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Bioactive alkaloids from *Brunsvigia radulosa*[☆]

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Abstract

A phytochemical investigation of the bulbs of *Brunsvigia radulosa* yielded the new alkaloid 1-*O*-acetylnorpluviine, together with the known structures 1-epideacetylbowdensine, crinamine, crinine, hamayne, lycorine, anhydrolycorin-6-one and sternbergine. All structures were established by spectroscopic evidence. Some of the ^{13}C assignments which were reported for crinamine and hamayne were corrected by means of 2D NMR techniques. In order to provide a further structure for biological testing, crinamine was converted to apohaemanthamine. The alkaloids were tested for activity against two strains of cultured *Plasmodium falciparum* and for cytotoxicity with BL6 mouse melanoma cells. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Amaryllidaceae; *Brunsvigia radulosa*; Alkaloids; 1-*O*-acetylnorpluviine; Apohaemanthamine; 1-Epideacetylbowdensine; Crinamine; Crinine; Hamayne; Lycorine; Anhydrolycorin-6-one; Sternbergine

1. Introduction

The genus *Brunsvigia*, subtribe Amaryllidinae, tribe Amaryllideae (Amaryllidaceae), is widely distributed over both summer- and winter- rainfall regions of the southern and southwestern regions of South Africa. Several Amaryllideae species have been used in the traditional medicine of the indigenous peoples of the country (Hutchings, Scott, Lewis & Cunningham, 1996). In a continuation of our phytochemical studies on genera from the subtribes Amaryllidinae and Crininae (Viladomat, Bastida, Codina, Nair & Campbell, 1997), we have investigated the taxon *Brunsvigia radulosa* Herb. The choice of this species was prompted by a report of the inhibitory activity of an extract of the plant against P 388 lymphocytic leukaemia in mice (Charlson, 1980), and by our own observation of moderate activity against two strains of *Plasmodium falciparum*. In the present study we describe the isolation and characterisation of the novel alkaloid 1-*O*-acetylnorpluviine (**1**), together with the known structures, 1-epideacetylbowdensine, crinamine, crinine, hamayne, lycorine, anhydrolycorin-6-one (**2**) and sternbergine. In addition, in order to provide a further structure for our studies on the antiplasmodial and cytotoxic activity of Amaryllidaceae alkaloids, crinamine was converted to the C-3/C-11 epoxide, apohaemanthamine (**3**).

2. Results and discussion

The alkaloids were isolated from an ethanol extract of the fresh bulbs of *Brunsvigia radulosa*. 1-*O*-acetyl-

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[☆] Part 7 in the series "Alkaloids from South African Amaryllidaceae". For part 6 see Nair et al., 1998. [Nair, J.J., Campbell, W.E., Gammon, D.W., Albrecht, C.F., Viladomat, F., Codina, C. & Bastida, J. (1998). *Phytochemistry*, 49, 2539].

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norpluviine (**1**) gave a M^+ ion at 315.1465 in the high resolution EI mass spectrum, which corresponded with a molecular formula, $C_{18}H_{21}NO_4$. The mass spectrum and the ^{1}H-NMR spectrum showed structural features similar to those of the known pyrrolo[de]phenanthridine alkaloid, 1-*O*-acetylcaranine (Pettit, Gaddamidi, Goswami & Cragg, 1984). The four singlets at δ 6.74, 6.64, 3.83, and 1.90 in the ^{1}H-NMR spectrum (Table 1) were assigned to the two aromatic protons (H-10 and H-7), the MeO group and an acetoxy methyl respectively. A correlation between the methyl protons of the MeO group and H-10 in the 2D NOESY spectrum allowed us to assign the group at C-9. The two doublets at δ2.76 and 2.66 ($J = 9.9$ Hz) were attributed to the two methine protons H-4a and H-10b, respectively. The large coupling constant supported a *trans*-fusion of the B/C rings. The location and axial configuration of the acetoxy group at C-1 were deduced from the COSY and NOESY experiments. The equatorial H-1 resonated as a double doublet at δ5.97 ($J =$ 5.9, 1.0 Hz) and was coupled in the COSY spectrum to H-10b and H-2β, and correlated with H-10 in the NOESY spectrum. The near zero value for $J_{1,\,10b}$ and the absence of coupling between H-1 and H-2α confirmed their orthogonal relationship. H-3 exhibited a doublet at δ5.39 ($J = 2.2$ Hz), and a COSY correlation was observed to H-2α, but not to the orthogonal H-2β. H-2α and H-2β were represented by multiplets at δ2.33 and δ2.62, respectively. Finally the doublets at δ4.13 and 3.50 were assigned to the AB system of the benzylic protons 6*β* and 6α, the *ddd* at δ3.34 to H-12β, the *m* at δ2.59 to H-11α and H-11β and the *m* at δ2.37 to H-12α. The ^{13}C-NMR spectrum (Table 3) together with the information from the DEPT spectrum, showed the presence of 18 carbon resonances which could be assigned to two methyl, four methylene, six methine and six quaternary carbon atoms, and from the HMQC experiment the protonated carbon resonances could be unambiguously assigned. From the HMBC spectrum, three bond correlations were observed for H-7 to C-9, H-10 to C-8, H-7 to C-10a, H-10 to C-6a and H-10b to C-4, enabling us to identify the resonances of the quaternary carbons, C-8, C-9, C-6a, C-10a and C-4. In addition a three bond coupling from the methyl protons to C-9 confirmed the NOESY result.

