ANTITUMOR AGENTS. 39.[1] BRUCEANTINOSIDE-A AND -B, NOVEL ANTILEUKEMIC QUASSINOID GLUCOSIDES FROM *BRUCEA ANTIDYSENTERICA*

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ABSTRACT.—The new antileukemic quassinoid glucosides bruceantinoside-A (**1**) and -B (**2**) were isolated from *Brucea antidysenterica*. The structure of **1** and **2** has been established from chemical transformation, correlations and spectral analyses, especially the ^{13}C-nmr spectral data.

An earlier investigation of the antileukemic constituents of the Ethiopian *Brucea antidysenterica* by Kupchan and associates has led to the isolation of bruceantin (**3**) and seven other related quassinoids (1). Bruceantin is currently in Phase II clinical trial by the National Cancer Institute (2–5).

As a result of our search for new/novel antitumor principles, two novel antileukemic quassinoid glycosides bruceoside-A (**4**) and -B (**5**) were isolated from a Chinese anticancer drug, *Brucea javanica* (6, 7). Both bruceoside-A and -B gave brusatol (**6**), which was also isolated from the chloroform extract of *B. javanica*[2] upon acid hydrolysis (6, 7).[3] The fact that brusatol (**6**) is structurally identical with bruceantin (**3**) except for the slight difference in the C–15 ester side chain, in which **6** has a senecioate whereas **3** bears a *trans*-3,4-dimethyl-2-pentenoate moiety, suggested that glycosides of **3** or related compounds might be found in the extract of *B. antidysenterica*. Subsequent careful examination of the thin layer chromatogram from a chloroform soluble fraction of *B. antidysenterica* revealed the presence of spots differing from those previously isolated by Kupchan *et al.* (1). Two of these spots showed R_f values slightly higher than those of bruceoside-A and -B. Isolation of these two compounds by an initial column chromatography of the fraction on silica gel followed by further purification of the corresponding resulting fractions with preparative thin layer chromatography and high performance liquid chromatography yielded the desired new glycosides, which were provisionally named bruceantinoside-A (**1**) and -B (**2**), as amorphous powders. Structural characterization of **1** and **2** was achieved by direct comparison of their hydrolysis products and ^{13}C nmr spectra with those of bruceoside-A (**4**) and related compounds.

RESULTS AND DISCUSSION

The ^{13}C nmr spectral difference between **1** and **4** corresponds very well to the difference between **3** and **6** (table 1). Thus the spectral data indicates that **1** is structurally identical with **4** except for the difference in the C–15 ester side chain in which **1** possesses a *trans*-3,4-dimethyl-2-pentenoate as found in **3**, and **4** contains a senecioate moiety as seen in **6**. This was further supported by the fact that the

[1]For part 38, see K. H. Lee, T. Ibuka, H. Furukawa, M. Kozuka, R. Y. Wu, I. H. Hall and H. C. Huang, *J. Pharm. Sci.*, **69**, 1050 (1980).

[2]K. H. Lee and M. Okano, unpublished data.

[3]K. H. Lee, M. Okano and I. H. Hall, *J. Pharm. Sci.*, submitted.

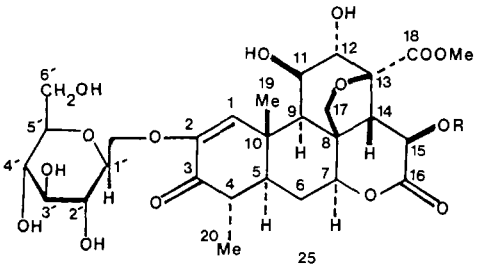
TABLE 1. Chemical shifts and assignments of quassinoids (6–8).[a]

