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ANTITUMOR AGENTS, 118.[1] THE ISOLATION AND CHARACTERIZATION OF BRUCEANIC ACID A, ITS METHYL ESTER, AND THE NEW BRUCEANIC ACIDS B, C, AND D, FROM *BRUCEA ANTIDYSENTERICA*

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ABSTRACT.—The known bruceanic acid A [**1**] and its methyl ester **2**, as well as the new bruceanic acids B [**3**], C [**4**], and D [**5**], have been isolated from *Brucea antidysenterica*. The structures of **1–5** were elucidated by spectral data. Compound **1** demonstrated cytotoxicity against KB and TE671 tumor cells. Compound **5** was cytotoxic against P-388 lymphocytic leukemia cells.

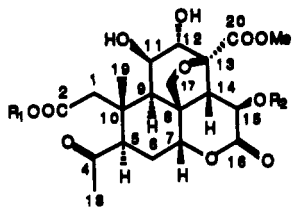
We reported previously the isolation and structural elucidation of six new antileukemic quassinoids, bruceantinosides A, B (1), and C (2) and bruceanols A, B (3), and C (4); three known compounds yadanziosides G, N (2), and M (5); and cytotoxic antileukemic alkaloids (6,7) from the stem of *Brucea antidysenterica* Mill. (Simaroubaceae). We now describe the isolation and characterization of the new compounds, bruceanic acids B [**3**], C [**4**], and D [**5**], along with the known compounds **1** and **2** from this same plant. Compound **1**, which is named as bruceanic acid A, was isolated previously from this same plant by Kupchan *et al.* (8). Compound **2**, prepared by methylation of **1** with CH_2N_2 by Kupchan *et al.* (8), was isolated for the first time from the $CHCl_3$ extract of *B. antidysenterica* by us. Compound **1** showed cytotoxicity against KB and TE671 tumor cells. Compound **5** was cytotoxic against P-388 leukemia cells.

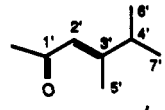
RESULTS AND DISCUSSION

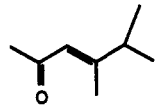
Bruceanic acid A [**1**], $C_{27}H_{36}O_{12}$, was obtained as a colorless amorphous solid. The ^{13}C-nmr signals of **1** were nearly identical with those of bruceantin [**6**] (9) except for the signals of C-1, C-2, C-4, C-5, C-9, C-18, and C-19 (Table 1). This evidence suggested that it was produced by a ring-A degradation of **6**. The ^1H-nmr (Table 2) and the eims spectra of **1** coincided very well with those of the degraded compound obtained previously by Kupchan *et al.* (8), and we named this compound **1** as bruceanic acid A, as it has never been named before.

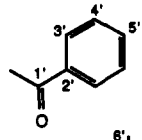
Further confirmation of the structural assignment of **1** for bruceanic acid A was achieved by spectral analysis. Analysis of the ^{13}C-nmr (Table 1) and ^1H-nmr (Table 2) spectra of **1** in ^1H-^1H COSY (Correlation Spectroscopy) and ^{13}C-^1H COSY suggested the presence of a geminal dimethyl (δ_H 1.05 and δ_C 20.7), an angular methyl (δ_H 1.39

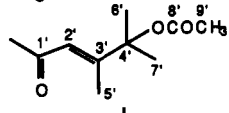
[1]For part 117, see D.J. Pan, C.Q. Hu, J.J. Chang, T.T. Lee, Y.P. Chen, H.Y. Hsu, D.R. McPhail, A.T. McPhail, and K.H. Lee, *Phytochemistry*, in press.

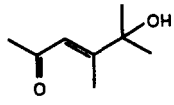


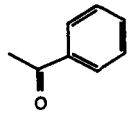
1 R_1=H, R_2= 

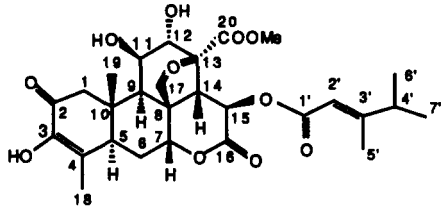
2 R_1=Me, R_2= 

3 R_1=H, R_2= 

4 R_1=H, R_2= 

5 R_1=H, R_2= 

7 R_1=Me, R_2= 



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and δ_C 19.2), a vinyl methyl (δ_H 2.10 and δ_C 16.8), an acetyl methyl (δ_H 2.21 and δ_C 31.8), a methoxyl (δ_H 3.71 and δ_C 52.6), three carboxyl (δ_C 165.8, 171.0, and 179.0), and a ketone (δ_C 212.5) group.

