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ANTITUMOR AGENTS, 74.[1] BRUCEANOL-A AND -B, TWO NEW ANTILEUKEMIC QUASSINOIDS FROM *BRUCEA ANTIDYSENTERICA*

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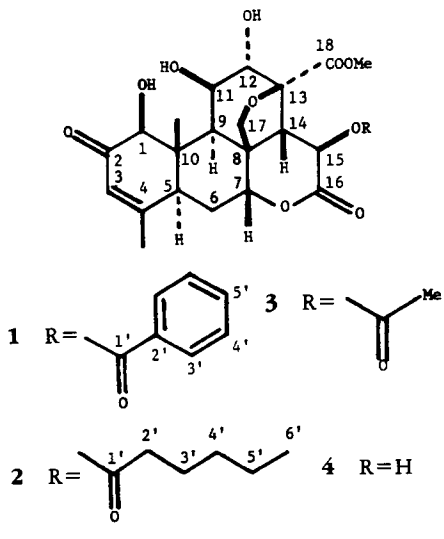
Eight quassinoids, including the antileukemic compound bruceantin, which was in phase II clinical trial by the National Cancer Institute, have been isolated from the Ethiopian tree *Brucea antidysenterica* Mill. (Simaroubaceae) by Kupchan and associates (1). In a previous paper, we reported the isolation and structural elucidation of two new antileukemic quassinoid glycosides, bruceantinoside-A and -B, from *B. antidysenterica* (2). We now describe the isolation and characterization of two additional new antileukemic isobruceine type quassinoids, bruceanol-A (**1**) and -B (**2**), from this same plant.

RESULTS AND DISCUSSION

Bruceanol-A (**1**) had the molecular formula $C_{28}H_{30}O_{11}$ from both field desorption and hrms. Compound **1** showed the presence of hydroxyl (3540, 3450, and 1030 cm^{-1}), phenyl (1600 and 1580 cm^{-1}), δ-lactone (1750 cm^{-1}), ester (1730 cm^{-1}), and α, β-unsaturated carbonyl (1675 cm^{-1}) groups in its ir spectrum. The ^{1}H-nmr spectrum of **1** is identical with that of isobruceine-B (**3**), except for the signals in the acid portion of the ester as well as the COOMe-13 and the H-15. These differences were observed in **1** as a phenyl ring [δ 8.0-8.05 (2H), H-3′ and 7.42-7.65 (3H), H-4′ and H-5′] and in **3** as a methyl group [δ 2.08 (3H, s)]. In **1**, the signal for COOMe-13 (δ 3.51) was shifted upfield by 0.32 ppm, and that for H-15 (δ 6.49) was shifted downfield by 0.19 ppm compared to the corresponding signals of **3** (δ 3.83 for COOMe-13 and δ 6.30 for H-15) due to the anisotropic effect of the aromatic ring in **1** (Table 1).



Added confirmation for these assignments was obtained by a comparison of the ^{13}C-nmr spectra of **1** and **3** (Table 2). The carbon signals for both compounds were superimposable except for the differences in the above mentioned ester side chains. Thus, **1** contained the aromatic carbons at δ 128.7 (s, C-2′), 128.5 (d, C-3′), 130.0 (d, C-4′), and 133.8 (d, C-5′), whereas **3** possessed a quartet methyl group at δ 20.5 (C-2′).

Bruceanol-B (**2**) was analyzed for $C_{27}H_{36}O_{11}$. Its ir spectrum was consistent with the presence of hydroxyl (3540, 3450, and 1030 cm^{-1}), δ-lactone (1750 cm^{-1}), ester (1730 cm^{-1}), and α,β-unsaturated carbonyl (1677 cm^{-1}) groups. Compound **2** showed

[1]For part 73, see H. Nozaki, H. Suzuki, T. Hirayama, R. Kasai, R.Y. Wu, and K.H. Lee, *Phytochemistry*, (in press).

also comparable ^{1}H-nmr (Table 1) and ^{13}C-nmr (Table 2) spectra to those of **3** except for the signals attributable to a hexanoyl group [δ 1.3 (m, H-3′, H-4′, and H-5′) and 0.91 (t, J=7.0 Hz, H-6′), and δ 33.6 (t, C-2′), 31.2 (t, C-3′), 24.2 (t, C-4′), 22.2 (t, C-5′), and 13.8 (q, C-6′)] in **2** and an acetyl [δ 2.08 (s) and δ 20.5 (q, C-2′)] moiety in **3**.

Deacetylisobruceine-B (**4**) was obtained by alkaline hydrolysis of **1**,**2**, and **3**. Thus, **1** and **2** were structurally identical with **3** except for the slight differences in the C-15 ester side chain, in which **1** and **2** have a benzoate and an hexanoate, respectively, whereas **3** bears an acetate moiety.

an internal standard. Mass spectra were recorded on a JEOL JMS-D100 (EI) and/or a Hitachi M-80 (EI and FD) instruments. Silica gel (Merck, type 60, 70-230 mesh was used for column chromatography, and precoated silica gel plates (Merck, 60 F-254, 0.25 and 2mm) were used for analytical and preparative tlc, respectively. Detection of components was made by use of a uv lamp. Hplc was performed on a Waters Associates Model ALC/GPC 244 liquid chromatograph using a μBondapak C18 column (3.9 mm×300 mm) for analytical, and M and S PAK C18-A (20 mm×250 mm) for preparative purposes.

