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ANTITUMOR AGENTS, 90.[1] BRUCEANTINOSIDE C, A NEW CYTOTOXIC QUASSINOID GLYCOSIDE FROM *BRUCEA ANTIDYSENTERICA*

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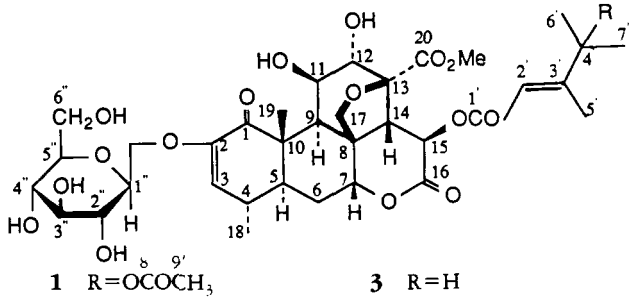
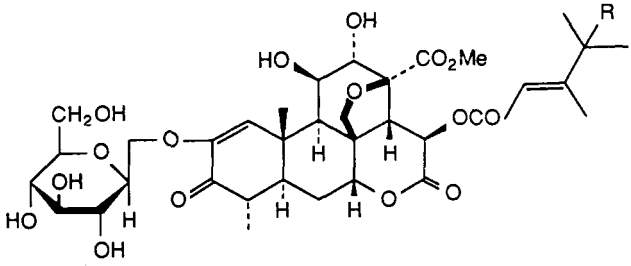
ABSTRACT.—Three cytotoxic, quassinoid glycosides, the new bruceantinoside C [**1**] and the known yadanziosides G [**2**] and N [**3**] were isolated from the stem of *Brucea antidysenterica*. The structures of **1-3** were determined from their spectral data.

Kupchan and associates (1) isolated eight quassinoids, including the antileukemic compound bruceantin, which was in phase II clinical trial at the National Cancer Institute, from the Ethiopian tree *Brucea antidysenterica* Mill. (Simaroubaceae).

Recently we reported on the isolation and structural elucidation of four new antileukemic quassinoids, bruceantinosides A and B (2) and bruceanols A and B (3) from the stem of *B. antidysenterica*. We now describe the isolation and characterization of



1 R=$OCOCH_3$ **3** R=H



2 R=$OCOCH_3$ **4** R=H

[1]For part 89, see T. Hayashi, F. Smith, and K.H. Lee, *J. Med. Chem.*, in press.

three cytotoxic quassinoid glycosides, bruceantinoside C [**1**], yadanziosides G [**2**] and N [**3**] from this same plant. Among these, **1** is a new compound. Although **2** and **3** have been obtained recently by Takahashi and associates (4,5) from *Brucea javanica*, these compounds were isolated for the first time from *B. antidysenterica*.

RESULTS AND DISCUSSION

Bruceantinoside C [**1**] was obtained as a colorless, amorphous powder. Fdms showed a peak at *m/z* 791 ($[M+Na]^+$), which suggested the molecular formula to be $C_{36}H_{48}O_{18}$. A comparison of the ^{1}H-nmr signals (Table 1) among **1**, **2**, and **3** clearly indicated that the signals for H-3, Me-4, and Me-10 in **1** and **3** are nearly superimposable, while the signals for the two methyl groups attached to C-4′ in **1** and **3** are quite different. On the other hand, the two methyl groups and one acetoxyl group attached to C-4′ in **1** and **2** are coincident. This evidence demonstrated the presence of an identical cyclohexanone ring A structure in **1** and **3** as well as an identical ester side chain in **1** and **2**. The comparable ^{13}C-nmr signals (Table 2) of C-1∼C-3′ and C-1″∼C-6″ in **1** and **3**, coupled with the different signals of C-4′∼C-9′ in these compounds, indicates that compounds **1** and **3** have a different C-15 ester side chain. Compound **1** must possess the same ester side chain as **2**, since the ^{13}C-nmr signals of C-1′∼C-9′ for both compounds are identical. The aforementioned data led to the unambiguous assignment of structure **1** for bruceantinoside C.

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

Protons	Compounds			
	1	**2**	**3**	**4**
H-3	6.16d(2)	[a]	6.11d(2)	[a]
H-4	[a]	[a]	2.24brt	[a]
H-5	[a]	[a]	[a]	[a]
H-7	5.18brs	[a]	5.15brs	[a]
H-9	2.97d(4)	[a]	2.95d(4)	[a]
H-11	6.32d(4)	[a]	6.20d(4)	[a]
H-12	4.95brs	[a]	4.91brs	[a]
H-15	6.98br	[a]	6.94br	[a]
H-20	5.16d(8)	[a]	5.13d(7)	[a]
Me-4	0.88d(7)	1.17d(5)	0.87d(7)	1.13d(6)
Me-10	1.90s	1.59s	1.87s	1.59s
COOMe	3.92s	3.76s	3.78s	3.76s
H-2′	[a]	[a]	5.90s	[a]
Me-3′	2.30s	2.26s	2.18s	2.14s
Me-4′	1.40s	1.39s	0.84d(7)	0.84d(6)
	1.46s	1.43s	—	—
OAc-4′	1.95s	1.95s	—	
H-1″	5.49d(7)	[a]	5.46d(7)	[a]
H-6″	4.58d(11)	[a]	4.56d(11)	[a]

[a]Not measured.

