C_2H_2O) more than that of **5**. The ^{13}C NMR spectrum for **6** was similar to that of **5**, except for C-5 and C-6 in the β-glucopyranosyl moiety at C-21. The ^1H NMR and ^{13}C NMR data of the glucose unit in **6** were analogous to those of **4**, suggesting an acetyl group at the C-6 position. Thus, daedaleaside E was 16α-acetoxy-24-methylene-3-oxolanosta-8-en-21-oic acid 21-*O*-6-*O*-acetyl-β-D-glucopyranoside.

Daedaleanic acid B (**7**) had the molecular formula of $C_{30}H_{48}O_5$ (HRFABMS). The ^{13}C NMR spectrum for **7** was similar to that of tumulosic acid,[12] which had a β-hydroxy group at C-3 and α-hydroxy group at C-16, except for the

Table 2. ^{13}C NMR Spectroscopic Data (δ) for **2**−**6** and **8** (in pyridine-d_5, 150 MHz)

	2	**3**	**4**	**5**	**6**	**8**
acyl moiety	16-*O*-Ac	16-*O*-Ac	16-*O*-Ac	16-*O*-Ac	16-*O*-Ac	3-*O*-malonyl
	21.3	21.2	21.7	22.0	21.3	167.7
	170.5	170.4	170.4	170.4	170.4	43.0
			Glc-6-*O*-Ac		Glc-6-*O*-Ac	166.9
			20.7		20.7	
			170.7		170.7	
sugar moiety						
Glc-1	95.9	95.9	95.7	95.9	95.6	
2	74.0	73.9	73.7	73.9	73.7	
3	78.9	79.3	79.1	79.2	79.1	
4	71.2	71.3	71.0	71.3	71.0	
5	79.3	79.3	76.0	78.9	76.1	
6	62.5	62.5	64.2	62.5	64.3	

Table 3. Effects of the Isolated Compounds **1**−**6** and **9** on the Growth of HL-60 and HCT-15 Cells (IC_{50} μM)[a]

compound	HL-60	HCT-15
1	36.0	206.0
2	27.9	32.3
3	9.4	55.0
4	12.0	ND[b]
5	14.0	60.0
6	15.0	ND
9	45.2	155.2
etoposide	25.0	—[c]

[a] IC_{50} based on duplicate five points. [b] Not determined. [c] Not measured.

chemical shift for C-3 (δ 75.0 in **7**, δ 78.0 in tumulosic acid) and the additional ketone function (δ 213.8). The broad singlet proton at δ 3.61, which was correlated with the carbon at δ 75.0 in the HMQC experiment, showed NOEs between H_3-28/29 and was determined to be the β-hydrogen at C-3. The ketone group was determined to be at C-24 in the side chain by HMBC correlations from H_3-26/27. From the analysis of these data and from biosynthetic considerations, daedaleanic acid B was identified as 3α,16α-dihydroxy-24-oxolanost-8-en-21-oic acid.

The molecular formula, $C_{34}H_{52}O_8$, of daedaleanic acid C (**8**) was deduced from HRFABMS. The ^1H NMR and ^{13}C NMR data showed that **8** was related to carboxyacetylquercinic acid (**9**).[6] The main difference was a proton signal at δ 4.35 (dd, J = 7.7, 7.7 Hz), which was correlated with an oxymethine carbon at δ 71.8 in the HMQC experiment and was also assigned H-12 by HMBC experiment. The coupling constants for H-12 and the NOE observed between H-12 and H_3-30 (δ 0.88) validated the β-equatorial orientation of HO-12. Thus, the structure of daedaleanic acid C was assigned as **8**. The stereochemistry of C-25 was not determined.