	9	6	3	2	8	7	4	1
C-1	42.4	42.4	42.4	44.1	130.1	130.1	129.2	129.6
C-2	193.0	192.9	193.0	199.7	150.1	150.2	148.8	148.8
C-3	145.7	145.9	145.9	146.4	195.0	195.1	194.6	194.8
C-4	128.5	128.3	128.3	125.8	43.8	43.8	43.7	43.8
C-5	42.4	42.0	42.1	40.8	40.9	40.9	40.3	40.4
C-6	29.6	29.5	29.6	29.6	30.0	29.9	29.8	29.9
C-7	83.1	83.5	83.6	83.0	82.9	82.9	84.3	83.4
C-8	45.7	46.0	46.1	46.6	46.3	46.3	46.5	46.6
C-9	42.2	42.0	42.1	42.1	41.2	42.1	41.3	41.3
C-10	41.3	41.3	41.4	48.8	39.6	39.6	39.5	39.6
C-11	72.8	73.0	73.1	71.3	72.2	71.1	71.1	71.3
C-12	76.8	75.7	75.8	76.2	77.1	77.0	75.9	76.0
C-13	82.6	82.6	82.7	82.6	82.6	82.6	82.5	82.5
C-14	52.3	52.3	52.4	52.3	52.3	52.3	52.3	52.3
C-15	66.1	68.2	68.3	68.7	66.2	66.2	68.0	68.3
C-16	172.7	168.2	168.3	168.4	172.6	172.7	168.2	168.2
C-17	74.1	73.6	72.3	73.6	74.1	74.1	73.4	73.3
C-18	173.5	171.2	171.3	171.1	173.5	173.6	171.1	171.1
C-19	15.6	15.7	15.7	18.9	17.7	17.6	17.8	17.8
C-20	13.4	13.4	13.4	14.5	12.4	12.4	12.5	12.5
COOMe	55.2	50.0	50.1	50.7	55.2	55.2	50.2	50.2
C-21		165.3	165.8	165.9			165.3	165.7
C-22		115.8	113.4	113.4			115.9	113.4
C-23		158.5	167.2	167.3			158.5	167.1
C-24		27.0	38.1	38.1			27.0	38.1
C-25		20.1	20.7	20.7			20.1	20.7
C-26			20.7	20.7				20.7
C-27			16.7	16.7				16.7
C-1'				100.7	102.0	102.0	101.9	102.0
C-2'				74.5	74.4	74.4	74.5	74.6
C-3'				78.7	78.7	78.7	78.8	78.8
C-4'				71.3	71.2	71.1	71.1	71.3
C-5'				78.3	78.2	78.1	78.4	78.3
C-6'				62.4	62.2	62.3	62.2	62.3

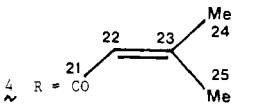
[a]Run in pyridine-d_5, and the δ values are in parts per million downfield from Me_4Si. The assignment of the carbon resonances for the basic quassinoid skeleton and the glucopyranosyl moiety were comparable to those reported by Polonsky (9) and Kasai (10), respectively.

^{13}C nmr spectrum of de-3,4-dimethyl-2-pentenoyl bruceantinoside-*A* (**7**) resulting from an alkaline hydrolysis of **1** coincided with that of desenecioyl bruceoside-A (**8**) which was obtained by an analogous alkaline hydrolysis of **4** (table 1). Acid hydrolysis of **1** with 3N sulfuric acid-methanol (1:1) yielded D-glucose, identified by gas liquid chromatography as its trimethylsilyl derivative, and the aglycon, which was identified as bruceantin (**3**) by a direct comparison (comparable tlc, ir and nmr spectral analyses) with an authentic sample. The above evidence led to the structural assignment of bruceantinoside-A as **1**.

Bruceantinoside-B (**2**) gave, upon the same acid hydrolysis as described above for **1**, the identical D-glucose and bruceantin (**3**), suggesting that **2** is a glucoside of **3** and that the D-glucose moiety of **2** might be located at either C-3, C-11 or C-12 position. The similarity of the chemical shifts for the C-11 and C-12 signals between **1** and **2** (table 1) indicated that D-glucose should be in position C-3. This is further supported by the comparable chemical shifts for C-1, C-2, C-3, and C-4 between **2** and **3**, leading to the structural assignment of bruceantinoside-B as bruceantin 3-β-D-glucopyranoside (**2**). The carbon resonances of the sugar moiety (C-1' to C-6') of **2** is comparable to those of a β-anomer of D-glucose as reported by Kasai *et al.* (10).



