Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytochemistry 65 (2004) 3143–3149
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Augustamine type alkaloids from *Crinum kirkii*

Alex K. Machocho [a], Jaume Bastida [b], Carlos Codina [b], Francesc Viladomat [b], Reto Brun [c], Sumesh C. Chhabra [a,*]

[a] *Department of Chemistry, Kenyatta University, PO Box 43844, 00100 GPO, Nairobi, Kenya*
[b] *Departament de Productes Naturals, Facultat de Farmàcia, Universitat de Barcelona, Avda. Diagonal 643, E-08028 Barcelona, Spain*
[c] *Swiss Tropical Institute, Protozoology Laboratory, Socinstraße 57, CH-4002 Basel, Switzerland*

Received 8 March 2004; received in revised form 17 September 2004

Abstract

Sixteen more Amaryllidaceae alkaloids have been isolated from bulbs of *Crinum kirkii* Baker of which noraugustamine and 4a,*N*-dedihydronoraugustamine are hitherto unknown. Their structures and those of earlier known alkaloids have been established by physical and spectroscopic analysis. Application of 2D NMR techniques was used for complete characterization of the alkaloids as well as of 3-*O*-acetylsanguinine. 1,2-Diacetyllycorine and 3-*O*-acetylsanguinine showed activity against *Trypanosoma brucci* rhodesiense, the parasite associated with sleeping sickness. 3-*O*-acetylsanguinine also showed some activity against *Trypanosoma cruzi*.

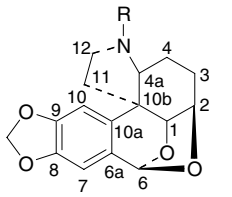
Keywords: *Crinum kirkii*; Amaryllidaceae; Alkaloids; Kirkine; Sanguinine; Zephyranthine; 1,2-*O*-Diacetylzephyranthine; Augustamine; Noraugustamine; 4a,*N*-Dedihydronoraugustamine

1. Introduction

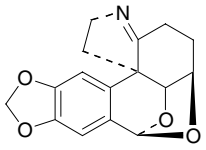
Crinum, the largest genus in the subtribe Crininae (Tribe Amaryllideae; Family Amaryllidaceae), has a wide geographical distribution in the temperate and subtropical regions (Snijman and Linder, 1996). The members of the genus have been used in treatment of various ailments and are considered capable of causing dermatitis (Hutchings et al., 1996). Continuing our phytochemical studies of the Amaryllidaceae, the Kenyan *Crinum kirkii* Baker, a species with a variety of traditional medicinal uses (Kokwaro, 1993; Watt and Breyer-Brandwijk, 1962) was revisited. In the previous study five alkaloids were reported (Bastida et al., 1995). In the present investigation, we describe the characterization of a further two new alkaloids together with 14 others already known. The new alkaloids, noraugustamine (**1**) and 4a,*N*-dedihydronoraugustamine (**2**), are related to augustamine, a rare Amaryllidaceae alkaloid that has only been reported once from *Crinum augustum* Rox (Ali et al., 1983). Lycorine, the main alkaloid, has various biological and pharmacological activities (Bastida et al., 1998), and recent reports indicate that it has antimalarial (Campbell et al., 1998), anti-inflammatory (Çitoglu et al., 1998) and antitumour (Yui et al., 1998) activities. Hippadine has been reported to produce reversible inhibition of fertility in male rats (Chattopadhyay et al., 1983), while 1,2-*O*-diacetyllycorine exhibited both antimalarial and cytotoxic activity (Campbell et al., 1998). The other main alkaloids were sanguinine, 3-*O*-acetylsanguinine (**3**), amabiline and zephyranthine. It was interesting to note high yields of the galanthamine related alkaloids, sanguinine and its acetate (**3**).

* Corresponding author. Tel.: +254 20 810901; fax: +254 20 811575.
E-mail addresses: scchhabra@avu.org, scchhabra@yahoo.com (S.C. Chhabra).