Compounds **1**−**9** were evaluated for their cytotoxic activities against two human tumor cell lines (HL-60 and HCT-15) in a dose-dependent manner as determined by the MTT assay[13] (Table 3). Etoposide was used as positive control. Although the cytotoxicity of **2**, **3**, and **5** against HCT-15 was moderate (IC_{50} 32.3−60.0 μM), HL-60 was relatively sensitive to **3**−**6**, showing IC_{50} values ranging between 9.4 and 15.0 μM. We also examined whether these triterpenoids induced DNA fragmentation, which is considered the end of point of the apoptotic pathway. Actinomycin D (0.008 μM) was also used as positive control. DNA fragments formed by intranucleosomal hydrolysis of chromatin were evident after 8 h of treatment with **3**−**6** (35 μM) in the HL-60 cell line.

Experimental Section

General Experimental Procedures. Melting points were measured with a Yanagimoto micromelting point apparatus and were uncorrected. Optical rotations were taken on a JASCO DIP-360 polarimeter. UV spectra were recorded on a SHIMAZU UV-1650PC, IR spectra were recorded on a JASCO FT/IR-5300, and NMR spectra were recorded on a Varian UNITY 600 spectrometer in C_5D_5N using TMS as internal standard. NMR experiments included COSY, DEPT, HMQC, HMBC, and ROESY. Coupling constants (J values) are given in hertz. The FABMS was measured on a JEOL JMS-PX303 mass spectrometer. Column chromatography was carried out on silica gel (230−400 mesh, Merck). Analytical TLC was performed on precoated Merck F_{254} silica gel plates and visualized by spraying with 30% H_2SO_4. HPLC was carried out on a JASCO PU-1580 pump with a JASCO UV-970 detector and a CAPCELL PAK C18-AQ column (5 μm, 20 mm i.d. × 250 mm) (SHISEIDO).

Material. The fruit bodies of *D. dickinsii* were collected at Nagano, Japan, in autumn 2000. A voucher specimen (TB 2121) was deposited in the Herbarium of Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan.

Extraction and Isolation. The fresh fruit bodies (730 g) of *D. dickinsii* were extracted with 70% EtOH at room temperature for 6 weeks. The EtOH extract was partitioned between EtOAc and H_2O. The EtOAc soluble portion (50.0 g) was subjected to silica gel column chromatography with hexanes−EtOAc (30:1−0:10) to afford fractions 1−7. Fraction 2 (4.29 g) was passed through silica gel with hexanes−EtOAc (5:1−1:3) and purified by preparative HPLC (75−85% MeOH, flow rate 8 mL/min, length of gradient 150 min) to afford daedaleanic acid A (**1**, 9.7 mg). Fraction 3 (2.0 g) was purified by preparative HPLC (75−100% MeOH, flow rate 8 mL/min, length of gradient 180 min) to afford carboxyacetylquercinic acid (**9**, 83.8 mg). Fraction 4 (2.14 g) was passed through silica gel with hexanes−EtOAc (1:1) and purified by preparative HPLC (70−100% MeOH, flow rate 8 mL/min, length of gradient 180 min) to afford daedaleanic acid B (**7**, 25.3 mg). Fraction 5 (1.72 g) was passed through silica gel with $[(Me)_2CH]_2O$-EtOAc (1:1−2:8) and preparative HPLC (70−100% MeOH, flow rate 8 mL/min, length of gradient 180 min) to afford daedaleasides A (**2**, 18.6 mg), C (**4**, 9.0 mg), and E (**6**, 8.9 mg). Fraction 6 (1.96 g) was passed through silica gel $[(Me)_2CH]_2O$−EtOAc (10:1−1:1) and purified by preparative HPLC (70−100% MeOH, flow rate 8 mL/min, length of gradient 180 min) to afford daedaleasides B (**3**, 21.9 mg), D (**5**, 31.8 mg), and daedaleanic acid C (**8**, 145.0 mg).