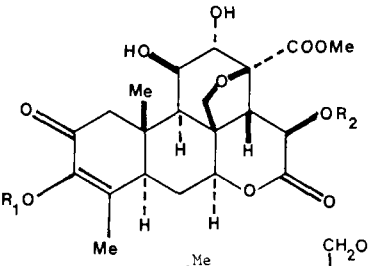
1 R = CO (Bruceantinoside-A)

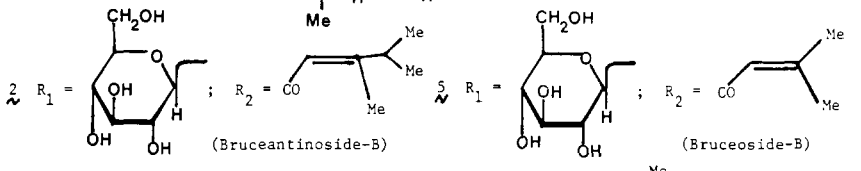


4 R = CO (Bruceoside-A)

7 R = H (De-3,4-dimethyl-2-pentenoyl bruceantinoside-A)

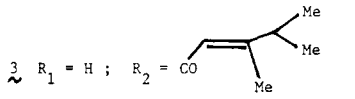
8 R = H (Desenecioyl bruceoside-A)



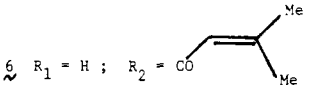


2 R_1 = ; R_2 = CO (Bruceantinoside-B)

5 R_1 = ; R_2 = CO (Bruceoside-B)



3 R_1 = H ; R_2 = CO (Bruceantin)



6 R_1 = H ; R_2 = CO (Brusatol)

9 R_1 = R_2 = H (Bruceolide)

EXPERIMENTAL[4]

[4]Melting points were determined on a Thomas-Hoover melting point apparatus and were uncorrected. Specific rotations were obtained on a Rudolph Autopol III automatic polarimeter (l=0.5 dm). Infrared (ir) spectra were recorded on a Perkin-Elmer 257 grating ir spectrometer. Proton nuclear magnetic resonance (pmr) spectra were determined on a Varian XL-100 spectrometer (Me_4Si as an internal standard). ^{13}C-nmr spectra were recorded on a Varian XL-100 spectrometer functioning at 25.20 MHz. All nmr spectra were obtained with the use of the Fourier transform technique. Mass spectra were determined on an A.E.I. MS-902 instrument at 70 eV using a direct inlet system. Gas-liquid chromatography (glc) was performed on a Varian model 3700 gas chromatograph. Silica gel (Merck silica gel 60PF-254) was used for column chromatography, and precoated silica gel (Merck silica gel 60 F-254, 2 mm) was used for preparative thin layer chromatography (ptlc). Detection of components was made either by spraying with 1% cerium sulfate-10% sulfuric acid solution followed by heating or by use of a uv lamp. High performance liquid chromatography (hplc) was performed on a Waters Associates Model ALC/GPC 244 liquid Chromatograph using a Whatman Partisil M9 10/50 column.

PLANT MATERIAL AND EXTRACTION.—The ground wood of *Brucea antidysenterica* (Simaroubaceae) (4228 lbs) was percolated three times with methanol. The combined methanol extracts were concentrated to 440 lbs, dissolved in a mixture of water-methanol (78:22, 116 gal.) and extracted with methylene dichloride (86;55;55 gal.). The combined methylene dichloride extracts were concentrated to 32 gal. and partitioned between 10% aqueous methanol (232 gal.) and *n*-hexane (186 gal.). The aqueous methanol extract was diluted with water (63 gal.) and extracted three times with carbon tetrachloride (136, 110, and 110 gal., respectively). The aqueous methanol layer was further diluted with water (83 gal.) and extracted four times with chloroform (55 gal. each time) to give rise to the chloroform extract.

CHROMATOGRAPHY OF THE CHLOROFORM FRACTIONS.—The crude chloroform fraction (181 g) was subjected to column chromatography on silica gel (1 kg, 7 x 60 cm and eluted with chloroform-methanol-water, 50:14:3, v/v) to yield four fractions: Fraction 1 (brown oil, 32.5 g), Fraction 2 (brown viscous oil, 68.6 g), Fraction 3 (amorphous, 51.1 g) and Fraction 4 (amorphous, 12.0 g). Ir spectra of Fraction 3 and Fraction 4 showed characteristic absorption bands of glycosides similar to those of bruceoside-A at 3400, 1060 and 1040 cm^{-1}.

