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BRUCEANOLS G AND H, CYTOTOXIC QUASSINOIDS FROM *BRUCEA ANTIDYSENTERICA*

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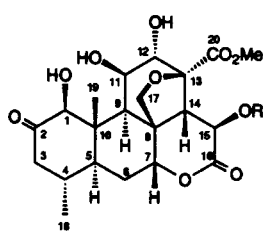
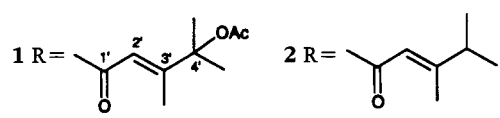
ABSTRACT.—Two new quassinoids, bruceanols G [**1**] and H [**4**], were isolated from *Brucea antidysenterica*, and their structures were elucidated by spectral evidence and chemical transformation. Bruceanol G exhibited significant cytotoxicity against the COLO-205 and KB neoplastic cell lines with ED_{50} values of 0.44 and 0.55 μM, respectively.

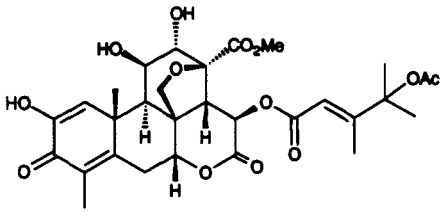
In previous papers, we have reported the isolation and structural elucidation of nine new antileukemic quassinoids, namely, bruceantinosides A, B (1), and C (2) and bruceanols A, B (3), C (4), D, E, and F (5), three known compounds, yadanziosides G, N (2), and M (6), cytotoxic antileukemic alkaloids (7,8), and three new degradation products, bruceanic acids B, C, and D (9), from the stems of *Brucea antidysenterica* Mill. (Simaroubaceae). We now describe the isolation and characterization of two new quassinoids, which have been given the trivial names bruceanol G [**1**] and bruceanol H [**4**]. Three known quassinoids, bruceanols E [**2**] and F [**5**] (5), and dehydrobruceantinol [**3**], which were also isolated from this plant, were useful in the structural elucidation of the new compounds. The evaluation of bruceanols G and H against three cancer cell lines is also reported herein.

Bruceanol G [**1**] was obtained as colorless needles. Its ir spectrum showed the presence of hydroxy (3450 cm^{-1}), δ-lactone and ester (1740 cm^{-1}), and α,β-unsaturated ester (1720 and 1640 cm^{-1}; groups. The uv spectrum of **1** exhibited an absorption maximum at 220 nm due to the presence of a conjugated enone system. The sims spectrum of **1** showed pseudo-molecular ion peaks of $[M+Na]^+$ and $[M+H]^+$ at *m/z* 631 and 609, respectively, suggesting a molecular formula of $C_{30}H_{40}O_{13}$. The hreims confirmed the molecular formula as $C_{30}H_{40}O_{13}$.

As shown in Table 1, the ^{1}H-nmr







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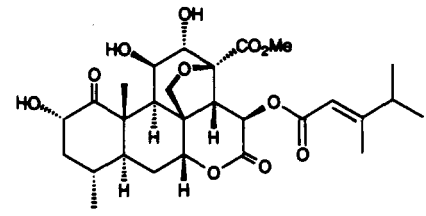

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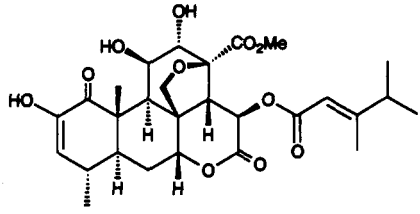