Daedaleanic Acid A (1): amorphous powder; $[\alpha]^{25}_D$ +8.2° (c 0.4, MeOH); FT-IR (dry film) ν_{max} 3400, 1710, 1070, 1040 cm^{-1}; ^1H NMR (C_5D_5N) δ 0.99 (6H, d, J = 6.9 Hz, Me-26, 27), 1.02 (6H, d, J = 6.9 Hz, Me-28, 29), 1.05 (3H, s, Me-18), 1.59 (3H, s, Me-30), 2.25 (3H, s, Me-19), 2.94 (1H, m, H-20), 2.96 (1H, m, H-17), 4.62 (1H, dd, J = 8.0, 4.9 Hz, H-16), 4.85, 4.99 (each 1H, brs, H_2-31), 6.96 (1H, d, J = 7.7 Hz, H-7), 7.07 (1H, d, J = 7.7 Hz, H-6); ^{13}C NMR (C_5D_5N) see Table 1; HREIMS m/z 482.3399 (calcd for $C_{31}H_{46}O_4$, 482.3396).

Daedaleaside A (2): amorphous powder; $[\alpha]^{25}_D$ −1.9° (c 0.7, MeOH); FT-IR (dry film) ν_{max} 3395, 1730, 1235, 1040 cm^{-1}; ^1H NMR (C_5D_5N) δ 1.00 (6H, d, J = 6.9 Hz, Me-26, 27), 1.01

(3H, s, Me-18), 1.01 (6H, d, J = 6.9 Hz, Me-28, 29), 1.26 (3H, s, Me-30), 2.16 (3H, s, Ac), 2.23 (3H, s, Me-19), 2.98 (1H, dd, J = 11.3, 6.0 Hz, H-17), 3.02 (1H, ddd, J = 11.3, 10.8, 3.3 Hz, H-20), 4.82, 4.88 (each 1H, brs, H_2-31), 5.52 (1H, dd, J = 8.0, 6.3 Hz, H-16), 6.82 (1H, d, J = 7.7 Hz, H-7), 7.02 (1H, d, J = 7.7 Hz, H-6), 6.45 (1H, d, J = 8.4 Hz, H-1 of GLC); ^{13}C NMR (C_5D_5N) see Tables 1 and 2; FABMS *m/z* [M − H]$^-$ 685; HRFABMS *m/z* 709.4070 (calcd for $C_{39}H_{58}O_{10}$ + Na, 709.4046).

Daedaleaside B (3): colorless needles, mp 210−211°C; $[\alpha]^{25}_D$ +3.7° (*c* 1.0, MeOH); UV (MeOH) λ max (log ϵ) 230 (3.98), 237 (4.03), 244 (3.88); FT-IR (film) ν_{max} 3400, 1720, 1700, 1030 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 0.96 (3H, s, Me-18), 1.01 (6H, d, J = 6.9 Hz, Me-26, 27), 1.03 (3H, s, Me-29), 1.05 (3H, s, Me-19), 1.09 (3H, s, Me-28), 1.14 (3H, s, Me-30), 2.11 (3H, s, Ac), 2.90 (1H, dd, J = 11.3, 6.3 Hz, H-17), 3.01 (1H, ddd, J = 11.3, 11.3, 3.6 Hz, H-20), 4.82, 4.89 (each 1H, brs, H_2-31), 5.45 (1H, dd, J = 8.2, 6.3 Hz, H-16), 5.49 (2H, d, J = 6.0 Hz, H-7, 11), 6.47 (1H, d, J = 8.2 Hz, H-1 of GLC); ^{13}C NMR (C_5D_5N) see Tables 1 and 2; HRFABMS *m/z* 709.3911 (calcd for $C_{39}H_{58}O_{10}$ + Na, 709.3928).

