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BRUCEANOLS D, E, AND F. THREE NEW CYTOTOXIC QUASSINOIDS FROM *BRUCEA ANTIDYSENTERICA*

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ABSTRACT.—Three new quassinoids, bruceanols D [**1**], E [**2**], and F [**3**], were isolated from *Brucea antidysenterica*, and their structures were elucidated by spectral evidence and chemical transformation. All of these compounds exhibited cytotoxicity against five human tumor cell lines, malignant melanoma (RPMI-7951), lung carcinoma (A-549), ileocecal adenocarcinoma (HCT-8), epidermoid carcinoma of the nasopharynx (KB), and medulloblastoma (TE-671), and against murine lymphocytic leukemia (P-388).

Previously we reported the isolation and structural elucidation of six new antileukemic quassinoids, bruceantinosides A, B (1), and C (2) and bruceanols A, B (3), and C (4); three known compounds, yadanziosides G, N (2), and M (5); cytotoxic antileukemic alkaloids (6,7); and three new degradation products, bruceanic acids B, C, and D (8), from the stems of *Brucea antidysenterica* Mill. (Simaroubaceae). We now describe the isolation and characterization of three new quassinoids, which have been given the trivial names bruceanol D [**1**], E [**2**], and F [**3**]. Three known quassinoids, yadanzioside N [**4**] (2), bruceantin [**5**] (10), and isobruceine B [**6**] (3), which we also isolated from this plant, were useful in structural elucidation of the new compounds.

RESULTS AND DISCUSSION

Bruceanol D [**1**] was obtained as a colorless amorphous solid. Its ir spectrum showed the presence of a hydroxyl group (3450 cm^{-1}), δ-lactone and ester groups (1740 cm^{-1}), and α,β-unsaturated ester (1720 cm^{-1}), and an α,β-unsaturated carbonyl group (1670 and 1650 cm^{-1}). The uv spectrum of **1** exhibited maximum absorption at 223 nm due to a conjugated enone system. The fdms spectrum of **1** showed a molecular ion peak at *m/z* 548, suggesting a molecular formula of $C_{28}H_{36}O_{11}$, and a fragment ion peak due to the C-15 side chain at *m/z* 111 ($C_7H_{11}O$). The hrsims spectrum of **1** confirmed the molecular formula.

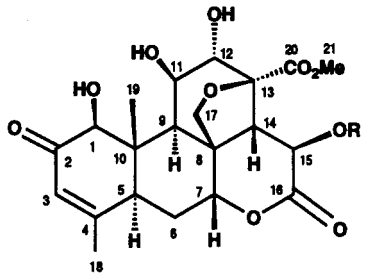
The proton signals of H-2′, H-4′, 3′-Me, and 4′-Me in **1** coincide with those of **5** (Table 1), and the carbon signals of C-1′ to C-7′ also coincide with those of **5** (Table 2). Together with the presence of the fragment ion peak at *m/z* 111, these data suggest that **1** and **5** have identical side chains. However, the remaining proton signals in **1** coincide with those of **6** (Table 1), and the carbon signals of C-1 to C-21 also coincide with those of **6** (Table 2). Thus, the structures of **1** and **6** differ only in the identity of the C-15 side chain. The stereochemistry of **1** was confirmed by the nOe correlations.

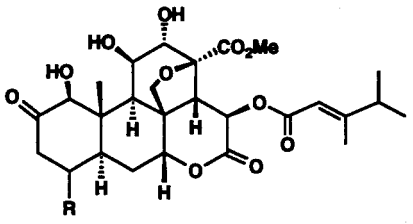
Bruceanol E [**2**] was obtained as colorless needles. Its ir, uv, and ms spectra were similar to those of **1**. The ir spectrum showed the presence of hydroxyl (3420 cm^{-1}), δ-lactone and ester (1750 cm^{-1}), and α,β-unsaturated ester (1720 cm^{-1}) groups; however, the α,β-unsaturated carbonyl in **1** was absent. The uv spectrum showed maximum absorption at 220 nm due to a conjugated enone system. The eims spectrum of **2** showed