The structures of 1-epideacetylbowdensine, crinamine, crinine, hamayne, lycorine and sternbergine were confirmed by a comparison of their ^{1}H and ^{13}C-NMR and EIMS data with authentic compounds isolated in our own laboratories. (Viladomat et al., 1997). However, the HMQC spectra for crinamine and hamayne indicated that previous ^{13}C assignments for C-6 and C-12 should be interchanged (Viladomat, Bastida, Codina, Campbell & Mathee, 1994; Frahm, Ali & Ramadan, 1985; Kobayashi, Tokumoto, Kihara, Imakura, Shingu & Taira, 1984). The IR, ^{1}H-NMR and MS for anhydrolycorin-6-one (**2**) were in agreement with the literature (Hänsel & Thober, 1982). The ^{13}C spectrum (Table 3), however, is reported for the first time, and all resonances were confirmed by the HMQC and HMBC techniques.

Of the Amaryllidaceae alkaloids previously shown to have significant antimalarial and cytotoxic activity, augustine with an oxirane ring between C-1 and C-2, has been the most active (Likhitwitayawuid, Angerhofer, Chai, Pezzuto & Cordell, 1993). In order to test whether other structures with the epoxide functionality are similarly active, crinamine was converted to the C-3, C-11 epoxide, apohaemanthamine (**3**), using the procedure of Fales and Wildman (Fales & Wildman, 1960). No spectroscopic data for this alkaloid have been reported. A significant feature of the ^{1}H-NMR spectrum (Table 2) was the *dd* ($J = 8.6$, 5.3 Hz) at δ6.77 which was

Table 1
^{1}H-NMR assignments for compound **1** (*J* in Hz in parentheses)

H	δ($CDCl_3$)
1	5.97 *dd* (5.9, 1.0)
2α	2.33 *m*
2*β*	2.62 *m*
3	5.39 *d* (2.2)
4a	2.76 *d* (9.9)
6α	3.50 *d* (14.5)
6*β*	4.13 *d* (14.5)
7	6.64 *s*
10	6.74 *s*
10b	2.66 *d* (9.9)
11α, *β*	2.59 *m*
12α	2.37 *m*
12*β*	3.34 *ddd* (11.3, 8.4, 4.5)
9-OMe	3.83 *s*
1-OAc	1.90 *s*

Table 2
^{1}H-NMR assignments for compound **3** (*J* in Hz in parentheses)

H	δ($CDCl_3$)
1	6.64 *dd* (8.6, 1.5)
2	6.77 *dd* (8.6, 5.5)
3	4.41 *ddt*(5.5, 3.8, 1.5)
4α	1.82 *ddd* (14.0, 10.0, 1.5)
4*β*	1.89 *ddd* (14.0, 3.8, 2.0)
4a	3.06 *dd* (10.0, 2.0)
6α	3.72 *d* (16.8)
6*β*	4.31 *d* (16.8)
7	6.49 *s*
10	6.84 *s*
11_{endo}	3.72 *dd* (4.5, 1.5)
12_{endo}	3.09 *ddd* (13.5, 4.5, 1.5)
12_{exo}	3.29 *d* (13.5)
–OCH_2O–	

assigned to H-2. Formation of the epoxide forces ring C to adopt a twist-boat conformation, H-3 then becomes pseudoequatorial with a dihedral angle between H-2 and H-3 of *ca* 0°. Relative to crinamine, the lone pair electrons on oxygen induced a deshielding effect on both H-1 ($\Delta\delta 0.43$) and H-2 ($\Delta\delta 0.61$). The ^{13}C resonances were assigned from the connectivities in the HMQC and HMBC spectra (Table 3).