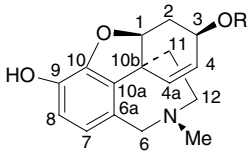
0031-9422/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2004.10.004



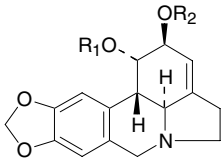
Noraugustamine (**1**), R=H
Augustamine, R=Me



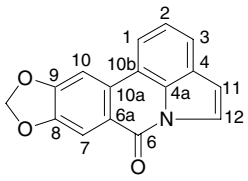
4a,*N*-Dedihydronoraugustamine (**2**)



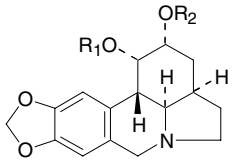
Sanguinine, R=H
3-*O*-Acetylsanguinine (**3**), R=Ac



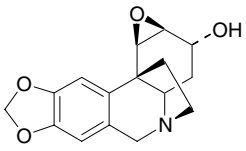
Lycorine, R_1=R_2=H
1-*O*-Acetyllycorine, R_1=Ac; R_2=H
2-*O*-Acetyllycorine, R_1=H; R_2=Ac
1,2-*O*-Diacetyllycorine, R_1=R_2=Ac



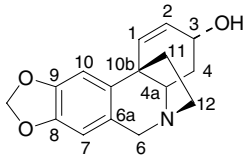
Hippadine



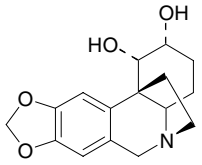
Zephyranthine, R_1=R_2=H
1,2-*O*-Acetylzephyranthine, R_1=R_2=Ac



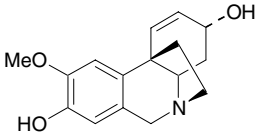
Flexinine



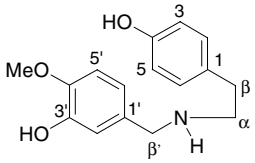
Crinine



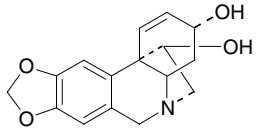
Amabiline



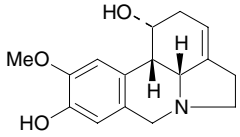
Macowine



O-Methylnorbelladine



Hamayne



Kirkine

2. Results and discussion

Flexinine, amabiline and macowine are the additional crinane type alkaloids reported here apart from crinine and hamayne. Their absolute configurations were deduced from their Circular Dichroism (CD) curves, which displayed a maximum around 250 nm and a minimum around 290 nm for β configuration of the 5,10b-ethano bridge. Hamayne on the other hand showed the opposite α configuration (Ali et al., 1984; Wagner et al., 1996).

Compounds **1** ($C_{16}H_{17}NO_4$) and **2** ($C_{16}H_{15}NO_4$) were identified as augustamine type alkaloids and the names noraugustamine and 4a,*N*-dedihydronoraugustamine were proposed, respectively. EIMS of **1** exhibited a base peak at *m*/*z* 287 which represented the molecular ion. Compound **2** on the other hand showed a peak representing the molecular ion at *m*/*z* 285, and was confirmed by the peak *m*/*z* 286 for $[M + 1]^+$ in the CIMS. The other main fragments of the two compounds were in agreement with the fragmentation pattern of augustamine (Ali et al., 1983), except for the peaks at m/z 257 and 256 in **2** which are associated with loss of C_2H_4 by a retro Diels–Alder reaction from $[M]^+$ and $[M - 1]^+$ respectively, involving C-11 and C-12. The two compounds have similar IR spectra, and the absorption peaks closely compared with the published data of augustamine (Ali et al., 1981a) where the bands associated with the aromatic methylene-dioxy were observed at around 1618, 1484 and 939 cm^{-1}. However, **2** exhibited a strong absorption band at 1657 cm^{-1} which suggested presence of an imine group.