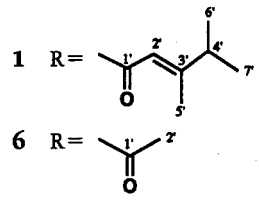
the same fragment peak found for **1** at *m/z* 111 ($C_7H_{11}O$) due to the C-15 side chain. This spectrum also showed a molecular ion peak at *m/z* 550 ($C_{28}H_{38}O_{11}$); this formula is 2H larger than that of **1** and was confirmed by the hreims spectrum.

The proton signals in **2** were nearly identical to those of **1**, except for the signals of H-3, H-4, H-5, and 4-Me (Table 1). The carbon signals in **1** and **2**, except for those of C-3 and C-4, were also quite similar (Table 2). These nmr data and the ms results indicate that the structure of **2** differs from that of **1** only at the C-3–C-4 double bond. The proton signals (δ_H 2.21, 2.49, and 1.68) of H-3α, H-3β, and H-4 in Table 1, and the carbon signals (δ_C 47.5 and 32.1) of C-3 and C-4 in Table 2 indicate that **2** has a saturated C-3–C-4 bond. Catalytic hydrogenation of **1** afforded two products, **7** and **8**; synthetic **7** was identical with isolated **2** as shown by comparison of their ^{1}H-nmr spectra and hplc retention times. Compounds **7** and **8** were found to be stereoisomers with different configurations at the C-4 position as shown by their nOe correlations. The nOe correlations of **7** are shown in Figure 1 (the nOe correlations of **2** were identical to those of **7**) and those of **8** were recognized between H-1α and H-3α, H-3α and H-3β, H-4α and H-4β, 4β-Me and 10β-Me, 10β-Me and H-6β, H-5α and H-9α, H-9α and H-11α, H-7β and CH_2-17.

Bruceanol F [**3**] was obtained as a colorless amorphous solid. Its ir spectrum was similar to that of **1** showing a hydroxyl group (3450 cm^{-1}), δ-lactone and ester groups (1740 cm^{-1}), an α,β-unsaturated ester (1720 cm^{-1}), and an α,β-unsaturated carbonyl

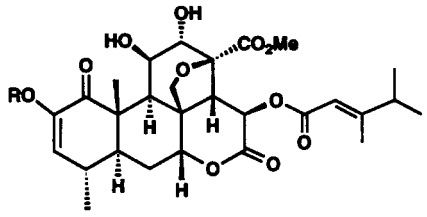


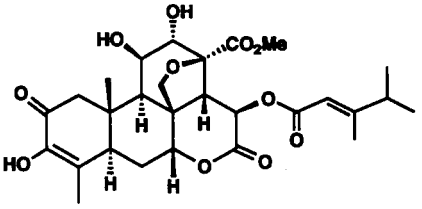




2=7 R=

8 R=





3 R=H

4 R=

5

TABLE 1. ^{1}H-nmr Spectra[a] of Compounds **1–8**.