Compounds 1-*O*-acetylnorpluviine (**1**), 1-epideacetylbowdensine, crinamine, hamayne, anhydrolycorin-6-one (**2**), sternbergine and apohaemanthamine (**3**) were tested for activity against the chloroquine-sensitive (D 10) and chloroquine-resistant (FAC 8) strains of cultured *Plasmodium falciparum*, and for cytotoxicity with BL6 mouse melanoma cells. The results are summarised in Table 4. Previous studies have shown that lycorine exhibits significant antimalarial and cytotoxic activity, while crinine is inactive (Campbell et al., 1998; Likhitwitayawuid et al., 1993). Among the pyrrolophenanthridines, 1-*O*-acetylnorpluviine (**1**) demonstrated strong cytotoxic and moderate antiplasmodial activity, while anhydrolycorin-6-one (**2**) and sternbergine gave moderate activity in both tests. The 5,10b-ethanophenanthridine structures with the α-bridge, crinamine and hamayne were moderately active, the antiplasmodial result for crinamine agreeing with that previously published (Likhitwitayawuid et al., 1993). 1-Epideacetylbowdensine with a β-5,10b-ethano bridge was inactive. The inactivity of apohamaemanthamine (**3**) which has an epoxide function and an α-bridge was not anticipated and could be due to the conformation of ring C.

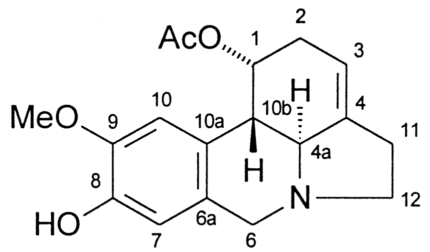
3. Experimental

3.1. General

Mps are uncorr. IR spectra were recorded in KBr discs. EIMS were obtained at 70 eV. NMR spectra were measured using Varian VXR 400 and VXR 500 instruments, in the solvents specified and with TMS as int. standard. Chemical shifts are reported in δ units (ppm) and coupling constants (J) in Hz. Silica gel 60 F_{254} (Merck) was used for analytical (0.25 mm) TLC and flash cc was carried out on Silica gel N for thin layer chromatography (Macherey Nagel). Spots on chromatograms were detected under UV (254 and 366 nm).

3.2. Plant material

Bulbs of *B. radulosa* Herb. were collected in April, 1994 near the H.F. Verwoed Dam in the Orange Free State, South Africa. A voucher specimen (du Preez s.n.) has been deposited in the Herbarium at the National Museum, Bloemfontein, South Africa.



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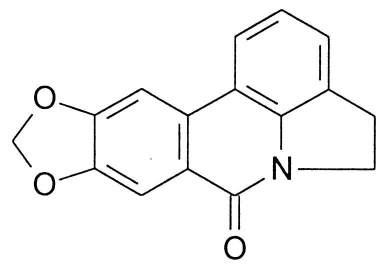

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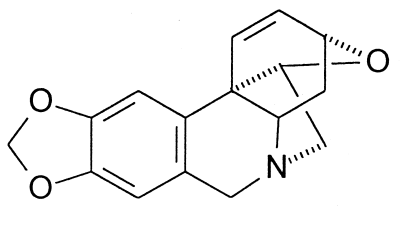

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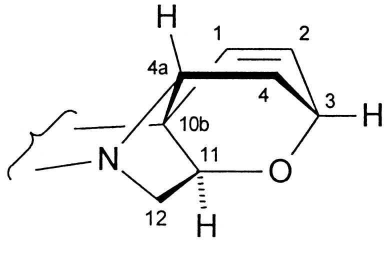

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Table 3
^{13}C-NMR assignments for compounds **1**, **2** and **3**

C	1	2	3
1	66.1 *d*	119.4 *d*	129.7 *d*
2	33.4 *t*	123.2 *d*	137.6 *d*
3	114.3 *d*	123.8 *d*	65.2 *d*
4	139.2 *s*	130.8 *s*	35.5 *t*
4a	61.2 *d*	139.8 *s*	61.0 *d*
6	56.4 *t*	159.5 *s*	60.1 *t*
6a	128.6 *s*	129.0 *s*	126.7 *s*
7	113.1 *d*	106.8 *d*	106.6 *d*
8	144.0 *s*	148.2 *s*	146.7 *s*
9	145.2 *s*	151.7 *s*	146.8 *s*
10	107.3 *d*	100.9 *d*	106.4 *d*
10a	125.7 *s*	130.9 *s*	131.3 *s*
10b	43.1 *d*	116.8 *s*	47.3 *s*
11	28.5 *t*	27.4 *t*	81.1 *d*
12	53.7 *t*	46.5 *t*	61.9 *t*
–OCH$_2$O–	56.2 *q*	102.0 *t*	100.9 *t*
9-OMe	21.3 *q*	–	–
1-0Ac	170.8 *s*	–	–