^1H NMR spectrum data of **1** (Table 1) closely compared with the data of augustamine except that the former lacked the N–Me peak. The two downfield signals appearing as singlets at δ 6.72 and 6.60 were assignable to the two aromatic *para* oriented H-10 and H-7, respectively. This placement was supported by the ROESY spectrum where H-10 showed contours with H-4a (δ 3.36) and H-11*endo* (δ 2.05). Additionally, HMBC experiment (Bax and Summers, 1986) showed strong correlations between H-10 and C-8, C-6a and C-10b. On the other hand H-7 had strong contours with C-6, C-9 and C-10a. The spectrum also displayed methylenedioxy

Table 1
^{1}H NMR, HMQC and HMBC data of noraugustamine (**1**)

H	^{1}H NMR (J in Hz)		Correlated C-atoms	
			HMQC	HMBC
1	4.24	*d* (4.5)	77.0 *d*	C-2, C-6, C-10a, C-10b, C-11
2	4.19	*dt* (4.5, 4.0)	74.8 *d*	C-1, C-4, C-10b
3*ax*	1.99	*dddd* (15.0, 10.0, 4.5, 4.0)	21.2 *t*	C-1, C-4, C-10a, C-10b
3*eq*	1.73	*dddd* (15.0, 4.5, 4.0, 4.0)	21.2 *t*	C-1, C-2, C-4, C-4a
4*ax*	1.38	*dddd* (14.5, 10.0, 4.5, 4.5)	19.5 *t*	C-2, C-3, C-4a, C-10b
4*eq*	1.44	*dddd* (14.5, 4.0, 4.0, 4.0)	19.5 *t*	C-2, C-3, C-4a, C-10b
4a	3.36	*dd* (4.5, 4.0)	66.1 *d*	C-1, C-3, C-10a, C-10b
6	5.84	*s*	100.2 *d* 133.4 *s* (C-6a)	C-1, C-2, C-6a, C-7, C-10a
7	6.60	*s*	104.7 *d* 145.2 *s* (C-8) 147.8 *s* (C-9)	C-6, C-8, C-9, C-10a
10	6.72	*s*	106.7 *d* 130.9 *s* (C-10a) 47.1 *s* (C-10b)	C-6a, C-8, C-9, C-10b
11*endo*	2.05	*ddd* (13.0, 10.0, 7.5)	40.8 *t*	C-1, C-4a, C-10a, C-10b, C-12
11*exo*	2.36	*ddd* (13.0, 8.5, 4.0)	40.8 *t*	C-1, C-4a, C-10b
12*endo*	3.30	*ddd* (10.5, 10.0, 4.0)	44.1 *t*	C-10b, C-11
12*exo*	3.21	*ddd* (10.5, 8.5, 7.5)	44.1 *t*	C-4a, C-11
OCH_2O	5.91–5.90	2*d* (1.5)	101.0 *t*	C-8, C-9

signals appearing as two doublets (J = 1.5 Hz) at δ 5.91 and 5.90. The singlet at δ 5.84 was assigned to the benzylic H-6 and its upfield shift shift to higher could be associated with the benzaldehyde acetal moiety (Ali et al., 1983). The spatial correlations with H-6 in the ROESY experiment and the three-bond connectivities with C-10a, C-7, C-1 and C-2 in the HMBC spectrum allowed the assignment of this proton. The two other significant chemical shifts centred at δ 4.24 and 4.19 were assigned to H-1 and H-2, respectively, with assistance of COSY and HMBC spectra. Both protons showed spatial correlations in the ROESY spectrum with H-3 axial (δ 1.99) and additionally, H-1 with H-11*exo*. This allowed for H-1 and H-2 to be assigned the axial and equatorial orientations, respectively. The remaining shifts were assigned accordingly with the help of COSY, ROESY, HMQC (Bax and Subramanian, 1986) and HMBC experiments (Tables 1 and 2).

The ^{13}C NMR spectrum of **1** indicated 16 carbon atoms, which fitted for the basic biosynthetic skeleton of Amaryllidaceae alkaloids (Bastida et al., 1998) and closely compared with the data of augustamine (Ali et al., 1983). The DEPT experiment of the alkaloid showed presence of six methine and five methylene carbons. The doublet at δ 100.2 corresponding to C-6 helped in ruling out the existence of benzylic hydroxyl group as carbon shift for the latter appears around δ 85.0 (Machocho et al., 1999). The two doublets at δ 77.0 and 74.8 were assignable to C-1 and C-2, respectively, and the placement was in agreement to with cycloaliphatic methine ether carbons of augustamine (Ali et al., 1983). The doublet and triplet at δ 66.1 and δ 44.1 assigned to C-4a and C-12, respectively, appeared rather upfield as compared to their counterparts in augustamine and can be attributed to lack of N–Me group in **1.** The placement for the quaternary carbons was corroborated by the HMBC experiment. Thus, the signals at δ 147.8 and 145.2 were assigned to C-9 and C-8, respectively, as the former showed strong correlations with H-7 while the latter with H-10. The shifts at δ 133.4 and 130.9 were for C-6a and C-10a, respectively. The former showed strong contours with H-10 while the latter with showed correlations with H-1, H-7 and the protons of C-11. Finally, the upfield shift at δ 47.1 was for C-10b as justified by the three bond correlations with H-10, H-1 and protons of C-4 among others.