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spectrum obtained for **1** was similar to that of **2**, except for the signals of the ester side-chain at C-15; compound **1** showed two singlets at 1.39 and 1.44 ppm due to the two 4′-methyls and a singlet at 1.94 ppm for the OAc-4′, while **2** showed a doublet for the two 4′-methyls at 0.85 ppm and a multiplet at 2.14 ppm for H-4′. The proton assignments for **1** were based on ^{1}H-^{1}H and ^{13}C-^{1}H COSY spectra. Also, because the mol wt of **1** is larger than that of **2** by 58 atomic mass units, these data suggested that **1** possesses an acetoxy group at C-4′. The signals of the H-2′, Me-3′, two Me-4′, and OAc-4′ in **1** coincided with those in **3**; thus, the side-chain at C-15 was also assumed to

TABLE 1. ^{1}H-Nmr Spectral Data of Compounds **1–5**.[a]

Proton(s)	Compound				
	1[b]	**2**[c]	**3**[c]	**4**[d]	**5**[c]
H-1	—	—	7.10 s	—	—
H-1α	4.26 br s	2.23 br s	—	—	—
H-2	—	—	—	5.07 dd (12.0, 4.4)	—
H-3	—	—	—	—	5.87 s
H-3α	2.21 dd (13.8, 12.6)	2.21 dd (13, 13)	—	2.93 dd (13.2, 12.0)	—
H-3β	2.50 dd (13.8, 4.6)	2.49 d (13)	—	2.24 dd (13.2, 4.4)	—
H-4	1.70 m	1.68 m	—	1.73 m	2.30 m
H-5	2.01 br s	1.96 br d (2)	—	2.55 dd (12.8, 2.0)	2.17
H-6α	2.16 dd (12.6, 2)	2.13 dd (12, 2)	3.35 dd	2.17 dd (12.8, 2.0)	2.20 dd (13, 13)
H-6β	1.53 dd (13, 12.6)	1.52 ddd (12, 12, 2)	2.82 d	1.48 ddd (12.8, 12.8, 2.0)	1.63 dd (13, 13)
H-7	5.00 br s	4.99 dd (2, 2)	5.31 d	4.91 br s	4.95
H-9	2.72 d (3.8)	2.70 d (4)	2.60 d	3.53 br s	2.99
H-11	5.34 br s	5.34 d (4)	[e]	5.03 d (4.0)	6.52 d (5)
H-12	5.15 br s	5.14 br d	[e]	4.62 d (4.0)	5.16
H-14	4.04 br s	4.01 br d	4.13 br d	4.04 br s	4.04 br d
H-15	6.70 br s	4.68 br d	[e]	6.39 d (4.8)	4.90 br d
H-17α	3.88 d (8.0)	3.87 d (7)	4.07 d	3.93 d (6.8)	3.92 d (7.5)
H-17β	4.99 d (8.0)	4.98 d (7)	5.32 d	5.09 d (6.8)	5.17 d (7.5)
H-2′	6.10 s	5.88 s	6.03 s	5.83 s	5.87 s
H-4′	—	2.14 m	—	1.80 m	2.14 m
Me-4	0.85 d (6.5)	0.84 d (6)	2.23 s	0.81 d (6.4)	0.98 d (7)
Me-10	1.36 s	1.35 s	1.92 s	1.54 s	1.91 s
Me-3′	2.26 s	2.17 s	2.24 d	2.11 s	2.17 s
Me-4′	1.39 s	0.85 d (7.5)	1.33 s	0.78 d (6.4)	0.85 d (7)
	1.44 s	0.85 d (7.5)	1.41 s	0.78 d (6.4)	—
OAc-4′	1.94 s	—	1.94 s	—	—
OMe-20	3.86 s	3.75 s	3.90 s	3.75 s	3.76 s

[a]Values are in δ ppm. The coupling constants (*J* values) in parentheses are in Hz.
[b]Measured at 270 MHz in C_5D_5N.
[c]Measured at 500 MHz in C_5D_5N.
[d]Measured at 400 MHz in C_5D_5N.
[e]Not assignable.

be a 4′-acetoxy-3′,4′-dimethyl-2-pentenoyl group as is present in **3**.