3.3. Extraction and isolation of alkaloids

Bulbs (3.1 kg) were macerated and stirred with cold EtOH (5 l) for 48 h The extract was evapd under red. pres. and the residue dissolved in H$_2$O and acidified to pH 4. After removal of neutral material with Et$_2$O, the acid solution was extracted with CH$_2$Cl$_2$ to give fraction A(3.74 g). Subsequent basification to pH 8–9, followed by extraction firstly with CH$_2$Cl$_2$ and then with CH$_2$Cl$_2$/MeOH (3:2) gave fraction B (9.72 g). Fractions A and B were combined and repeatedly chromatographed by flash chromatography on silica gel, using CH$_2$C$_{12}$–EtOH of increasing polarity, to give **1** (15 mg), 1-epideacetylbowdensine (21 mg), crinamine (1.1 g), crinine (1.3 g), hamayne (33 mg), lycorine (25 mg), **2** (10 mg) and sternbergine (11 mg).

3.4. 1-O-acetylnorpluviine (1)

White amorphous powder from acetone–hexane, mp 185–187°. $[\alpha]_D^{22}$-67° (EtOH; *c* 0.25). IR ν_{max}^{KBr}cm^{-1}: 1726(CO), 1504, 1486, 1378, 1265, 1250, 1034. ^{1}H-NMR: Table 1. ^{13}C-NMR: Table 3. EIMS *m*/*z* (rel. int.): 315[M]$^+$(72), 272(2), 254(55), 229(33), 228(45), 126(8), 115(12), 97(8), 69(26), 43(100); HRMS *m*/*z* 315.1465[M]$^+$ (C$_{18}$H$_{21}$NO$_4$ requires: 315.1471).

1-Epideacetylbowdensine, crinamine, crinine, hamayne, lycorine, sternbergine (Viladomat et al., 1997) were identified by a comparison of their chromatographic and spectroscopic data with those of authentic compounds isolated from other plant sources. For anhydrolycorin-6-one, IR, MS and proton shifts were in agreement with reference data (Hänsel & Thober, 1982). As no carbon shifts could be found, these data are added (Table 3).

3.5. Conversion of crinamine to apohaemanthamine (3)

Crinamine (106 mg) was dissolved in 6M HCl (3 ml) and heated at 100°C for 1 h. The soln was poured into 100 ml H$_2$O, basified with aqueous NH$_3$ and extracted with CHCl$_3$ (3 × 20 ml). The dried extract was concentrated to give an oil (71 mg) which was chromatographed over silica gel, eluting with benzene–EtOAc (9:1) to give **3** (30 mg). Colourless crystals from cyclohexane, mp 145–147° $[\alpha]_D^{22}$ +198° (CHCl$_3$; *c* 0.64). IR ν_{max}^{KBr} cm^{-1}: 2932, 1504, 1482, 1383, 1321, 1232, 1034, 934, 819, 749, 721. ^{1}H- NMR: Table 2. ^{13}C-NMR: Table 3. EIMS *m*/*z* (rel. int.): 269[M]$^+$ (97), 240(7), 214(20), 186(18), 181(24), 129(4), 115(4), 107(8), 94(27), 86(90), 73(27), 69(65), 57(76), 43(100).

3.6. Antiplasmodial and cytotoxicity assays

The biological evaluations for cytotoxic and antiplasmodial activities of the alkaloids were carried out

Table 4
Biological activities of the alkaloids

Alkaloid	Antiplasmodial activity IC$_{50}$ (μg/ml)		Cytotoxic activity IC$_{50}$ (μg/ml)
	Strain D10	Strain FAC8	
1-*O*-acetylnorpluviine (**1**)	28.3	34.2	1.6
1-Epideacetylbowdensine	> 50	> 50	> 100
Crinamine	2.8	3.4	1.8
Hamayne	15.6	18.2	9.4
Lycorine	0.6	0.7	1.8
Anhydrolycorin-6-one (**2**)	6.1	6.4	3.3
Sternbergine	3.9	4.8	3.5
Apohaemanthamine (**3**)	> 50	> 50	> 100
Chloroquine	0.002	0.01	20.9
Daunomycin	–	–	0.43

according to methods previously described (Campbell, Gammon, Smith, Abrahams & Purves, 1997; Theron, Albrecht, Kruger, Jenkins & van der Merwe, 1994).

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