Proton	Compound							
	1[b]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**6**[b]	**7**[c]	**8**[b]
H-1α	4.3 brs	2.23 brs	—	—	3.31 d (16)	4.31 s	4.25 brs	4.31 brs
H-1β	—	—	—	—	2.57 d (16)	—	—	—
H-3	6.12 s	—	5.87 s	6.10	—	6.14 s	—	—
H-3α	—	2.21 dd (13, 13)	—	—	—	—	2.24 dd (13, 13)	2.37 d (13)
H-3β	—	2.49 d (13)	—	—	—	—	2.50 d (13)	2.81 dd (13, 7)
H-4	—	1.68 m	2.30 m	2.23 m	—	—	1.68 m	2.10 m
H-5	3.06 brd (2)	1.96 brd (2)	2.15	1.87 brd (13)	3.10 brd	3.04 brd (2)	1.97 brd (2)	2.54 brd
H-6α	2.26 dd (14, 2)	2.13 dd (12, 2)	2.20 dd (13, 13)	2.02 d (13)	2.31 dd (14, 3)	2.36 dd (12, 2)	2.11 dd (13, 2)	2.00 d (13)
H-6β	1.76 ddd (14, 2, 2)	1.52 ddd (12, 12, 12)	1.63 dd (13, 13)	1.56 dd (13, 13)	1.77 ddd (14, 14, 3)	1.77 ddd (12, 2, 2)	1.52 dd (13, 13, 2)	1.81 d (13)
H-7	5.07	4.99 dd (2, 2)	4.95	4.90 brt	5.13	5.04 d (2)	5.00	5.00
H-9	2.89 d (5)	2.70 d (4)	2.99	2.95 brd	2.63 d (4)	2.88 d (4)	2.71 d (5)	2.68 d (5)
H-11	5.52 dd (5, 5)	5.34 d (4)	6.52 d (5)	6.20 brd	4.82 d (4)	5.51 brd (4)	5.36 d (5)	5.31 d (5)
H-12	5.11	5.14 brd	5.12	5.14	5.09	5.11 d (2)	5.17 brd	5.15
H-14	4.04 brd	4.01 brd	4.04 brd	4.01 br	4.04 brd	3.93 brd	4.05 brd	4.07 brd
H-15	6.70 brd	4.68 brd	4.90 brd	6.90 br	6.55 br	6.72 brd	6.77 brd	6.98 brd
H-17α	3.90 d (7)	3.87 d (7)	3.92 d (7.5)	3.90 d (7)	3.95 d (7)	3.90 d (7)	3.89 d (7)	3.89 d (7)
H-17β	5.13 d (7)	4.98 d (7)	5.17 d (7.5)	5.12 d (7)	5.11 d (7)	5.13 d (7)	5.00	4.93 d (7)
H-2′	5.85 s	5.88 s	5.87 s	5.88 s	5.87 s	—	5.89 s	5.88 s
H-4′	2.14 m	2.14 m	2.14 m	2.12 m	2.14 m	—	2.16 m	2.14 m
4-Me	1.72 s	0.84 d (6)	0.98 d (7)	0.87 d (6)	1.96 s	1.74 s	0.84 d (6)	0.85 d (6)
10-Me	1.44 s	1.35 s	1.91 s	1.87 s	1.65 s	1.44 s	1.36 s	1.48 s
3′-Me	2.15 s	2.17 s	2.17 s	2.17 s	2.17 s	—	2.18 s	2.19 s
4′-Me	0.83 d (7.5)	0.85 d (7.5)	0.85 d (7)	0.85 d (7)	0.85 d (7)	—	0.85 d (6)	0.85 d (6)
20-OMe	3.74 s	3.75 s	3.76 s	3.77 s	3.79 s	3.77 s	3.76 s	3.76 s
15-OAc	—	—	—	—	—	2.11 s	—	—
H-1″	—	—	—	5.46 d (6)	—	—	—	—
H-2″	—	—	—	4.25 m	—	—	—	—
H-3″	—	—	—	4.29 m	—	—	—	—
H-4″	—	—	—	4.29 m	—	—	—	—
H-5″	—	—	—	4.01 m	—	—	—	—
H-6″	—	—	—	4.34 d (12)	—	—	—	—

[a]Values are in ppm.
[b]500 MHz.
[c]270 MHz. The coupling constants (*J* values) in parentheses are in Hz.

TABLE 2. ^{13}C-nmr Spectra[a] of Compounds **1–6**.