Table 2
Scalar and spacial correlations of the protons of noraugustamine (**1**)

H	COSY	ROESY
1	H-2	H-2, H-3α, 11*exo*
2	H-1, H-3α, H-3β	H-1, H-3α
3*ax*	H-2, H-3β, H-4α, H-4β	H-3β, H-4α, H-4
3*eq*	H-2, H-3, H-4α, H-4β	H-3α, H-4α, H-4β
4*ax*	H-3α, H-3β, H-4α	H-3α, H-3β, H-4β
4*eq*	H-3α, H-3β, H-4β	H-3α, H-3, H-4β
4a	H-4α, H-4β	H-4α, H-4β, H-10
6		H-7
7		H-6
10		H-4a, 11*endo*
11*endo*	H-11*exo*, H-12*endo*, H-12*exo*	H-10, H-11*exo*, H-12*endo*, H-12*exo*
11*exo*	H-11*endo*, H-12*endo*, H-12*exo*	H-11*endo*, H-12*endo*, H-12*exo*
12*endo*	H-11*endo*, H-11*exo*, H-12*exo*	H-11*endo*, H-11*exo*, H-12*exo*
12*exo*	H-11*endo*, H-11*exo*, H-12*endo*,	H-11*endo*, H-11*exo*, H-12*endo*,
OCH_2O		

^{1}H NMR spectrum of **2** (Table 2) closely compared with the one of alkaloid **1,** but the doubles of double assigned to H-4a in the latter was lacking in **2**. Pronounced deshielding of the protons of C-12 (δ 4.12 for H-12*endo* ; δ 4.07 for H-12*exo*) and C-4 (δ 2.26 for H-4ax; δ 1.98 for H-4eq) as compared to their counterparts in **1** further suggested an imine group between C-4a and the nitrogen atom. The other chemical shifts were assigned as in **1** with some variations attributable to the structural differences between the two alkaloids (Table 3). The ^{13}C NMR spectrum of **2** also compared with that of **1**, but the doublet at δ 66.1 for C-4a in the latter was replaced with a downfield singlet at δ 178.5 in the former, for the imine carbon atom. The triplets at δ 60.6, 26.2 and 22.6 for C-12, C-3 and C-4, respectively, doublet at δ 83.0 for C-1 and singlet at δ 58.9 for C-10b appeared downfield when compared with those of their counterparts in **1**.

3-*O*-Acetylsanguinine **3**, has been isolated earlier from *Haemanthus multiflorus* as a derivative of chlidanthine (Abdallah et al., 1989), and ^{1}H and ^{13}C NMR data were in close agreement with the published information. However, we report here the complete assignment of both ^{1}H and ^{13}C NMR spectral data by application of multipulse and 2D NMR techniques, thus providing the missing coupling constants, confirming and making correct assignments for C-4 (δ 122.9, *d*), C-4a (δ 130.3, *d*), C-6a (δ 126.8, *s*), C-10a (δ 131.7, *s*), C-9 (δ 140.7, *s*) and C-10 (δ 145.3 *s*).

Biological activity tests by in vitro against parasitic protozoa were performed on some of the isolated alkaloids. 1,2-*O* -Diacetyllycorine and 3-*O*-acetylsanguinine (**3**) showed activity against *Trypanosoma brucei rhodesiense* (strain STIB 900, stage trypoma-stigotes, std Melarsoprol) with an IC_{50} of 1.0 and 1.1 μg/ml, respectively. However, hippadine, noraugustamine (**1**), sanguinine, amabiline and kirkine showed very low activities with an IC_{50} values of 8.4, 18.7, 22.5, 31.9 and 90 μg/ml, respectively. Alkaloid **3** also showed some activity against *Trypanosoma cruzi* (strain Tulahuen C4, stage trypomastigotes, std Benznidazole) with an IC_{50} of 2.3 μg/ml. All other tested alkaloids showed no activity. No activity was reported against *Plasmodium falciparum* (strain K1 and NF54, stages IEF, stds Chloroquine and Qinghaosu) and *Leishmania donovani* (strain MHOM-ET-67, stage amastigotes, std Pentostam).