ISOLATION OF BRUCEANTINOSIDE-A (**1**).—Rechromatography of Fraction 3 (51.1 g) on silica gel (1 kg, 7 x 60 cm) with elution by chloroform-methanol-water (50:14:3, v/v) gave fractions (10.1 g, amorphous) which showed slightly higher R_f value compared to that of bruceoside-A (6). Subsequent purification of this fraction by ptlc (chloroform-acetone 1:1) afforded an amorphous compound (5.6 g). This amorphous compound was further subjected to hplc (1-butanol-ethyl acetate-water 4:1:2, upper layer) to yield pure bruceantinoside-A (**1**, amorphous): mp ca. 150° (dec.); $[\alpha]^{25}D$ +7.8° (c 0.6, pyridine); ir (KBr) 3410, 1060, 1040 (glycosidic OH), 1725 (δ-lactone and ester CO), 1678 (α,β-unsaturated CO), 1635 and 800 (C=C) cm^{-1}; Pmr (pyridine-d_5) δ 0.84 (6H, d, J=7 Hz, Me-24), 1.13 (3H, d, J=6 Hz, Me-4), 1.59 (3H, s, Me-10), 2.14 (3H, s, Me-23), and 3.76 (3H, s, COOMe).

The presence of a glucose moiety in **1** was also revealed by the characteristic ions of the trimethylsilyl ether of the sugar moiety at m/e 271.1184 (calcd for $C_{12}H_{23}O_6Si_2$: 217.1184) and 361 ($C_{15}H_{33}O_4Si_3$) in the mass spectrum of a trimethylsilyl ether of **1**.

DE-3,4-DIMETHYL-2-PENTENOYL BRUCEANTINOSIDE-A (**7**).—A solution of **1** (709 mg) in 0.1 N potassium hydroxide-methanol (20 ml) was added with 1N potassium hydroxide-methanol (10 ml). After the mixture was stirred at room temperature for 22 hr, it was neutralized with cation exchange resin (Dowex 50 W-X2) and filtered. The filtrate was methylated with diazomethane-ether[5] in the usual manner. The methylated product was evaporated *in vacuo* and purified by ptlc (chloroform-methanol-water, 50:14:3, v/v) to yield **7** (126 mg, amorphous): mp ca. 190° (dec.); ir (KBr) 3400, 1068, 1042 (glycosidic OH), 1725 (δ-lactone and ester CO), and 1675 (α,β-unsaturated CO) cm^{-1}; pmr ($CDCl_3$+CD_3OD) δ 1.17 (3H, d, J=6 Hz, Me-4), 1.62 (3H, s, Me-10), 3.87 (3H, s, COOMe), 5.21 (1H, d, J=13 Hz, H-15), and 6.99 (1H, s, H-1).

DESENECIOYL BRUCEOSIDE-A (**8**) FROM BRUCEOSIDE-A (**4**).—Bruceoside-A (**4**, 1.36 g) was dissolved in 1N potassium hydroxide-methanol (34 ml) at 0°. The mixture was stirred at 0° for 20 min. and neutralized with 3N sulfuric acid and then subjected to ptlc (chloroform-methanol-water, 50:14:3, v/v) to give an amorphous compound (**8**, 533 mg): mp ca. 200° (dec.). The pmr spectrum of **8** is exactly identical to that of **7** described above.

BRUCEANTIN (**3**) AND D-GLUCOSE FROM BRUCEANTINOSIDE-A (**1**).—A solution of **1** (96 mg) in 3N sulfuric acid-methanol (1:1, v/v, 20 ml) was refluxed for 7 hr and then extracted with chloroform. The chloroform extract was dried (anhydrous magnesium sulfate), filtered, and evaporated *in vacuo* to give a product which was subjected to ptlc (chloroform-acetone, 1:1, v/v) to yield a pure **3** (17 mg): ms m/e 548.2250 (calcd for $C_{28}H_{36}O_{11}$, 548.2256). The identity of **3** was confirmed by a direct comparison (mixed mp, tlc, specific rotation, ir, pmr, ^{13}C-nmr, and mass spectra) with an authentic sample of bruceantin (1, 8).