Carbon	Compound					
	1	**2**	**3**	**4**	**5**	**6**
C-1	83.1 (CH)	83.9 (CH)	201.5 (C=O)	199.8 (C=O)	50.2 (CH_2)	82.9 (CH)
C-2	198.4 (C=O)	209.5 (C=O)	146.3 (C)	146.4 (C)	193.1 (C=O)	198.4 (C=O)
C-3	125.2 (CH)	47.5 (CH_2)	120.8 (CH)	124.9 (CH)	146.1 (C)	125.2 (CH)
C-4	163.0 (C)	32.1 (CH)	31.0 (CH)	31.5 (CH)	128.4 (C)	162.9 (C)
C-5	43.8 (CH)[b]	44.7 (CH)[b]	44.5 (CH)[b]	44.1 (CH)[b]	42.5 (CH)[b]	43.7 (CH)[b]
C-6	28.5 (CH_2)	29.5 (CH_2)	28.8 (CH_2)	28.7 (CH_2)	29.6 (CH_2)	28.4 (CH_2)
C-7	83.9 (CH)	83.8 (CH)	83.2 (CH)	83.0 (CH)	83.7 (CH)	83.6 (CH)
C-8	48.4 (C)	48.7 (C)	48.4 (C)	48.9 (C)	46.1 (C)	48.4 (C)
C-9	42.7 (CH)[b]	43.2 (CH)[b]	37.2 (CH)[b]	36.9 (CH)[b]	42.1 (CH)[b]	42.8 (CH)[b]
C-10	46.5 (C)	47.0 (C)	47.0 (C)	46.7 (C)	41.4 (C)	46.5 (C)
C-11	75.5 (CH)	75.7 (CH)	75.3 (CH)	75.1 (CH)	73.1 (CH)	75.4 (CH)
C-12	75.9 (CH)	76.0 (CH)	76.5 (CH)	76.3 (CH)	75.8 (CH)	75.9 (CH)
C-13	82.6 (C)	82.5 (C)	83.0 (C)	83.0 (C)	82.7 (C)	82.7 (C)
C-14	50.0	50.5	50.7	51.0	50.1	50.5
C-15	68.6	68.5	68.9	68.9	68.4	69.0
C-16	167.4 (C=O)	167.1 (C=O)	167.3 (C=O)	167.4 (C=O)	167.4 (C=O)	168.1 (C=O)
C-17	78.6 (CH_2)	73.2 (CH_2)	73.8 (CH_2)	73.7 (CH_2)	73.8 (CH_2)	73.5 (CH_2)
C-18	22.2 (Me)	19.8 (Me)	15.0 (Me)	18.9 (Me)	13.4 (Me)	22.2 (Me)
C-19	11.5 (Me)	12.6 (Me)	19.5 (Me)	14.6 (Me)	15.8 (Me)	11.4 (Me)
C-20	171.3 (C=O)	171.3 (C=O)	171.3 (C=O)	171.2 (C=O)	171.3 (C=O)	171.4 (C=O)
C-21	52.4 (Me)	52.3 (Me)	52.4 (Me)	52.4 (Me)	52.4 (Me)	52.4 (Me)
C-1′	166.0 (C=O)	166.2 (C=O)	166.0 (C=O)	166.1 (C=O)	165.9 (C=O)	179.0 (C=O)
C-2′	113.5 (CH)	113.5 (CH)	113.6 (CH) 1	113.5 (CH)	113.4 (CH)	20.7 (Me)
C-3′	168.4 (C)	168.4 (C)	168.4 (C)	168.3 (C)	168.4 (C)	—
C-4′	38.2 (CH)	38.1 (CH)	38.2 (CH)	38.2 (CH)	38.2 (CH)	—
C-5′	16.7 (Me)	16.7 (Me)	16.7 (Me)	16.8 (Me)	16.7 (Me)	—
C-6′	20.7 (Me)	20.7 (Me)	20.7 (Me)	20.7 (Me)	20.7 (Me)	—
C-7′	20.7 (Me)	20.7 (Me)	20.7 (Me)	20.7 (Me)	20.7 (Me)	—
C-1″	—	—	—	100.7 (CH)	—	—
C-2″	—	—	—	74.6 (CH)	—	—
C-3″	—	—	—	78.6 (CH)	—	—
C-4″	—	—	—	71.4 (CH)	—	—
C-5″	—	—	—	79.0 (CH)	—	—
C-6″	—	—	—	62.4 (CH_2)	—	—