Each carbon signal, except for C-15 and the quaternary ones, was assigned based on the ^{13}C-^1H COSY spectral data. In the ^1H-^1H COSY spectrum of **1**, there are mutual relations between H-1α and H-1β, H-5 and H-6, H-9 and H-11, H-11 and H-12, H-6 and H-7, H-6 and 10-Me, H-6 and H-17α, H-6 and H-17β, H-7 and H-14, H-7 and H-17, H-17α and H-17β in the ring. In addition, there are also mutual relations in the side chain: H-2′ and 3′-Me, H-2′ and 4′-Me, 3′-Me and 4′-Me, and H-4′ and 4′-Me. This also suggested that **1** has the same structure as bruceantin [**6**] except for the difference in ring A. Moreover, the lack of an α,β-unsaturated carbonyl absorption in the ir and uv spectra, coupled with a molecular formula of $C_{27}H_{36}O_{12}$, which was

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

Carbon	Compound					
	1[b]	**2**[c]	**3**[c]	**4**[c]	**5**[c]	**6**[d]
C-1	45.5 t	41.3 t	45.1 t	44.8 t	45.0 t	48.7 t
C-2	179.0 s	172.1 s	177.3 s	176.9 s	177.2 s	192.2 s
C-3	—	—	—	—	—	144.2 s
C-4	212.5 s	209.9 s	211.3 s	209.9 s	211.5 s	127.9 s
C-5	38.2 d	37.9 d	36.6 d	38.2 d	40.2 d	41.2 d
C-6	29.0 t	29.5 t	29.8 t	29.8 t	29.5 t	29.2 t
C-7	75.7 d	76.0 d	76.7 d	76.7 d	76.5 d	75.9 d
C-8	45.0 s	46.1 s	46.7 s	46.5 s	46.2 s	45.5 s
C-9	36.1 d	36.2 d	36.5 d	36.2 d	36.0 d	41.9 d
C-10	39.7 s	40.3 s	40.5 s	40.5 s	40.2 s	41.2 s
C-11	71.7 d	73.4 d	73.5 d	73.6 d	73.4 d	71.1 d
C-12	81.4 d	82.4 d	83.7 d	83.4 d	82.7 d	82.4 d
C-13	82.7 s	82.5 s	83.1 s	82.9 s	83.1 s	81.4 s
C-14	49.8 d	49.6 d	50.2 d	50.0 d	49.8 d	51.7 d
C-15	66.5 d	68.1 d	69.6[e]	69.0[e]	68.6[e]	66.0 d
C-16	169.3 s	170.8 s	168.2 s	168.3 s	168.3 s	167.0 s
C-17	73.4 t	73.2 t	73.7 t	73.7 t	73.0 t	74.1 t
C-18	31.8 q	31.1 q	32.2 q	32.1 q	32.0 q	13.3 q
C-19	19.2 q	19.3 q	19.8 q	19.9 q	19.6 q	15.5 q
C-20	171.0 s	172.1 s	171.3 s	171.2 s	171.0 s	171.8 s
2-OMe	—	50.9 q	—	—	—	—
20-OMe	52.6 q	52.0 q	52.3 q	52.4 q	52.1 q	52.9 q
C-1′	165.8 s	167.2 s	165.8 s	166.0 s[f]	166.7 s	165.0 s
C-2′	112.1 d	113.1 d	130.4 s	113.5 d	112.6 d	111.8 d
C-3′	168.2 d	167.8 s	130.2 d	169.6 s	168.2 s	169.6 s
C-4′	38.2 d	37.9 d	128.8 d	82.4 s	73.2 s	38.4 d
C-5′	16.8 q	16.4 q	133.7 d	14.5 q	15.3 q	17.0 q
C-6′	20.7 q	20.4 q	—	25.7 q	28.5 q	20.8 q
C-7′	20.7 q	20.4 q	—	26.4 q	28.6 q	20.8 q
C-8′	—	—	—	163.7 s[f]	—	—
C-9′	—	—	—	21.4 q	—	—

[a]Signal assignments of **1**, **2**, and **5** were carried out by ^{13}C-^{1}H COSY spectra, and those of **3** and **4** were done by DEPT.