CHROMATOGRAPHY OF THE $CHCl_3$ FRACTIONS.—The crude $CHCl_3$ fraction (372 g), which is part of the $CHCl_3$ extract of the ground wood of *B. antidysenterica* (4228 lbs) reported previously (2), was subjected to column chromatography on silica gel (2 kg, 10 cm×60 cm and

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

	Bruceanol-A (**1**)[a]	Bruceanol-B (**2**)[b]	Isobruceine-B (**3**)[c]	Deacetylisobruceine-B (**4**)[d]
H-1	4.32 s	4.28 br.s	4.26 s	4.85 br.s
H-3	6.13 br.s	6.1 br.s	6.11 br.s	6.13 br.s
H-7	4.8 m	4.75 br.s	4.75 m	5.54 t (4.9)[e]
H-11	4.57 s	4.54 br.s	4.75 m	5.08 br.s
H-12	4.20 s	4.18 br.s	4.12 br.s	4.35 br.s
H-15	6.49 d (13)[e]	6.29 d (13)[e]	6.30 d (13)[e]	6.12 d (12.2)[e]
OMe	3.51 s	3.83 s	3.83 s	3.79 s
Me-4	1.98 s	1.96 s	1.95 s	1.72 s
Me-10	1.21 s	1.19 s	1.20 s	1.44 s
2′	—	—	2.08 s	—
3′			—	—
4′	8.0-8.05 (2H)	1.3 m	—	—
5′	7.42-7.65 (3H)		—	—
6′	—	0.91 t (7)[e]	—	

[a]Measured at 200 MHz in $CDCl_3$.
[b]Measured at 400 MHz in $CDCl_3$.
[c]Measured at 400 MHz in $CDCl_3$ by Polonsky *et al.* (3).
[d]Measured at 400 MHz in C_5D_5N.
[e]Coupling constant in Hz.

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 Yanagimoto OR-50 polarimeter (L=0.5 dm). Ir and uv spectra were recorded on a Hitachi 215 grating ir spectrometer and a Hitachi 320-S uv spectrometer, respectively. ^{1}H-nmr and ^{13}C-nmr spectra were determined on a JEOL JNM-FX 200 (200 MHz for ^{1}H nmr and 50.1 MHz for ^{13}C nmr) and/or a JEOL JNM-GX400 (400 MHz for ^{1}H nmr and 100 MHz for ^{13}C nmr) using TMS as

eluted first with $CHCl_3$ and then with increasing amounts of MeOH in $CHCl_3$ to yield 28 fractions. Fraction 20 (31 g) was further subjected to column chromatography on silica gel (500 g, 4 cm×90 cm and eluted with EtOAc-Et_2O, 1:1, v/v) to afford a pale yellow gum (6.7 g) which showed the presence of isobruceine-B (**3**), bruceanol-A (**1**), and bruceanol-B (**2**) on analytical hplc (MeOH-H_2O, 1:1, v/v).

ISOLATION OF BRUCEANOL-A (**1**).—The pale yellow gum (6.7 g) was subjected to preparative hplc using a mixed solvent of MeOH-H_2O (1:1, v/v) to give a colorless amorphous compound (1.1

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

	Bruceanol-A (**1**)[a]	Bruceanol-B (**2**)[b,c]	Isobruceine-B (**3**)[d]	Deacetylisobruceine-B (**4**)[e]
C-1	80.7 d	80.6 d	81.3 d	82.9 d
C-2	196.7 s	196.9 s	197.6 s	198.4 s
C-3	124.3 d	124.3 d	124.5 d	125.0 d
C-4	163.0 s	162.9 s	162.6 s	163.2 s
C-5	43.5 d	43.5 d	43.4 d	43.9 d
C-6	28.6 t	28.5 t	28.2 d	28.6 t
C-7	81.3 d	81.1 d	81.7 d	83.1 d
C-8	45.8 s	45.7 s	45.8 s	46.2 s
C-9	42.8 s	42.7 d	42.4 d	43.4 d
C-10	47.6 s	47.5 s	47.7 s	48.4 s
C-11	72.7 d	72.5 d	74.3 d	75.2 d
C-12	75.8 d	75.8 d	75.1 d	76.9 d
C-13	82.9 s	82.8 s	81.7 s	82.6 s
C-14	51.4 d	51.5 d	52.3 d	55.5 d
C-15	67.4 d	66.5 d	67.8 d	66.3 d
C-16	166.7 s	166.9 s	167.6 s	172.8 s
C-17	73.3 t	73.3 t	73.0 t	74.0 t
C-18	172.0 s	172.4 s	169.5 s	173.5 s
OMe	52.9 q	52.8 q	49.8 q	52.3 q
Me-4	22.5 q	22.5 q	22.4 q	22.2 q
Me-10	11.6 q	11.5 q	11.3 q	11.4 q
C-1′	164.8 s	171.9 s	170.7 s	—
C-2′	128.7 s	33.6 t	20.5 q	—
C-3′	128.5 d	31.2 t	—	—
C-4′	130.0 d	24.2 t	—	—
C-5′	133.8 d	22.2 t	—	—
C-6′	—	13.8 q	—	—

[a]Measured at 50.1 MHz in $CDCl_3$.