3. Experimental

3.1. General

Mp uncorr. Optical rotations: Perkins–Elmer 241 Polarimeter. IR spectra: Perkin–Elmer 1600 FTIR series Spectrometer in dry film. CD: Jasco J-700 Spectropolarimeter. EIMS and CIMS: Hewlett Parkard 5989A Mass Spectrometer at 70 eV. ^{1}H NMR, ^{13}C NMR, DEPT, COSY, ROESY, HMQC and HMBC spectra: Varian VXR 500 in $CDCl_3$ and CD_3OD. Chemical shifts are reported in δ (ppm) units relative to TMS signal and coupling constants (*J*) in Hz. Silica gel SDS chromagel 60 A CC (6–35 μm) was used for VLC, and silica gel 60 F_{254} (Macherey–Nagel) for analyt. (0.25) and preparation (0.25) TLC. Spots on chromatograms were detected under UV light (254 nm) and by Dragendorff's reagent.

Table 3
^{1}H NMR, HMQC and HMBC data of 4a,*N*-dedihydronoraugustamine (**2**)

H	^{1}H NMR (*J* in Hz)		Correlated C-atoms	
			HMQC	HMBC
1	4.35	*d* (4.5)	83.0 *d*	C-2, C-6, C-10a, C-10b, C-11
2	4.22	*dt* (4.5, 3.5)	74.6 *d*	
3*eq*	2.29	*dddd* (14.0, 5.0, 5.0, 3.5)	26.2 *t*	C-4a
3*ax*	1.78	*dddd* (14.0, 13.0, 5.0, 3.0)	26.2 *t*	C-4
4*eq*	1.98	*dddd* (13.0, 5.0, 5.0, 2.0)	22.6 *t*	
4*ax*	2.26	*ddd* (13.0, 13.0, 5.0)	22.6 *t*	C-2, C-3, C-4a, C-10b
			178.5 *s* (C-4a)	
6	5.94	*s*	100.4 *d*	C-1, C-2, C-6a, C-7, C-10a
			131.6 *s* (C-6a)	
7	6.62	*s*	104.5 *d*	C-6, C-8, C-9, C-10, C-10a
			146.1 *s* (C-8)	
			148.4 *s* (C-9)	
10	6.40	*s*	106.8 *d*	C-6a, C-7, C-8, C-9, C-10b
			130.8 *s* (C-10a)	
			58.9 *s* (C-10b)	
11*endo*	2.34	*ddd* (14.0, 9.0, 5.0)	40.3 *t*	C-1, C-4a, C-10a, C-10b, C-12
11*exo*	2.44	*ddd* (14.0, 9.0, 7.0)	40.3 *t*	C-1, C-4a, C-10a, C-10b
12*endo*	4.12	*dddd* (16.0, 9.0, 5.0, 2.5)	60.6 *t*	C-4a
12*exo*	4.07	*dddd* (16.0, 9.0, 7.0, 2.5)	60.6 *t*	C-4a
OCH_2O	5.92-5.91	2*d* (1.5)	101.3 *t*	C-8, C-9

3.2. Plant material

Bulbs of *C. kirkii* Baker were collected in Mt. Kenya forest, Karatina, Kenya in September 2001 during flowering period. Mr. Simon Mathenge of the Herbarium of the Botany Department of University of Nairobi, Kenya authenticated the sample. A specimen voucher (SM/118/01) has been deposited in the above mentioned herbarium.