[b]Measured at 125.7 MHz in $CDCl_3$.

[c]Measured at 125.7 MHz in C_5D_5N.

[d]Measured at 22.5 MHz in $CDCl_3$. Data are from Sakaki *et al.* (9).

[e]Small and broad signal.

[f]Very small signal.

suggested by a pseudo molecular ion peak at *m/z* 575 $[M + Na]^+$ and a molecular ion at *m/z* 552 $[M]^+$ in the fdms, supported the structure of **1** for bruceanic acid A.

The methyl ester **2** of **1**, $C_{28}H_{38}O_{12}$, was obtained as a colorless amorphous solid from the same extract. The ^{13}C-nmr signals of **2** were nearly identical with those of **1**, except for a signal of 2-OMe (50.9, q) as seen in Table 1. The differences of the ^{1}H-nmr spectra (Table 2) between **1** and **2** might be due to the use of different solvents.

Detailed analysis of the ^{13}C-nmr (Table 1) and ^{1}H-nmr (Table 2) spectra of **2** in ^{1}H-^{1}H COSY and ^{13}C-^{1}H COSY spectra suggested the presence of a geminal dimethyl (δ_H 0.85 and δ_C 20.4), a vinyl methyl (δ_H 2.19 and δ_C 16.4), an acetyl methyl (δ_H 2.21 and δ_C 31.1), two methoxyl (δ_H 3.56, δ_H 3.76 and δ_C 50.9, δ_C 52.0), two carboxyl (δ_C 167.2, δ_C 171.1), and a ketone (δ_C 209.9) group. In the ^{1}H-^{1}H COSY spectrum of **2**, there are mutual relations in the ring: H-1α and H-1β, H-1α and H-5, H-5 and H-9,

TABLE 2. ^{1}H-nmr Spectra of Compounds **1–6**.

Proton	Compound					
	1[a]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**6**[c]
H-1α	2.35	2.90 d (17)	2.83 d (16)	3.10 d (16.5)	3.01 d (16.5)	[d]
H-1β	2.68 d (17)	3.25 d (17)	3.44	3.45 d (16.5)	3.44 d (16.5)	[d]
H-5	2.35	2.19				
H-6	1.93 2.03	2.3 [d]	2.0–2.4	2.2–2.4	2.2–2.4	[d]
H-7	4.26	5.1	5.35 [d]	5.14 br	5.12 br	[d]
H-9	2.80	3.22 d (4.5)		3.59 br	3.59 br	[d]
H-11	4.44	5.03	5.1	5.28 d (4.5)	5.27 d (4)	[d]
H-12	4.66	5.1	5.1	5.21	5.18	[d]
H-14	3.68	3.93 dd (12.5, 2.5)	4.13	4.30 d (12)	4.29 d (12)	[d]
H-15	6.16 brd	6.83 brd	6.45 br	6.90 br	6.90 br	6.21 d (13)
H-17α	4.66	5.13 d (7.5)	5.17	5.18 d (7.5)	5.18 d (7)	[d]
H-17β	3.68	3.97 d (7.5)	3.97 d (7)	3.98 d (7.5)	3.96 d (7)	[d]
4-Me	2.21 s	2.21 s	2.26 s	2.30 s	2.40 s	1.89 brs
10-Me	1.39 s	1.89 s	1.88 s	1.93 s	1.94 s	1.44 s
2-OMe	—	3.56 s	—	—	—	—
20-OMe	3.71 s	3.76 s	3.45 s	3.88 s	3.70 s	3.76 s
H-2′	5.64 s	5.87 s	—	6.08 s	6.75 s	5.71 s
H-3′	—	—	8.23 d (8)	—	—	—
H-4′	2.38	2.19 s	7.36 t (8)	—	—	[d]
H-5′	—	—	7.49 t (8)	—	—	—
3′-Me	2.10 s	2.19 s	—	2.26 s	2.30 s	2.18 s
4′-Me	1.05 d (7)	0.85 d (7)	—	1.34 s	1.40 s	1.12 d (6.5)
				1.42 s	1.42 s	
OAc	—	—	—	1.94 s	—	—

[a]Measured at 500 MHz in $CDCl_3$.
[b]Measured at 500 MHz in C_5D_5N.
[c]Measured at 100 MHz in $CDCl_3$. Data are from Okano *et al.* (1).
[d]Not measured.

