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ANTITUMOR AGENTS, 93.[1] BRUCEANOL C, A NEW CYTOTOXIC QUASSINOID FROM *BRUCEA ANTIDYSENTERICA*

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Kupchan and associates (1) isolated eight quassinoids, including the antileukemic bruceantin that reached phase II clinical trials at the National Cancer Institute, from the Ethiopian *Brucea antidysenterica* Mill. (Simaroubaceae).

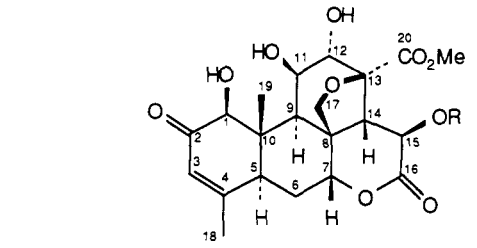
Recently we reported on the isolation and structural elucidation of four new antileukemic quassinoids, bruceantinoside A and B (2), bruceanol A and B (3), and cytotoxic antileukemic alkaloids (4,5) from the stem of *B. antidysenterica*. We now describe the isolation and characterization of a new, potent, cytotoxic quassinoid, bruceanol C [**1**], from this same plant.

Bruceanol C [**1**] was obtained as a colorless amorphous powder. The ^{1}H-nmr (Table 1) signals for H-1, H-3, H-7, H-12, H-15, Me-4, Me-10, and OMe of **1** were coincident with those of bruceanol B [**2**] and isobruceine B [**3**]. On the other hand, the signals for H-2′, Me-3′, Me-4′, and OAc-4′ of **1** were very similar to those of yadanzioside K [**4**] (6). Two singlets for Me-4′ at 1.51 and 1.53 ppm are due to the OAc substitution at 4′ position of the C-15 side chain. The ^{13}C-nmr signals for C-1∼Me-10 of **1** coincided with those of **2** and **3**. On the other hand, the signals for C-1′∼C-9′ of **1** were coincident with those of **4**. These data led to the assignment of structure **1** for bruceanol C. The ir spectrum also supported this structure. From this structure, the molecular formula should be $C_{30}H_{38}O_{13}$ (mol wt 606); however, eims showed the highest peak at *m/z* 546 instead of *m/z* 606. High resolution eims showed a peak at *m/z* 546.2105, which corresponds to $C_{28}H_{34}O_{11}$ ($[M-C_2H_4O_2]^+$), indicating the elimination of HOAc from **1**.

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. ^{1}H-nmr and ^{13}C-nmr spectra were determined on a JEOL JNM-GX400 (400 MHz for ^{1}H nmr and 100.40 MHz for ^{13}C nmr) in $CDCl_3$ using TMS as an internal standard, and the results are shown in Tables 1 and 2, respectively. The assignments of the carbon signals were made by off-resonance decoupling. Mass spectra were recorded on a Hitachi M80 instrument. Si gel (Merck, type 60, 70–230 mesh) was used for cc, and precoated Si gel plates (Merck, 60F-254, 0.25 mm) were used for analytical tlc. Detection of components was made by use of an uv lamp. Analytical hplc was performed on a Waters Associates Model ALC/GPC 244 liquid chromatograph equipped with Radial Pak Liquid Chromatography Cartridge (8NVC18). Preparative hplc was done on a Gilson preparative liquid chromatograph equipped with an M&S PACK C18-A column (20×250 mm).

[1]For part 92, see Y.C. Wu, S.T. Lu, J.J. Chang, and K.H. Lee, *Phytochemistry*, in press.



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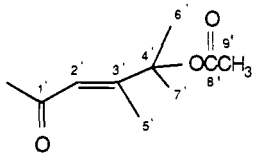

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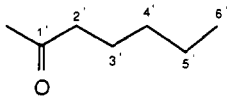

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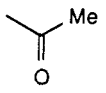

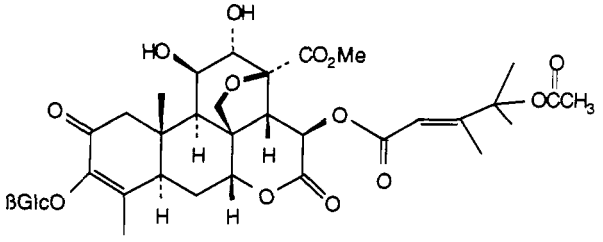

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TABLE 1. ^{1}H-nmr Spectra of Quassinoids **1**, **2**, **3**, and **4**.

Proton	Compound			
	Bruceanol C [**1**][a]	Bruceanol B [**2**][a]	Isobruceine B [**3**][b]	Yadanzioside K [**4**][c]
H-1	4.29 s	4.28 br s	4.26 s	[f]
H-3	6.10 br s	6.1 br s	6.11 br s	[f]
H-7	4.8 m	4.75 br s	4.75 m	[f]
H-11	4.75 br s	4.54 br s	4.75 m	[f]
H-12	4.15 s	4.18 br s	4.12 br s	[f]
H-15	6.3 d (13)[d]	6.29 d (13)[d]	6.30 d (13)[d]	6.85 d (13)[d]
Me-4	1.96 s	1.96 s	1.95 s	2.04 s
Me-10	1.18 s	1.19 s	1.20 s	1.68 s
OMe	3.79 s	3.83 s	3.83 s	3.89 s
H-2′	5.78 s	[e]	2.08 s	6.06 s
Me-3′	2.13 s	[e]	—	2.26 s
Me-4′	1.51 s	[e]	—	1.42 s
	1.53 s	[e]	—	1.47 s
OAc-4′	2.02 s	[e]	—	1.98 s

[a]Measured at 400 MHz in $CDCl_3$.
[b]Measured at 400 MHz in $CDCl_3$ by Moretti *et al.* (7).
[c]Measured at 90 MHz in C_5D_5N by Sakaki *et al.* (6).
[d]Coupling constant in Hz.
[e]The signals of H-2′–H-6′ were found at 1.3 ppm (m) and 0.91 ppm (t, J = 7 Hz).
[f]Not measured.