The aqueous layer was passed through a column of anion exchange resin (Amberlite IR-45) and evaporated under reduced pressure to give a residue. Part of this residue (ca. 1 mg) was treated with one drop of TRISIL at 80° for 1 hr, and the product was extracted with hexane. hexane extract was analyzed by glc (3% OV-17 on chromosorb, 80–100 mesh, 3 mm X 2 m, 170°, N_2, 15 ml/min) to reveal two peaks at 9.6 and 13.8 min. which were identified as trimethylsilyl D-glucose (α-, and β-) by comparison with those of an authentic sample prepared from a trimethylsilylation of D-glucose.

ISOLATION OF BRUCEANTINOSIDE-B (**2**).—Preparative thin layer chromatography of Fraction 4 (12.0 g) on silica gel furnished an amorphous compound (6.9 g) showing a R_f value somewhat higher than that of bruceoside-B (**5**) on tlc (chloroform-methanol-water, 50:14:3, v/v). This compound was further chromatographed twice on sephadex (Pharmacia Sephadex LH-20, 35 mm X 75 cm) to yield pure **2** (2.1 g, amorphous): mp ca. 200° (dec.); $[\alpha]^{25}D$ −3.6° (c 0.5, pyridine); ir (KBr) 3440, 1070, 1035 (glycosidic OH), 1725 (δ lactone and ester CO), 1675 (α,β-un-

[5]Further methylation of C-13 COOMe was needed as it had been partially hydrolyzed.

saturated CO), 1635 and 800 (C=C) cm^{-1}; pmr (pyridine-d_5) δ 0.89 (6H, d, J=7 Hz, Me-24), 1.40 (3H, s, Me-10), 1.86 (3H, s, Me-4), 2.17 (3H, s, Me-23), and 3.78 (3H, s, COOMe).

BRUCEANTIN (**3**) and D-GLUCOSE FROM BRUCEANTINOSIDE-B (**2**).—A solution of **2** (246 mg) in 3N sulfuric acid-methanol (1:1, 50 ml v/v) was refluxed for 7 hr and worked up in a manner similar to that described above for the hydrolysis of **1** to yield a D-glucose (identified by glc as the trimethylsilyl derivative) and an aglycon **3**, which was shown to be identical with bruceantin.

BIOLOGICAL ACTIVITY.—*In vivo* activity was assayed by the standard National Cancer Institute procedures described in literature reference 11. Bruceantinoside-A (**1**) and -B (**2**) showed significant (T/C≥125%) antileukemic activity in P-388 lymphocytic leukemia (e.g. **1** at 0.5, 1, 6, 10, and 20 mg/kg afforded T/C % values of 110, 106, 118, 152 and 172, respectively, and **2** at 10 and 20 mg/kg afforded T/C % 132 and 160, respectively).

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LITERATURE CITED

1. S. M. Kupchan, R. W. Britton, J. A. Lacadie, M. F. Ziegler and C. W. Sigel, *J. Org. Chem.*, **40**, 648 (1975).
2. M. Suffness and J. Douros, "Methods in Cancer Research," V. T. DeVita and H. Busch, eds., Academic Press, New York, 1979, Vol. XVI, Chapter III.
3. *National Cancer Institute—DCT Bulletin*, July, P. 4 (1979).
4. A. Y. Bedikian, M. Valdivieso, G. P. Bodey, W. K. Murphy and E. J. Freireich, *Cancer Treatment Reports*, **63**, 1843 (1979).
5. M. B. Garnick, R. H. Blum, G. P. Canellos, R. J. Mayer, L. Parker, A. T. Skarin, F. P. Li, I. C. Henderson and E. Frei, *Cancer Treatment Reports*, **63**, 1929 (1979).
6. K. H. Lee, Y. Imakura and H. C. Huang, *J. Chem. Soc.-Chem. Comm.*, 69 (1977).
7. K. H. Lee, Y. Imakura, Y. Sumida, R. Y. Wu and I. H. Hall, *J. Org. Chem.*, **44**, 2180 (1979).
8. M. Okano and K. H. Lee, *J. Org. Chem.*, **46**, 1138 (1981).
9. J. Polonsky, Z. Baskévitch, H. E. Gottlieb, E. W. Hagaman and E. Wenkert, *J. Org. Chem.*, **40**, 2499 (1975).
10. R. Kasai, M. Okihara, J. Asakawa, K. Mizutani and O. Tanaka, *Tetrahedron Letters*, **35**, 1427 (1979).
11. R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher and B. J. Abbott, *Cancer Chemother. Rep., Part* **3**, **3**, 1 (1972).