H-9 and H-11, H-11 and H-15, H-6 and H-7, H-6 and 10-Me, H-6 and H-17β, and H-17α and H-17β. The following are mutual relations in the side chain: H-2′ and 3′-Me, and 3′-Me and 4′-Me. In addition to these data, the lack of an α,β-unsaturated carbonyl absorption in its ir and uv spectra, as well as the possession of a molecular formula of $C_{28}H_{38}O_{12}$, which was suggested by a molecular ion peak at *m/z* 566 in the eims, led to the assignment of **2** for the structure of bruceanic acid A methyl ester.

Bruceanic acid B [**3**] ($C_{27}H_{30}O_{12}$) was obtained as a colorless amorphous solid. The ^{13}C-nmr (Table 1) and ^{1}H-nmr (Table 2) spectra of **3** showed the presence of a benzoate moiety [δ_H 8.23 d (2H), δ_H 7.36 t (2H), and δ_H 7.49 (1H); δ_C 165.8 s (1C), δ_C 130.4 d (1C), δ_C 130.2 d (2C), δ_C 128.8 d (2C), and δ_C 133.7 d (1C)], and other signals which are identical with those of **1**, except for the signals corresponding to the C-15 side chain. Each carbon signal, except for the quaternary ones, was assigned based on the DEPT spectral data. Moreover, the following mutual relations are found in the ^{1}H-^{1}H COSY spectrum: H-1α and H-1β, H-6α and H-6β, H-7 and H-14, H-17α and H-17β, and H-3′ and H-4′. This also suggested that structures **1** and **3** are identical except for the difference in their side chains.

Further evidence for the structural assignment of **3** was obtained from the eims of the methyl ester **7** of **3**, which was prepared from **3** by CH_2N_2 methylation. The eims of **7** showed a molecular ion peak at *m/z* 560.1901 (calcd for $C_{28}H_{32}O_{12}$, 560.1910)

and other pertinent peaks at *m/z* 560 $[M]^+$, 105 $[C_6H_5CO]^+$, 77 $[C_6H_5]^+$, and 43 $[Ac]^+$.

Bruceanic acid C [**4**], $C_{29}H_{38}O_{14}$, was obtained as a colorless amorphous solid. The ^{13}C-nmr (Table 1) and ^1H-nmr (Table 2) spectra of **4** coincided with those of **1**, except for the portion of the C-15 side chain. The spectra also suggested the presence of 4′-Me (δ_H 1.34 s, δ_H 1.42 s, δ_C 25.7 q, and δ_C 26.4 q) and 4′-OAc (δ_H 1.96 s, δ_C 163.7 s, and δ_C 21.4 q) in the side chain. Except for the quaternary ones, each carbon signal was assigned based on the DEPT spectral data. Moreover, the following mutual relation found in the ^1H-^1H COSY spectrum of **4** substantiated this assignment: H-1α and H-1β, H-6β and H-7, H-9 and H-11, H-17α and H-17β, H-2′ and 3′-Me, and 3′-Me and 4′-Me. Thus, the structure of **4** is the same as that of **1**, except for the 4′-OAc group of the C-15 side chain. The pseudo molecular ion peak revealed at *m/z* 633 $[M + Na]^+$ in the fdms of **4** further confirmed its structural assignment.