TABLE 2. ^{13}C-nmr Spectra of Quassinoids **1**, **2**, **3**, and **4**.

Carbon	Compound			
	Bruceanol C [**1**][a]	Bruceanol B [**2**][a]	Isobruceine B [**3**][b]	Yadanzioside K [**4**][c]
C-1	80.5 d	80.6 d	81.3 d	51.1 t
C-2	196.9 s	196.9 s	197.6 s	193.6 s
C-3	124.2 d	124.3 d	124.5 d	148.0 s
C-4	163.1 s	162.9 s	162.6 s	146.6 s
C-5	43.5 d	43.5 d	43.4 d	43.4 d
C-6	28.5 t	28.5 t	28.2 t	29.3 t
C-7	81.2 d	81.1 d	81.7 d	83.4 d
C-8	45.5 s	45.7 s	45.8 s	46.0 s
C-9	42.6 d	42.7 d	42.4 d	41.9 d
C-10	47.5 s	47.5 s	47.7 s	40.8 s
C-11	72.4 d	72.5 d	74.3 d	73.0 d
C-12	75.7 d	75.8 d	75.1 d	75.9 d
C-13	82.2 s	82.8 s	81.7 s	82.6 s
C-14	51.3 d	51.5 d	52.3 d	50.2 d
C-15	66.4 d	66.5 d	67.8 d	68.4 d
C-16	167.0 s	166.9 s	167.6 s	168.0 s
C-17	73.3 t	73.3 t	73.0 t	73.5 t
C-18	22.6 q	22.5 q	22.4 q	15.2 q
C-19	11.6 q	11.5 q	11.3 q	15.8 q
C-20	172.1 s	172.4 s	169.5 s	171.2 s
OMe	53.1 q	52.8 q	49.8 q	52.5 q
C-1′	165.2 s	171.9 s	170.7 s	165.7 s
C-2′	111.9 d	33.6 t	20.5 q	113.5 d
C-3′	169.5 s	31.2 t	—	169.5 s
C-4′	82.7 s	24.2 t	—	82.3 s
C-5′	14.6 q	22.2 t	—	14.3 q
C-6′	26.0 q	13.8 q	—	25.8 q
C-7′	26.3 q	—	—	26.3 q
C-8′	164.7 s	—	—	163.4 s
C-9′	21.6 q	—	—	21.4 q

[a]Measured at 100.40 MHz in $CDCl_3$.
[b]Measured at 22.63 MHz in $CDCl_3$ by Moretti *et al.* (7).
[c]Measured at 22.5 MHz in C_5D_5N by Sakaki *et al.* (6).

CHROMATOGRAPHY OF THE $CHCl_3$ FRACTIONS.—The crude $CHCl_3$ fraction (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 $CHCl_3$ and then with increasing amounts of MeOH in $CHCl_3$ to yield 19 fractions. Concentration of fraction 11 by evaporation of the solvent afforded a brown gum (7.55 g).

ISOLATION OF BRUCEANOL C [**1**].—The foregoing brown gum (7.55 g) was subjected to analytical hplc using a mixed solvent of MeOH-H_2O (1:1, v/v), and a new peak was found by comparing the retention times with those of known quassinoids and alkaloids. Therefore, the gum was subjected twice to preparative hplc using an M&S PACK C18-A column (20 mm×25 cm), a mixed solvent of MeOH-H_2O (3:1, v/v; flow rate 2 ml/min), and Gilson Model 111B uv detector at 254 nm. This led to a yellow, resinous substance (397 mg) that was purified by repeated recrystallization from EtOAc/CH_2Cl_2/Et_2O to afford the pure compound **1** (34 mg, 0.0000017%). This compound was characterized as follows:

BRUCEANOL C [**1**].—Colorless, amorphous powder, mp 125–127°; $[\alpha]^{25}D$ +38° (c=0.24, EtOH); uv λ max (EtOH) 225 nm (ε 14000); ir (KBr) 3560 and 3450 (OH), 1735 (ester and lactone C=O), 1675 (α,β-unsaturated C=O), 1645 (C=C), 1385 and 1375 (*gem*-demethyl), 1262 (acetate), 1060 (OH), 965 (C=C) cm^{-1}; ^{1}H nmr and ^{13}C nmr see Tables 1 and 2, respec-

tively; hreims *m/z* 546.2105 (calcd for $C_{28}H_{34}O_{11}$ $[M-C_2H_4O_2]^+$, 546.2100).

BIOLOGICAL ACTIVITY.—The in vitro tissue culture cytotoxicity assay was carried out according to standard National Cancer Institute procedure (8).[2] Bruceanol C demonstrated potent cytotoxicity against human KB, A-549 lung carcinoma, and HCT-8 colon tumor as well as murine P-388 lymphocytic leukemia with ED_{50} values of <0.04, 0.48, <0.40, and 0.56 μg/ml, respectively.

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