[a]Values are in ppm.
[b]Interchangeable.

group (1680 and 1640 cm^{-1}). The uv spectrum of **3** exhibited maximum absorption at 220 and 265 nm due to a conjugated enone system. The cims spectrum of **3** showed a peak at *m/z* 549 $[M+H]^+$ and a fragment ion peak due to the C-15 side chain at *m/z* 111 ($C_7H_{11}O$). This same fragment ion peak was also observed in the eims spectrum of **3**

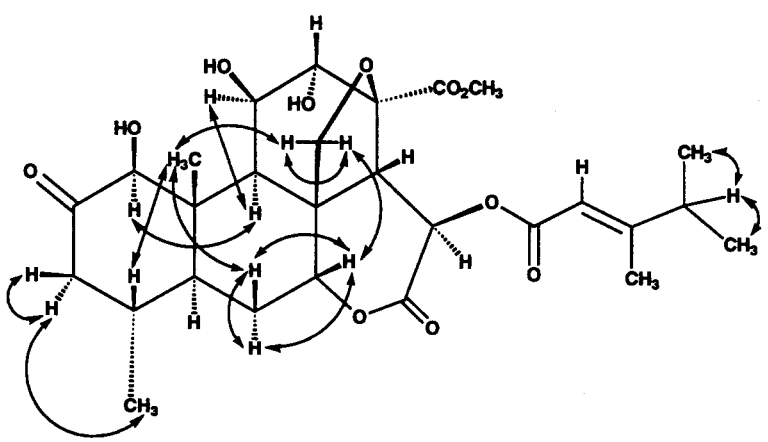


FIGURE 1. NOe correlations of compound **7**.

together with a peak at *m/z* 530 $[M-H_2O]^+$. From these mass spectra and an hrsims spectrum of **3**, the molecular formula was determined to be $C_{28}H_{36}O_{11}$.

Except for the absence of the signals for the glucose moiety, the proton and carbon signals of **3** coincide with those of **4** as shown in Table 1 and 2. Thus, **3** is the aglycone of **4**. Acid hydrolysis of **4** afforded of colorless amorphous solid; its ^{1}H- and ^{13}C-nmr spectra coincided with those of isolated **3**. The stereochemistry of **3** was confirmed by the nOe correlations, which were recognized between H-4β and 10β-Me, 10β-Me and CH_2-17, CH_2-17 and H-7β, H-4β and 4α-Me, 4α-Me and H-6α, H-5α and H-9α, H-9α and H-11α. Sakaki *et al.* (9) also previously obtained **3** by hydrolysis of **4**; however, our isolation of **3** from *B. antidysenterica* is the first time this compound has been obtained from the plant extract.

These three new compounds were tested for in vitro cytotoxicity in five human tumor cell lines (KB, A-549, HCT-8, RPMI-7951, TE-671) and a murine lymphocytic leukemia (P-388). The results are shown in Table 3. All of the compounds showed significant activity in each of the cell lines tested.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on an MRK air-bath melting point apparatus and are uncorrected. Specific rotations were obtained on a JASCO-DIP-370 digital

TABLE 3. Cytotoxicity of Bruceanols D [**1**], E [**2**], and F [**3**] Against Various Tumor Cell Lines.

Compound	Cell Line (ED_{50}, μg/ml)					
	KB	A-549	HCT-8	P-388	TE-671	RPMI-7951
1	0.08	0.55	0.09	0.09	0.08	0.09
2	0.55	3.75	0.37	0.57	0.12	0.11
3	0.43	0.55	0.13	0.36	0.09	0.09