Bruceanic acid D [**5**] ($C_{27}H_{36}O_{13}$, colorless amorphous solid) showed ^{13}C-nmr (Table 1) and ^1H-nmr (Table 2) spectra nearly identical with those of **1**, except for the signals of C-4′ (δ_C 73.2 s in **5** vs. 38.2 d in **1**) and 4′-Me (δ_H 1.40 s, δ_H 1.42 s, δ_C 28.5 q, and δ_C 28.6 q in **5**). These signals were nearly identical with those found in **4**, except for the difference of an acetoxyl group (δ_H 1.94, δ_C 163.7 s, and δ_C 21.4 q) as seen in **4**. The signal of δ_C 73.2 s in **5** suggested that its C-4′ has a hydroxyl group besides the geminal dimethyl moiety. Each carbon signal, except for the quaternary ones, was assigned based on its ^{13}C-^1H COSY spectral data. The mutual relations which were found in the ^1H-^1H COSY spectrum of **5** (H-1α and H-1β, H-6 and H-7, H-9 and H-11, H-14 and H-17β, H-17α and H-17β, H-2′ and 3′-Me, and 3′-Me and 4′-Me) further supported its structural assignment. Thus, the structures of **5**, **4**, and **1** are identical except for the differences in their side chains.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on an MRK air-bath type melting point apparatus and were uncorrected. Specific rotations were obtained on a YANAKO OR-50D polarimeter (L = 0.1 dm). Ir and uv spectra were recorded on a JASCO IR-810 spectrometer and a Hitachi 320-S spectrometer, respectively. ^1H- and ^{13}C-nmr spectra were determined on a VARIAN VXR-500 (500 MHz for ^1H nmr and 125.7 MHz for ^{13}C nmr) in $CDCl_3$ or C_5D_5N, using TMS as an internal standard, and the results are shown in Tables 2 and 1, respectively. The assignments of the carbon signals were made by DEPT (Distortionless Enhancement by Polarization Transfer) and/or ^{13}C-^1H COSY spectra. Mass spectra were recorded on a Hitachi M80 instrument. 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 the plates of 1 mm and 2 mm thickness for preparative tlc. Detection of components was made by using an uv lamp. Low pressure cc using a Kusano Lober column (ODS) was carried out for preparative purposes before doing preparative tlc and hplc. Analytical hplc was performed on a liquid chromatograph of Waters Associates (pump 6000A, uv monitor 441) and/or TOSOH (pump CCPE, uv monitor UV-8011) equipped with a reversed-phase column (TSK-gel ODS-80T_M) at 254 nm. Mixed solvents of MeOH-H_2O (1:1) and/or MeOH-H_2O-HOAc (50:50:1) were used as eluents. Preparative hplc was carried out on a liquid chromatograph of GILSON (pump 302, uv monitor 111B) equipped with a reversed-phase column (M & S PACK C_{18}A and/or TSK-gel ODS-80T_M) at 254 nm. The solvents used for the analytical hplc were also used for the preparative hplc.

ISOLATION OF BRUCEANIC ACID A [**1**] AND ITS METHYL ESTER **2**.—The crude $CHCl_3$ fraction (Code no. BA-d2, 266 g), which is a part of the extract of the ground wood of *B. antidysenterica* (1922 kg) reported previously (1), was subjected to cc on Si gel (2 kg), eluting at first with EtOAc-Et_2O (1:1) and then with $CHCl_3$-MeOH gradient (10:1, 10:2, 10:3, and 1:1) to yield 10 and 7 fractions, respectively.

Fractions 2 and 3 of the gradient elution, each of which showed four major spots including a tailing one on tlc, were combined (39.8 g) and subjected to a Kusano Lober column (ODS), eluting with MeOH-H_2O (1:1) to afford 25 fractions. Fractions 11–19 of the Lober column, which showed two spots on tlc [R_f 0.53 (tailing) and 0.75, $CHCl_3$-MeOH-H_2O (50:14:3), were combined to furnish a pale yellow gum (4.58 g). The gum was subjected to repeated preparative tlc and preparative hplc to give two colorless amorphous solids: compounds **1** (61.2 mg, 0.000000032%) and **2** (46.8 mg, 0.000000024%).

ISOLATION OF BRUCEANIC ACIDS B [**3**] AND C [**4**].—The crude $CHCl_3$ fraction (Code no. BA-dl, 372 g), which is a part of the extract of the ground wood of *B. antidysenterica* (1922 kg) reported previously (1), was subjected to cc on Si gel (2 kg), eluting with $CHCl_3$-MeOH (10:1) and $CHCl_3$-MeOH (1:1), to yield 9 and 18 fractions, respectively.

A part (24.37 g) of Fraction 12 of the $CHCl_3$-MeOH (1:1) elution (128 g) was column chromatographed on Si gel (500 g, 4 × 90 cm), and eluted with EtOAc-Et_2O (2:1) to give 15 fractions. Of these, fraction 13 (5.50 g) was subjected to preparative hplc with a M & S PACK C_{18}A column (20 mm × 25 cm) and uv detector (254 nm) and eluted with MeOH-H_2O (1:1) to provide 7 fractions. An analytical tlc for fraction 6 (1.53 g) of these 7 fractions, using a mixed solvent of $CHCl_3$-MeOH-H_2O (50:14:3), showed two major tailing spots (R_f 0.58 for **4** and 0.50 for **3**). Subsequent preparative tlc and hplc of this fraction led to the isolation of two colorless amorphous solids: Compounds **3** (147.3 mg, 0.000000077%) and **4** (53.7 mg, 0.000000028%).