As shown in Table 2, the ^{13}C-nmr spectrum of **1** was also similar to that of **2**, except for the signals of the ester side-chain (C-1′, C-2′, C-3′, Me-3′, Me-4′, and OAc-4′), which coincided with those in **3**. The assignments for **1** were based on ^{13}C-^{1}H COSY and DEPT nmr spectra.

Bruceanol H [**4**] was obtained as colorless needles. Its ir spectrum showed the presence of hydroxy (3450 cm^{-1}), δ-lactone (1745 cm^{-1}), and α,β-unsaturated ester (1710 and 1640 cm^{-1}) groups. The uv spectrum of **4** exhibited an absorption maximum at 220 nm due to a conjugated enone system being present. The eims spectrum of **4** showed a molecular ion peak at *m/z* 550, suggesting a molecular formula of $C_{28}H_{38}O_{11}$, and a fragment ion peak at *m/z* 111 ($C_7H_{11}O_{10}$) due to the C-15 side-chain. The hreims confirmed the molecular formula to be $C_{28}H_{38}O_{11}$.

The ^{1}H-nmr spectrum of **4** was similar to that of **5**, except for the signals of H-2 and H-3; compound **4** showed a double doublet signal at δ 5.08 ppm for H-2 consistent with a methine bearing an OH group, while **5** did not show a corresponding signal. Also, **4** showed two doublets at δ 2.93 and 2.24 ppm, for H-

TABLE 2. ^{13}C-Nmr Spectral Data of Compounds **1–5**.[a]

Carbon	Compound				
	1[b]	**2**[c]	**3**[c]	**4**[c]	**5**[c]
C-1	83.9 (CH)	83.1 (CH)	124.3 (CH)	212.2 (C=O)	201.5 (C=O)
C-2	209.4 (C=O)	209.5 (C=O)	148.3 (C)	73.1 (CH)	146.3 (C)
C-3	47.5 (CH_2)	47.5 (CH_2)	183.0 (C=O)	45.3 (CH_2)	120.8 (CH)
C-4	32.1 (CH)	32.1 (CH)	130.9 (C)	32.6 (CH)	31.0 (CH)
C-5	44.7 (CH)	44.7 (CH)	157.5 (C)	38.7 (CH)	44.5 (CH)
C-6	29.5 (CH_2)	29.5 (CH_2)	32.9 (CH_2)	29.5 (CH_2)	28.8 (CH_2)
C-7	83.6 (CH)	83.8 (CH)	85.2 (CH)	83.6 (CH)	83.2 (CH)
C-8	48.7 (C)	57.0 (C)	46.7 (C)	46.5 (C)	48.4 (C)
C-9	43.2 (CH)	43.2 (CH)	41.9 (CH)	34.7 (CH)	37.2 (CH)
C-10	47.0 (C)	48.7 (C)	44.3 (C)	44.7 (C)	47.0 (C)
C-11	75.7 (CH)	75.7 (CH)	75.9 (CH)	76.4 (CH)	75.3 (CH)
C-12	75.9 (CH)	76.0 (CH)	76.0 (CH)	78.5 (CH)	76.5 (CH)
C-13	82.3 (C)	82.5 (C)	82.3 (C)	83.0 (C)	83.0 (C)
C-14	51.0 (CH)	50.5 (CH)	49.5 (CH)	50.2 (CH)	50.7 (CH)
C-15	69.0 (CH)	68.5 (CH)	69.0 (CH)	68.6 (CH)	68.6 (CH)
C-16	168.3 (C=O)	167.1 (C=O)	167.7 (C=O)	167.0 (C=O)	167.3 (C=O)
C-17	73.2 (CH_2)	73.2 (CH_2)	72.9 (CH_2)	73.9 (CH_2)	73.8 (CH_2)
C-18	19.8 (CH_3)	19.8 (CH_3)	11.2 (CH_3)	20.0 (CH_3)	15.0 (CH_3)
C-19	12.6 (CH_3)	12.6 (CH_3)	24.3 (CH_3)	14.4 (CH_3)	19.5 (CH_3)
C-20	171.3 (C=O)	171.3 (C=O)	171.1 (C=O)	171.5 (C=O)	171.3 (C=O)
OMe	52.6 (CH_3)	52.3 (CH_3)	52.8 (CH_3)	52.4 (CH_3)	52.4 (CH_3)
C-1′	165.8 (C=O)	166.2 (C=O)	166.0 (C=O)	166.0 (C=O)	166.0 (C=O)
C-2′	113.6 (CH)	113.5 (CH)	113.5 (CH)	113.5 (CH)	113.6 (CH)
C-3′	169.6 (C)	168.4 (C)	169.6 (C)	168.2 (C)	168.4 (C)
C-4′	82.5 (C)	38.1 (CH)	83.1 (C)	38.2 (CH)	38.2 (CH)
Me-3′	14.5 (CH_3)	16.7 (CH_3)	14.5 (CH_3)	16.7 (CH_3)	16.7 (CH_3)
Me-4′	25.8 (CH_3)	20.7 (CH_3)	25.6 (CH_3)	20.7 (CH_3)	20.7 (CH_3)
Me-4′	26.4 (CH_3)	20.7 (CH_3)	26.5 (CH_3)	20.7 (CH_3)	20.7 (CH_3)
OAc-4′	163.5 (C=O)	—	163.5 (C=O)	—	—
OAc-4′	21.4 (CH_3)	—	21.4 (CH_3)	—	—