polarimeter (L=0.5 dm). Ir and uv spectra were recorded on a JASCO IR-810 spectrometer and Hitachi 320-S spectrometer, respectively. ^{1}H- and ^{13}C-nmr spectra were determined on a Varian VXR-500, a JASCO GSX-500, or a JASCO GSX-270 in C_5D_5N using TMS as an internal standard. Mass spectra were recorded on a Hitachi M80 instrument. Hrsims spectra were obtained in a mixture of glycerol and thioglycerol. Si gel (Merck, type 60, 70–230 mesh) was used for cc. Precoated Si gel plates (Merck, $60F_{254}$) of 0.25 mm thickness were used for analytical tlc, and plates of 1 mm and 2 mm thickness were used for preparative tlc. Components were detected on tlc using a uv lamp. Low pressure liquid chromatography using a Kusano Lober column (ODS) and a mixed solvent of MeOH-H_2O (1:1) was carried out for preparative purposes before performing preparative tlc and hplc. Analytical hplc was performed on a Tosoh liquid chromatograph equipped with a uv detector at 254 nm and a reversed-phase column (TSK-gel ODS-80T_M) using mixed solvent of MeOH-H_2O (55:45, 50:50, 45:55, and 40:60). Preparative hplc was carried out on Tosoh, Waters, and/or Gilson liquid chromatographs equipped with a reversed-phase column (Dynamax-60A and/or Lichrosorb RP-18) at 254 nm using the same solvents as for analytical hplc.

CHROMATOGRAPHY OF THE $CHCl_3$ FRACTION.—The crude $CHCl_3$ fraction (Code no. BA-d2, 266 g), which was part of the $CHCl_3$ extract of the ground wood of *B. antidysenterica* (1918 kg) reported previously (1), was subjected to cc on Si gel (3 kg, 10×90 cm) and eluted first with EtOAc-Et_2O (1:1) to yield 10 fractions. The seventh fraction contained dehydrobruceantin as the major component and four minor components including bruceantin as shown by hplc analysis [MeOH-H_2O (1:1)]. This fraction gave a brown gum (35.7 g) after evaporation of solvent.

ISOLATION OF BRUCEANOLS D [**1**], E [**2**], AND F [**3**] AND BRUCEANTIN [**5**].—The brown gum (35.7 g) was subjected to low pressure lc using a Kusano Lober column (ODS) and a mixed solvent of MeOH-H_2O (1:1) to afford 29 fractions. Fractions 11–21 (9.16 g) contained three new compounds as shown by tlc [EtOAc-Et_2O (1:1)] and hplc analyses. These fractions were subjected to preparative tlc followed by repeated hplc to afford bruceanol D [**1**] (51.2 mg, 0.0000026%), bruceanol E [**2**], (9.4 mg, 0.00000049%), bruceanol F [**3**] (43.4 mg, 0.0000023%), and bruceantin [**5**] (10) (56.4 mg, 0.0000029%).

Bruceanol D [**1**].—Colorless amorphous solid: mp 140–142°; $[\alpha]^{22}D$ +64.7° (*c*=0.068, EtOH): uv λ max (EtOH) 223 (ε 15500) nm; ir (KBr) 3450 (OH), 1740 (ester and δ-lactone C=O), 1720, (α,β-unsaturated ester C=O), 1670 (α,β-unsaturated C=O), 1650 (C=C) cm^{-1}; ^1H nmr see Table 1: ^{13}C nmr see Table 2: fdms *m/z* $[M+1]^+$ 549 (100%), $[M]^+$ 548 (27%), $[C_7H_{11}O]^+$ 111 (side chain) (32%); hrsims *m/z* $[M+1]^+$ 549.2316 (calcd for $C_{28}H_{37}O_{11}$, 549.2325).

Bruceanol E [**2**].—Colorless needles: mp 260–262°; $[\alpha]^{27}D$ +48.8° (*c*=0.086, EtOH); uv λ max (EtOH) 220 (ε 13800) nm; ir (KBr) 3420 (OH), 1740 (ester and δ-lactone C=O), 1720 (α,β-unsaturated ester C=O), 1640 (C=C) cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* $[M]^+$ 550 (10%), $[M-H_2O]^+$ 532 (3%), $[C_7H_{11}O]^+$ 111 (side chain) (100%); hrsims *m/z* $[M]^+$ 550.2414 (calcd for $C_{28}H_{38}O_{11}$, 550.2411).