ISOLATION OF BRUCEANIC ACID D [**5**].—The crude $CHCl_3$ fraction (Code no. BAC, 705 g) which is a part of the extract of the ground wood of *B. antidysenterica* (1922 kg) reported previously (1), was subjected to cc, using Si gel (3 kg, 10 cm × 90 cm) and two mixed solvents [EtOAc-Et_2O (1:1) then $CHCl_3$-MeOH-H_2O (50:14:3)] to yield 9 and 16 fractions, respectively. Fractions 1–10 of the latter elution were combined to afford a brown gum (229.7 g). To remove the brown resinous substance, the gum was column chromatographed (Sephadex LH-20, 6 cm × 90 cm) in MeOH to yield a pale yellow gum (123.9 g).

The pale yellow gum was further subjected to low pressure cc using the Kusano Lober column (ODS) and a mixed solvent of MeOH-H_2O (1:1) for elution to give 25 fractions. Fractions 3–11 showed a new tailing spot [R_f 0.44, analytical tlc, $CHCl_3$-MeOH-H_2O (50:14:3)]. Purification of these fractions was achieved by use of preparative tlc and hplc to afford a colorless amorphous solid **5** (37.6 mg, 0.00000002%).

BRUCEANIC ACID A [**1**].—Colorless, amorphous solid: mp 177–179°; $[\alpha]^{20}D$ +40° (c = 0.4, EtOH); uv λ max (EtOH) 220 (ε 8800) nm; ir (KBr) 3450 (OH), 1740 (ester and δ-lactone C=O), 1715 (ketone and carboxyl C=O), 1640 (C=C) cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr see Table 1; fdms *m/z* $[M+Na]^+$ 575 (100%), $[M]^+$ 552 (58%), $[C_7H_{11}O]^+$ 111 (side chain) (82%).

METHYL ESTER **2** OF **1**.—Colorless, amorphous solid: mp 90–91°; $[\alpha]^{20}D$ +71° (c = 0.6, EtOH); uv λ max (EtOH) 220 (ε 7400) nm; ir (KBr) 3500 (OH), 1730 (ester and δ-lactone C=O) 1710 (ketone C=O), 1640 (C=C) cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr see Table 1; eims *m/z* $[M]^+$ 566 (2%) $[C_7H_{11}O]^+$ 111 (side chain) (100%).

METHYLATION OF **1**.—Compound **1** (2.0 mg) was suspended in Et_2O (1 ml), and then a few drops of MeOH were added to the supension to dissolve **1**. An Et_2O solution (0°) of CH_2N_2, whch was freshly prepared from nitrosomethylurea and KOH, was added to the solution of **1** at 0° until the reaction mixture became a yellow color. After the mixture was stirred at 0° for 1 h, the solvent was evaporated to afford a colorless and amorphous solid (ca. 2 mg). The R_f and Rt values of this compound coincided with those of **2** described above.

BRUCEANIC ACID B [**3**].—Colorless amorphous solid: mp 228–231° (dec); $[\alpha]^{16}D$ +46° (c = 0.26, EtOH); uv λ max (EtOH) 228 (ε 12000) nm; ir (KBr) 3450 (OH), 1730 (ester and δ-lactone C=O), 1710 (ketone and carboxyl C=O), 1640 (C=C) cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr see Table 1.

MENTHYLATION OF **3**.—A solution of **3** (32.6 mg) in MeOH (1 ml) was methylated with CH_2N_2 in a manner analogous to that described above for the methlyation of **1** to afford a colorless amorphous solid (27.7 mg), of which the R_f value (0.39) is higher than that (R_f 0.05) of **3** on tlc [EtOAc-Et_2O (1:1)]. Purification by the preparative tlc [EtOAc-Et_2O (1:1)] of the product afforded a colorless amorphous solid **7** (3.1 mg): eims *m/z* $[M]^+$ 560 (2%), $[C_6H_5CO]^+$ 105 (100%), $[C_6H_5]^+$ 77 (24%), $[Ac]^+$ 43 (58%); hreims *m/z* 560.1901 (calcd for $C_{28}H_{32}O_{12}$, 560.1910).