Compound **2** was also isolated as a colorless, amorphous powder. The fdms of **2** showed the same pattern as that of **1**. However, its ^{13}C-nmr (Table 2) signals around ring A were different from those of **1**, despite the fact that it showed identical ester side chain signals to **1**. Because the signals around ring A of **2** are superimposable with those of bruceantinoside A [**4**] (2), structure **2** was assigned to this compound. The identity of **2** with yadanzioside G (4) was established by their comparable physical constants and spectral data.

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

Carbon Atoms	Compounds			
	1	**2**	**3**	**4**
C-1	199.7s	129.3d	199.7s	129.6d
C-2	146.4s	148.9s	146.4s	148.8s
C-3	124.9d	194.5s	124.8d	194.8s
C-4	31.5d	43.8d	31.5d	43.8d
C-5	36.8d	40.3d	36.9d	40.4d
C-6	28.7t	30.1t	28.7t	29.9t
C-7	82.9d	83.5d	83.0d	83.4d
C-8	46.7s	46.6s	46.7s	46.6s
C-9	44.1d	41.4d	44.1d	41.3d
C-10	48.9s	39.6s	48.8s	39.6s
C-11	75.1d	73.6d	75.1d	71.3d
C-12	76.2d	76.0d	76.3d	76.0d
C-13	82.9s	82.6s	83.0s	82.5s
C-14	50.8d	52.6d	51.0d	52.3d
C-15	69.2d	71.3d	68.8d	68.3d
C-16	168.1s	168.1s	168.2s	168.2s
C-17	73.6t	73.7t	73.7t	73.3t
C-18	14.5q	12.6q	14.5q	12.5q
C-19	18.9q	18.0q	18.9q	17.8q
C-20	171.1s	171.1s	171.1s	171.1s
OMe	52.6q	50.2q	52.3q	50.2q
C-1′	165.9s	165.7s	166.5s	165.7s
C-2′	113.5d	113.6d	113.5d	113.4d
C-3′	169.5s	167.1s	167.3s	167.1s
C-4′	82.3s	82.3s	38.1d	38.1d
C-5′	14.5q	14.5q	16.7q	16.7q
C-6′	26.4q	26.4q	20.7q	20.7q
C-7′	25.7q	25.8q	20.7q	20.7q
C-8′	163.7s	169.5s	——	——
C-9′	21.4q	21.4q	——	——
C-1″	100.7d	102.1d	100.7d	102.0d
C-2″	74.6d	74.7d	74.6d	74.6d
C-3″	79.0d	79.0d	79.0d	78.8d
C-4″	71.4d	71.3d	71.4d	71.3d
C-5″	78.6d	78.5d	78.6d	78.3d
C-6″	62.3t	62.3t	62.4t	62.3t

Compound **3** was assigned a composition $C_{34}H_{46}O_{16}$ based upon its fdms peak at *m/z* 749 ($[M+K]^+$). Because its ^{13}C-nmr (Table 2) spectrum coincided with that of **1** except for the signals around the side chain and with that of **4** except for the signals around ring A, the structure of **3** was assumed to have the same moiety as that around ring A of **1** and that around the side chain of **4**. A comparison of the physical constants and spectral data of **3** and yadanzioside N (5) established their identity.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on an MRK air-bath melting point apparatus and were uncorrected. Specific rotations were obtained on a Yanako OR-50D polarimeter (L=1 dm). Ir and uv spectra were recorded on a Jasco IR-810 spectrometer and a Hitachi 320S spectrometer, respectively. ^{1}H-nmr and ^{13}C-nmr spectra were determined on a Varian XL-200 (200.06 MHz for ^{1}H nmr and 50.31 MHz for ^{13}C nmr) using TMS as an internal standard are shown in Tables 1 and 2, respectively. All the samples for nmr analyses were dissolved in pyridine-d_5. The assignments of the carbon signals were made by off-resonance decoupling and "distortionless enhancement by polarization transfer" (DEPT) methods. Fd mass spectra were recorded on a Hitachi M80 instrument. Si gel (Merck, type 60,

70-230 mesh) was used for column chromatography, and precoated Si gel plates (Merck, 60F-254, 0.25 mm) were used for analytical tlc. Detection of components was made by use of a uv lamp. Analytical hplc was performed on a Waters Associates Model ALC/GPC 244 liquid chromatograph equipped with a Radial-Pak Liquid Chromatography Cartridge (8NVC18) and a Waters Model 440 uv detector. Preparative low pressure liquid chromatography was carried out on a Kusano Model KHLC-201-25 liquid chromatograph equipped with an ODS column (25 mm×10 mm). Preparative hplc was done on a Gilson preparative liquid chromatograph equipped with a M&S PACK C18-A column (20 mm×250 mm) and a Gilson Model 111B UC detector.