[a]Values are in δ ppm.
[b]Measured at 68 MHz in C_5D_5N.
[c]Measured at 125.7 MHz in C_5D_5N.

3α and H-3β, respectively, while **5** showed only one singlet at δ 5.87 ppm for the H-3 olefinic proton. The proton assignments for **4** were based on ^{1}H-^{1}H and ^{13}C-^{1}H COSY nmr spectra. These results suggested that the structures of **4** and **5** differ only in the degree of saturation of the A ring. Also, the molecular formula of **4** was 2 atomic mass units higher than that of **5**.

The ^{13}C-nmr spectrum of **4** was also similar to that of **5**, except for the signals of C-2 and C-3; compound **5** showed a quaternary carbon signal at 146.3 ppm (C-2) and a methine signal at 120.8 ppm (C-3), both corresponding to sp^2 carbons, while **4** showed a methine signal at 73.1 ppm and a methylene signal at 45.3 ppm corresponding to C-2 and C-3, respectively. The carbon assignments for **4** were based on ^{13}C-^{1}H COSY and DEPT nmr spectra.

Thus, **4** was assumed to arise from saturation of the A ring double bond of **5**. Indeed, **4** was obtained chemically by catalytic hydrogenation of **5**. The product was identified as **4** by comparing its tlc and hplc behavior and its ir and ^{1}H-nmr spectra with those of the authentic compound.

The configurations of the protons attached to C-2 and C-4 were determined by differential nOe measurement. On saturation of the signal due to Me-10, increases in the area of signals due to H-2 and H-4 were observed, indicating H-2β and H-4β orientations in the molecule of **4**.

Bruceanols G [**1**] and H [**2**] were evaluated against three cancer cell lines: SK-MEL-5 (melanoma), COLO-205 (colon cancer), and KB (nasopharynx carcinoma). These compounds were only marginally cytotoxic in the melanoma cell line with ED_{50} values of 4.08 and 6.37 μM, respectively. However, bruceanol G [**1**] showed activity against the COLO-205 and KB cell lines with ED_{50} values of 0.44 and 0.55 μM, respectively.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on an MRK air-bath type melting-point apparatus and are uncorrected. Specific rotations were obtained on a Jasco DIP-370 digital 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 JNM-A400, or a Jasco GSX-270 instrument in C_5D_5N using TMS as internal standard. 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 plates of 1 mm and 2 mm thickness were used for prep. tlc. Components on tlc were detected by a uv lamp. Lplc using a Kusano Lober column (ODS) and a mixed solvent of MeOH-H_2O (1:1) was carried out before performing prep. 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) using mixed solvents of MeOH-H_2O (55:45–40:60). Prep. 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.