BRUCEANIC ACID C [**4**].—Colorless amorphous solid: mp 236–240° (dec); $[\alpha]^{16}D$ +89° (c = 0.18, EtOH); uv λ max (EtOH) 220 (ε 11000) nm; ir (KBr) 3480 (OH), 1735 (ester and δ-lactone C=O), 1710 (ketone and carboxyl C=O), 1640 (C=C) cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr see Table 1; fdms *m/z* $[M+Na]^+$ 633 (37%), $[C_7H_9O]^+$ 109 (12%), $[MeOCO]^+$ 59 (100%).

BRUCEANIC ACID D [**5**].—Colorless amorphous solid: mp 232–240° (dec); $[\alpha]^{16}D$ +100° (c = 0.04, EtOH); uv λ max (EtOH) 218 (ε 16000) nm; ir (KBr) 3420 (OH), 1735 (ester and δ-lactone C=O), 1710 (ketone and carboxyl C=O), 1640 (C=C) cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr see Table 1.

CYTOTOXICITY ASSAY.—The in vitro cytotoxicity assay was carried out according to procedures described in Geran *et al.* (10) and Ferguson *et al.* (11). The assay against KB (nasal pharynageal carcinoma), TE671 (human medulloblastoma), A-549 (human lung carcinoma), HCT-8 (human colon carcinoma),

and P-388 (murine leukemia) tumor cells was based on a method reported in Lee *et al.* (12). Bruceanic acid A [**1**] demonstrated cytotoxicity against KB (ED_{50} 4.16 μg/ml) and TE671 (ED_{50} 5.50 μg/ml), and bruceanic acid D [**5**] was cytotoxic against P-388 (ED_{50} 0.77 μg/ml). Neither compound **1** nor **5** was cytotoxic against A-549 and HCT-8 at ED_{50} 10 μg/ml. No cytotoxicity was shown by **2–4** at 10 μg/ml against KB, TE671, A-549, HCT-8, and P-388 tumor cells.

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LITERATURE CITED

1. M. Okano, K.H. Lee, I.H. Hall, and F.E. Boettner, *J. Nat. Prod.*, **44**, 470 (1981).
2. N. Fukamiya, M. Okano, K. Tagahara, T. Aratani, Y. Muramoto, and K.H. Lee, *J. Nat. Prod.*, **50**, 1075 (1987).
3. M. Okano, N. Fukamiya, T. Aratani, M. Ju-ichi, and K.H. Lee, *J. Nat. Prod.*, **48**, 972 (1985).
4. N. Fukamiya, M. Okano, K. Tagahara, T. Aratani, and K.H. Lee, *J. Nat. Prod.*, **51**, 349 (1988).
5. M. Okano, N. Fukamiya, T. Toyota, K. Tagahara, and K.H. Lee, *J. Nat. Prod.*, **52**, 398 (1989).
6. N. Fukamiya, M. Okano, T. Aratani, K. Negoro, A.T. McPhail, M. Ju-ichi, and K.H. Lee, *J. Nat. Prod.*, **49**, 428 (1986).
7. N. Fukamiya, M. Okano, T. Aratani, K. Negoro, Y.M. Lin, and K.H. Lee, *Planta Med.*, 140 (1987).
8. S.M. Kupchan, R.W. Britton, J.A. Lacadie, M.F. Ziegler, and C.W. Sigel, *J. Org. Chem.*, **40**, 648 (1975).
9. T. Sakaki, S. Yoshimura, T. Tsuyuki, T. Takahashi, T. Honda, and T. Nakanishi, *Bull. Chem. Soc. Jpn.*, **59**, 3541 (1986).
10. R.I. Geran, N.H. Greenberg, M.M. MacDonald, A.M. Schumacher, and B.J. Abbott, *Cancer Chemother. Rep., Part 3*, **3**(2), 1 (1972).
11. P.J. Ferguson, M.H. Fisher, J. Stephenson, D.H. Li, B.S. Zhou, and Y.C. Cheng, *Cancer Res.*, **48**, 5956 (1988).
12. K.H. Lee, Y.M. Lin, T.S. Wu, D.C. Zhang, T. Yamagishi, T. Hayashi, I.H. Hall, J.J. Chang, R.Y. Wu, and T.H. Yang, *Planta Med.*, **54**, 308 (1988).

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