CHROMATOGRAPHY OF THE $CHCL_3$ FRACTIONS.—The crude $CHCl_3$ fraction (705 g), which was part of the $CHCl_3$ extract of the ground wood of *B. antidysenterica* (4228 lbs) reported previously (1), was subjected to column chromatography on Si gel (3 kg, 10 cm×90 cm) and eluted at first with EtOAc-Et_2O (1:1, v/v) and then with $CHCl_3$-MeOH-H_2O (50:14:3, v/v) to yield 9 and 16 fractions, respectively. The 11th-16th fractions of the latter elution were combined (72.6 g) and subjected twice to column chromatography on Sephadex LH-20 (60 mm×90 cm) eluting with MeOH to remove dark brown resinous substance and afford a pale yellow gum (26.2 g).

ISOLATION OF BRUCEANTINOSIDE C [**1**], YADANZIOSIDE G [**2**], AND YADANZIOSIDE N [**3**].—The foregoing pale yellow gum (26.2 g) was further subjected to low pressure column chromatography using an ODS column and MeOH-H_2O (1:1, v/v) as eluent to give ten fractions. The 2nd-5th fractions were shown to contain three major components (retention time 9.8 min for **1**, 12.8 min for **2**, and 14.5 min for **3**) on analytical hplc with elution by MeOH-H_2O (1:1, v/v, 1 ml/min). Preparative hplc of these fractions with elution by MeOH-H_2O (1:1, v/v; flow rate 2 ml/min), led to the isolation of **1** (1.43 g, 0.000075%), **2** (200 mg, 0.000010%), and **3** (1.98 g, 0.000101%).

BRUCEANTINOSIDE C [**1**].—Compound **1** was obtained as a colorless, amorphous powder, mp 153-155°; $[\alpha]^{23}D$+12.7° (*c* 1.1, EtOH); uv (EtOH) 255 nm (ϵ 6300); ir (KBr) 3420 (OH), 1738 (ester C=O), 1682 (α,β-unsaturated C=O), 1640 (C=C), 1075, 1045, 1020 cm^{-1}; ^{1}H-nmr and ^{13}C-nmr spectra see Tables 1 and 2. Fdms *m/z* 791 ($[M+Na]^+$) and 546 ($M-C_2H_4O_2-C_6H_{10}O_5$); hreims *m/z* 546.2075 [calcd for $C_{28}H_{34}O_{11}$ ($M-C_2H_4O_2-C_6H_{10}O_5$): *m/z* 546.2100].

YADANZIOSIDE G [**2**].—This compound was obtained as a colorless, amorphous powder, mp 180-185°; $[\alpha]^{18}D$+18.4° (*c* 0.76, EtOH); uv (EtOH) 253 nm (ϵ 7460); ir (KBr) 3420 (OH), 1738 (ester C=O), 1682 (α,β-unsaturated C=O), 1650 (C=C), 1070, 1045, 1020 cm^{-1}; ^{1}H-nmr and ^{13}C-nmr spectra see Tables 1 and 2; fdms *m/z* 807 ($[M+K]^+$), 708 ($M-C_2H_4O_2$), 546 ($M-C_2H_4O_2-C_6H_{10}O_5$).

YADANZIOSIDE N [**3**].—This was also obtained as a colorless, amorphous powder, mp 175-180°; $[\alpha]^{14}D$+7.7° (*c* 1.42, EtOH); uv (EtOH) 255 nm (ϵ 6100); ir (KBr) 3420 (OH), 1740 (ester C=O), 1682 (α,β-unsaturated (C=O), 1640 (C=C), 1075, 1045, 1020 cm^{-1}; ^{1}H-nmr and ^{13}C-nmr spectra see Tables 1 and 2; fdms *m/z* 749 ($[M+K]^+$), 549 ($M-C_6H_{10}O_5+1$), 530 ($M-C_6H_{12}O_6$).

BIOLOGICAL ACTIVITY.—In vitro cytotoxicity assays were carried out according to standard National Cancer Institute procedures (6). Compounds **1**, **2**, and **3** showed significant (ED_{50}<4.0 μg/ml) cytotoxicity in vitro against P-388 and L-1210 lymphocytic leukemia tissue culture cells at ED_{50}=2.12 and 3.50, 1.25 and 2.58, and 2.24 and 4.56 μg/ml, respectively. The control drug, 5-fluorouracil, used in this assay showed ED_{50}=3.72 and 1.94 μg/ml, respectively.

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