CATALYTIC HYDROGENATION OF **1**.—Compound **1** (17.9 mg, 0.33 mmol) was hydrogenated at room temperature and pressure in MeOH (20 ml) with Pd/C (5%) as the catalyst. After 24 h, hplc analysis of the reaction mixture showed two products **7** and **8**; both were isolated by preparative hplc (**7** 0.89 mg, 4.97%; **8** 3.79 mg, 21.1%).

Compound **7**.—Colorless needles: mp 260–262°; $[\alpha]^{27}D$ +8.77° (*c*=0.011, EtOH); uv λ max (EtOH) 220 (ε 16900) nm; ir (KBr) 3430 (OH), 1740 (ester and δ-lactone C=O), 1710 (α,β-unsaturated ester C=O), 1630 (C=C) cm^{-1}; ^1H nmr see Table 1; eims *m/z* $[M]^+$ 550 (6%), $[M-H_2O]^+$ 532 (3%), $[C_7H_{11}O]^+$ 111 (side chain) (100%).

Compound **8**.—Colorless needles: mp 134–136°; $[\alpha]^{27}D$ +20.8° (*c*=0.058, EtOH); uv λ max (EtOH) 220 (ε 12900) nm; ir (KBr) 3440 (OH), 1740 (ester and δ-lactone C=O), 1710 (α,β-unsaturated ester C=O), 1630 (C=C) cm^{-1}; ^1H nmr see Table 1; eims *m/z* $[M]^+$ 550 (5%), $[M-H_2O]^+$ 532 (3%), $[C_7H_{11}O]^+$ 111 (side chain) (100%).

Bruceanol F [**3**].—Colorless amorphous solid: mp 138–140°; $[\alpha]^{26}D$ +29.3° (*c*=0.041, EtOH); uv λ max (EtOH) 220 (ε 15100), 265 (4490) nm; ir (KBr) 3450 (OH), 1740 (ester and δ-lactone C=O), 1720 (α,β-unsaturated ester C=O), 1680 (α,β-unsaturated C=O), 1640 (C=C) cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* $[M-H_2O]^+$ 530 (1%), $[C_7H_{11}O]^+$ 111 (side chain) (100%); cims *m/z* $[M+1]^+$ 549 (4%), $[C_7H_{11}O]^+$ 111 (side chain) 100%; hrsims *m/z* $[M+1]^+$ 549.2303 (calcd for $C_{28}H_{37}O_{11}$, 549.2325).

ACID HYDROLYSIS OF YADANZIOSIDE N [**4**].—Compound **4** (99.8 mg, 0.14 mmol) was dissolved in a mixture of MeOH (20 ml) and 10% H_2SO_4 (10 ml), and the reaction mixture was stirred at 70°. After 4 h, compound **4** (R_f 0.40) had disappeared, and a hydrolyzed product (R_f 0.76) was observed on analytical tlc [$CHCl_3$-MeOH-H_2O (50:14:3)] The product was extracted with $CHCl_3$ and purified by preparative hplc [MeOH-H_2O (1:1)] to afford pure compound **3** (26.3 mg, 0.048 mmol, 34.3%) as a colorless amorphous solid. Its identity was confirmed by comparison of its physical constants (mp, $[\alpha]D$) and spectral data (uv, ir, ^1H and ^{13}C nmr, and eims) with those of the isolated compound **3**.

CYTOTOXICITY ASSAY.—The in vitro cytotoxicity assays were carried out according to procedures described in Geran *et al.* (11) and Ferguson *et al.* (12). The cell lines were human tumor cell lines, malignant melanoma (PRMI-7951), lung carcinoma (A-549), ileocecal adenocarcinoma (HCT-8), epidermoid carcinoma of the nasopharynx (KB), and medulloblastoma (TE-671), and a murine cell line, lymphocytic leukemia (P-388); the method has been reported previously in Lee *et al.* (13).

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