[b]Measured at 100.40 MHz in $CDCl_3$.

[c]The characterizations of sec-C was based on the INEPT (Insensitive Nuclei Enhanced by Polarization Transfer).

[d]Measured at 22.63 MHz in $CDCl_3$ by Polonsky *et al.* (3).

[e]Measured at 100.40 MHz in C_5D_5N.

g), which was eluted after isobruceine-B. This amorphous compound was further subjected to preparative hplc to yield pure bruceanol-A (**1**, colorless and amorphous, 0.000058% yield); mp 174-177° (from CH_2Cl_2/Et_2O); $[\alpha]^{25}D$ +60° (c 0.5, EtOH); uv λ max (EtOH) 232 nm (ε 22400); The ir spectral data were discussed in the text. The ^{1}H-nmr and ^{13}C-nmr data were summarized in Table 1 and 2, respectively. Fdms *m/z* 543 (M^++1); eims *m/z* 542.1853 (calcd. for $C_{28}H_{30}O_{11}$: 542.1788, M^+), 524,1657 (calcd. for $C_{28}H_{28}O_{10}$: 524.1682, M^+-H_2O), 492.1418 (calcd. for $C_{27}H_{24}O_9$: 492.1420, M^+-MeOH), and 420.1438 (calcd. for $C_{21}H_{24}O_9$: 420.1420, M^+-C_6H_5COOH).

ISOLATION OF BRUCEANOL-B (**2**).—The pale yellow gum (6.7 g) was further eluted after the elution of bruceanol-A to afford a compound (0.54 g). This compound was purified by preparative hplc to yield pure bruceanol-B (**2**, colorless and amorphous, 0.000028% yield); mp 170-172° (from EtOAc/Et_2O); $[\alpha]^{25}D$ +32° (c 0.5, MeOH); uv λ max (EtOH) 236 nm (ε 8300); fdms *m/z* 537 (M^++1); Found: C, 60.49%; H, 6.74%. Calcd. for $C_{27}H_{36}O_{11}$: C, 60.43%, H, 6.76%.

ISOLATION OF ISOBRUCEINE-B (**3**).—Isobruceine-B (**3**) was obtained as a colorless and amorphous compound (0.1 g) by the preparative hplc of the foregoing pale yellow gum (6.7 g). It was also obtained in 4% yield from fraction 21 (88 g) in the same manner as described above. This compound was identified by comparing its ^{1}H-nmr (400 MHz) spectrum (Table 1) with that presented by Polonsky (3).

HYDROLYSIS OF ISOBRUCEINE-B (**3**).—To a solution of isobruceine-B (**3**, 25 mg) in MeOH (1.5 ml) was added 1N NaOH (0.45 ml). After stirring at −15° for 17 min, the reaction mixture was neutralized with 1N HCl (0.8 ml) and evaporated in vacuo to give a residue. The residue was subjected to preparative tlc to afford pure deacetylisobruceine-B (**4**) as colorless needles (8.2

mg); mp 279-282° (from EtOAc/Et_2O); ir (KBr) 3400, 1735 and 1670 cm^{-1}; eims *m/z* 438.1538 (M^+; calcd. for $C_{21}H_{26}O_{10}$: 438.1526); Found: C, 57.49%; H, 5.99%. Calcd. for $C_{21}H_{26}O_{10}$: C, 57.53%; H, 5.98%.

HYDROLYSIS OF BRUCEANOL-A (**1**).—Bruceanol-A (**1**, 46.6 mg) was hydrolyzed and worked up in the same manner described above to give colorless needles (6.2 mg) identified as deacetylisobruceine-B (**4**) by comparing its ^{1}H-nmr (400 MHz, C_5D_5N) spectrum with that of an authentic sample derived from the hydrolyzed product of isobruceine-B.

HYDROLYSIS OF BRUCEANOL-B (**2**).—Bruceanol-B (**2**, 25 mg) was hydrolyzed and worked up in the same manner as described above to yield colorless needles (7.2 mg) identified as deacetylisobruceine-B (**4**) by a direct ^{1}H-nmr comparison with an authentic sample.

BIOLOGICAL ACTIVITY.—In vivo antitumor assay was carried out according to a standard National Cancer Institute procedure described in the literature (4). Bruceanol-A (**1**) and -B (**2**) showed significant (T/C≧120%) antileukemic activity in vivo against P-388 lymphocytic leukemia (3-day dosing) at T/C=130% (0.5 mg/kg), 129% (1 mg/kg) and 134% (2 mg/kg), and 123% (0.5 mg/kg), respectively. The control, 5-fluorouracil, had T/C=135% (200 mg/kg, 1-day dosing).

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