Daedaleaside C (4): amorphous powder; $[\alpha]^{25}_D$ −20.1° (*c* 0.5, MeOH); UV (MeOH) λ max (log ϵ) 232 (4.06), 238 (4.24), 246 (3.87); FT-IR (film) ν_{max} 3490, 1740, 1700, 1245, 1075 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 0.95 (3H, s, Me-18), 1.04 (6H, d, J = 6.6 Hz, Me-26, 27), 1.06 (3H, s, Me-29), 1.08 (3H, s, Me-19), 1.09 (3H, s, Me-28), 1.18 (3H, s, Me-30), 1.97 (3H, s, Ac of H_2-6 of GLC), 2.11 (3H, s, Ac), 2.90 (1H, dd, J = 11.5, 6.6 Hz, H-17), 2.96 (1H, ddd, J = 11.5, 10.4, 3.3 Hz, H-20), 4.76 (1H, dd, J = 11.5, 4.6 Hz, H_2-6 of GLC), 4.89 (1H, dd, J = 11.5, 2.2 Hz, H_2-6 of GLC), 4.86, 4.91 (each 1H, brs, H_2-31), 5.44 (1H, dd, J = 8.0, 6.6 Hz, H-16), 5.50 (1H, d, J = 6.0 Hz, H-11), 5.52 (1H, d, J = 6.0 Hz, H-7), 6.38 (1H, d, J = 8.2 Hz, H-1 of GLC); ^{13}C NMR (C_5D_5N) see Tables 1 and 2; HRFABMS *m/z* 751.4009 (calcd for $C_{41}H_{60}O_{11}$ + Na, 751.4034).

Daedaleaside D (5): colorless needles, mp 217.2−219 °C; $[\alpha]^{25}_D$ +7.3° (*c* 1.4, MeOH); FT-IR (film) ν_{max} 3400, 1735, 1700, 1245 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 0.93 (3H, s, Me-18), 0.99 (3H, s, Me-29), 1.00 (6H, d, J = 7.1 Hz, Me-26, 27), 1.08 (3H, s, Me-19), 1.10 (3H, s, Me-28), 1.15 (3H, s, Me-30), 2.12 (3H, s, Ac), 2.83 (1H, dd, J = 11.5, 6.3 Hz, H-17), 2.99 (1H, ddd, J = 11.5, 10.4, 3.3 Hz, H-20), 4.81, 4.87 (each 1H, brs, H_2-31), 5.44 (1H, dd, J = 7.7, 6.3 Hz, H-16), 6.43 (1H, d, J = 8.0 Hz, H-1 of GLC); ^{13}C NMR (C_5D_5N) see Table 1; HRFABMS *m/z* 711.4085 (calcd for $C_{39}H_{60}O_{10}$ + Na, 711.4084).

Daedaleaside E (6): amorphous powder; $[\alpha]^{25}_D$ +7.5° (*c* 0.3, MeOH); FT-IR (film) ν_{max} 3410, 1720, 1700, 1030 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 0.95 (3H, s, Me-18), 1.03 (3H, s, Me-29), 1.05 (6H, d, J = 6.9 Hz, Me-26, 27), 1.06 (3H, s, Me-19), 1.11 (3H, s, Me-28), 1.18 (3H, s, Me-30), 2.08 (3H, s, Ac of H-6 of GLC), 2.11 (3H, s, Ac), 2.90 (1H, dd, J = 11.5, 6.6 Hz H-17), 2.96 (1H, ddd, J = 11.5, 10.4, 3.3 Hz, H-20), 4.76 (1H, dd, J = 11.5, 4.6 Hz, H_2-6 of GLC), 4.89 (1H, dd, J = 11.5, 2.2 Hz, H_2-6 of GLC), 4.86, 4.91 (each 1H, brs, H_2-31), 5.44 (1H, dd, J = 8.0, 6.6 Hz, H-16), 6.36 (1H, d, J = 8.2 Hz, H-1 of GLC); ^{13}C NMR (C_5D_5N) see Tables 1 and 2; HRFABMS *m/z* 753.4195 (calcd for $C_{41}H_{62}O_{11}$ + Na, 753.4191).

Daedaleanic Acid B (7): colorless needles, mp 188−190.1 °C; $[\alpha]^{25}_D$ +16.6° (*c* 0.4, CH_3Cl/MeOH = 1:1); FT-IR (film) ν_{max} 3410, 1705, 1020 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 0.92 (3H, s, Me-29), 0.99 (3H, d, J = 6.9 Hz, H-27), 1.02 (3H, s, Me-19), 1.03 (3H, d, J = 6.9 Hz, H-26), 1.12 (3H, s, Me-18), 1.21 (3H, s, Me-28), 1.31 (3H, s, Me-30), 2.78 (1H, dd, J = 11.3, 6.1 Hz H-17), 2.94 (1H, ddd, J = 11.3, 11.3, 2.7 Hz, H-20), 3.61 (1H, brs, H-3), 4.59 (1H, dd, J = 8.1, 6.1 Hz, H-16); ^{13}C NMR (C_5D_5N) see Table 1; HRFABMS *m/z* [M − H]$^-$ 487.3430 (calcd for $C_{30}H_{47}O_5$, 487.3423).