PLANT MATERIAL.—As reported previously (1). Bruceanol F [**5**] used for chemical transformation into bruceanol H [**4**] was obtained from *B. antidysenterica* (5).

EXTRACTION AND ISOLATION.—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* (1,915 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. The brown gum (35.7 g) was subjected to lplc using a Kusano Lober column (ODS) and a mixed solvent of MeOH-H_2O (1:1) to afford 29 fractions. Fractions 11–13 were shown to contain an unknown compound by hplc analysis. Repeated prep. hplc of fractions 11–13 gave a new quassinoid, bruceanol G [**1**] (29.5 mg, 0.0000015%). Fractions 15–21 were also revealed to contain an unknown compound by hplc analysis. Repeated prep. hplc of fractions 15–21 gave a new quassinoid, bruceanol H [**4**] (13.4 mg, 0.0000007%).

Bruceanol G [**1**].—Colorless needles; mp 138–140°; $[\alpha]^{28}D$ +5.5° (*c*=0.051, EtOH); uv λ max (EtOH) 220 (ε 17600) nm; ir ν max (KBr) 3450 (OH), 1740 (ester and δ-lactone C=O), 1720 (α,β-unsaturated ester C=O), 1640 (C=C) cm^{-1}; ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2; sims *m/z* $[M+Na]^+$ 631 (3.6), $[M+H]^+$ 609 (0.5), $[M-C_2H_4O_2+H]^+$ 549 (7); hrsims *m/z* $[M+H]^+$ 609.2542 (calcd for $C_{30}H_{41}O_{13}$, 609.2544).

Bruceanol H [**4**].—Colorless needles (MeOH); mp 152–156°; $[\alpha]^{27}D$ +32.6° (*c*=0.067, EtOH); uv λ max (EtOH) 220 (ε 15360) nm; ir ν max (KBr) 3450 (OH), 1740 (ester and δ-lactone C=O), 1720 (α,β-unsaturated ester C=O), 1640 (C=C) cm^{-1}; ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2; eims *m/z* $[M]^+$ 550 (6.4), $[M-H_2O]^+$ 532 (4.2), $[C_5H_{11}O]^+$ 111 (100); hrsims *m/z* $[M]^+$ 550.2393 (calcd for $C_{28}H_{38}O_{11}$, 550.2414).

CATALYTIC HYDROGENATION OF BRUCEANOL F [**5**] INTO BRUCEANOL H [**4**].—To a solution in MeOH (3 ml) of **5** (5.0 mg), palladium-carbon catalyst (6 mg) was added. The mixture was stirred under a H_2 atmosphere at room temperature for 5 min. After removing the catalyst by filtration, the solvent was evaporated to afford a crude product. The crude product was purified by prep. hplc [Lichrosorb RP-18, MeOH-H_2O (4:6)] to yield **4** (1.3 mg, 26.7% yield); mp 152–156°.

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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. Tagahura, T. Aratani, and K.H. Lee, *J. Nat. Prod.*, **51**, 349 (1988).
5. K. Imamura, N. Fukamiya, M. Okano, K. Tagahara, and K.H. Lee, *J. Nat. Prod.*, **56**, 2091 (1993).
6. M. Okano, N. Fukamiya, T. Toyota, K. Tagahara, and K.H. Lee, *J. Nat. Prod.*, **52**, 398 (1989).
7. N. Fukamiya, M. Okano, T. Aratani, K. Negoro, A.T. McPhail, M. Ju-ichi, and K.H. Lee, *J. Nat. Prod.*, **49**, 428 (1986).
8. N. Fukamiya, M. Okano, T. Aratani, K. Negoro, Y.M. Lin, and K.H. Lee, *Planta Med.*, **53**, 140 (1987).
9. T. Toyota, N. Fukamiya, M. Okano, K. Tagahara, J.J. Chang, and K.H. Lee, *J. Nat. Prod.*, **53**, 1526 (1990).

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