Daedaleanic Acid C (8): amorphous powder; $[\alpha]^{25}_D$ +2.6° (*c* 2.3, MeOH); FT-IR (film) ν_{max} 3400, 1730, 1030 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 0.86 (3H, s, Me-29), 0.88 (3H, s, Me-30), 0.98 (3H, s, Me-19), 1.01 (3H, s, Me-18), 1.05 (3H, s, Me-28), 1.14 (3H, d, J = 6.9 Hz, Me-31), 1.33 (3H, d, J = 7.1 Hz, Me-27), 1.33 (3H, d, J = 6.3 Hz, Me-21), 2.72 (1H, m, H-17), 2.94 (1H, m, H-20), 3.72 (2H, brs, H_2-2 of malonyl), 4.35 (1H, dd, J = 7.7, 7.7 Hz, H-12), 4.97 (1H, brs, H-3); ^{13}C NMR (C_5D_5N) see Tables 1 and 2; FABMS *m/z* [M − H]$^-$ 587; HRFABMS *m/z* 611.3578 (calcd for $C_{34}H_{52}O_8$ + Na, 611.3560).

Acid Hydrolysis of Daedaleaside A (2). A solution of **2** (3 mg) in 5% H_2SO_4−dioxane (1:1) was heated at 100 °C for 2 h. The reaction mixture was diluted with H_2O and then neutralized with Amberlite IRA-35 and evaporated in vacuo to dryness. The identification and the D or L configuration of sugars were determined by using RI detection (Waters 410) and chiral detection (Shodex OR-1) by HPLC [Shodex RSpak NH_2P-50 4E, CH_3CN−H_2O−H_3PO_4 (95:5:1), 1 mL/min, 47 °C] by comparison with an authentic sugar (10 mmol each of D-GLC and L-GLC). The sugar portion gave the peak of D-(+)-GLC at 20.7 min.

Acid Hydrolysis of Daedaleasides B (3)−E (6). A solution of each compound **3**−**6** (each 3−4 mg) was carried out in the same manner as described for **2** to give the peak of D-(+)-GLC at 20.7 min.

Cytotoxicity Assay. The MTT Cell Growth Assay Kit (Chemicon International Inc., Temecula, CA) was used in this assay. Cells were maintained in the RPMI-1640 medium (Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (ICN Biomedicals Inc., Solon, OH) in a humidified atmosphere of 5% CO_2 at 37 °C throughout the study. Cells (90 μL) at a density of 5 × 10^5 cells/mL in the exponential growth phase were plated in 96-well flat-bottomed microplates with various drug concentrations (10 μL). After 24 h (HL-60) or 48 h (HCT-15), 10 μL of 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution was added to each well. After a further 4 h of incubation, 100 μL of 2-propanol with 0.04 N HCl solution was added to each well, and the formazen crystals in each well were dissolved by stirring with a pipet. The optical density measurements were made using a microplate reader (BIO-RAD Co. Ltd., Tokyo, Japan) at 570 nm.

Determination of DNA Fragmentation. HL-60 cells were plated at 5 × 10^5 cells/mL medium/well into each of six well plates. After 1 h of growth, cells were treated with compounds for 8 h. The cells of each well were harvested and washed once with PBS(−). DNA of the cells was extracted by a Quick Apoptotic DNA Ladder Detection Kit (BioVision, Mountain View, CA). Extracted DNA was dissolved in Tris-EDTA buffer. Electrophoresis was carried out with tris-borate-EDTA as the running buffer on 2% agarose gel containing 0.5 μg/mL ethidium bromide. DNA in the gel was visualized and photographed under UV light.

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