3.3. Extraction and isolation of alkaloids

Fresh whole plants of *C. kirkii* (26 kg) were crushed and macerated with MeOH for 48 hr at room temp. and the process repeated three times. The crude alcoholic extracts were evaporated under reduced pressure the residue dissolved in H_2O and acidified with 5% H_2SO_4 to pH 3–4. Later the neutral materials were removed with Et_2O and hippadine (96 mg) crystallised out after the extract was dissolving in MeOH. The acidic solution was basified with 10% NH_3 solution to pH 8–9 and then extracted with EtOAc severally and later with EtOAc–MeOH (9:1). The extracts were combined to give a brown gummy residue (28 g), from which lycorine (2.6 g) crystallised directly in MeOH. The extract was dried and subjected to VLC on silica gel eluting initially with *n* -Hexane and increasing the polarity of the eluent with EtOAc and later up to EtOAc–MeOH (8:2). Six major fractions containing alkaloids were obtained. Fr. I (850 mg) was first treated by VLC on silica gel in a smaller scale, eluting with *n*-Hexane and increasing the polarity with EtOAc up to EtOAc (100%) where 1,2-*O*-diacetyllycorine (31 mg) crystallised out in MeOH. Preparation of TLC of the solution eluting with EtOAc–MeOH (9:1) afforded more 1,2-*O*-diacetyllycorine (43 mg), 1-*O*-acetyllycorine (38 mg), 2-*O*-acetyllycorine (42 mg) and augustamine (4.5 mg). Fr. II (1.4 g) was treated as fr. I by VLC on silica gel, where lycorine (256 mg) crystallised out in MeOH. Second crystallization afforded a mixture of lycorine and 3-*O* -acetylsanguinine (**3**), where the latter (215 mg) was removed as a $CHCl_3$ solution. Preparation of TLC of the solution of fr. II eluting with EtOAc in NH_3 atm. yielded more 3-*O*-acetylsanguinine (**3**) (46 mg), 1-*O*-acetyllycorine (27 mg), 1,2-*O*-diacetylzephyranthine (12 mg), noraugustamine (**1**) (10.5 mg) and 4a,*N*-dedihydronoraugustamine (**2**) (4.5 mg). More lycorine (51 mg) crystallised out from fr. III (1.9 g) in MeOH. The solution of this fraction was first cleaned by VLC on silica gel eluting with *n*-Hexane–EtOAC (1:1) and increasing the polarity up to EtOAC (100%). Earlier fractions afforded more noraugustamine (**1**) (5 mg) and 4a,*N*-dedihydronoraugustamine (**2**) (3.2 mg) after treatment of the sub-fraction by preparation. TLC eluted with EtOAc in NH_3 atm. More 3-*O*-acetylsanguinine (**3**) (130 mg) crystallised out in MeOH from the late fractions. Second crystallization indicated presence of two alkaloids which were separated by preparation. TLC eluted with EtOAc–MeOH (1:1) to give more 3-*O*-acetylsanguinine (**3**) (65 mg) and sanguinine (30 mg). The preparation of TLC of fr. III developed with EtOAc–MeOH (1:1) afforded more 3-*O* -acetylsanguinine (**3**) (29 mg) and sanguinine (48 mg), flexinine (21 mg) and *O*-methylnorbelladine (7.8 mg). Fr. IV (2.3 g) was first subjected to VLC on silica gel eluting with *n*-Hexane–EtOAc (1:1) increasing the polarity up to EtOAc–MeOH (9:1). Sanguinine (320 mg) crystallised out in MeOH from the early fractions of the column. Preparation of TLC of the late fractions developing with EtOAc–MeOH (19:1) in NH_3 atm. gave more sanguinine (41 mg), hamayne (14 mg), flexinine (57 mg), *O*-methylnorbelladine (11 mg) and crinine (36 mg). Fr. V (2.1 g) was treated as fr. IV above by VLC on silica gel where amabiline (178 mg) crystallised out in MeOH of combined fractions containing alkaloids. Preparation of TLC of the solution developing with EtOAc–MeOH (19:1) in NH_3 atm. yielded more amabiline (55 mg), crinine (43 mg) sanguinine (15 mg) hamayne (25 mg) and zephyranthine (12 mg). Finally, fr. VI (1.8 g) was first cleaned as fr. V above by VLC on silica gel eluting with EtOAc–MeOH (from 10:0 · to 8:2) where zephyranthine (102 mg) crystallised out in Me_2CO. Preparation of TLC of the solution developed with EtOAc–MeOH (9:1) in NH_3 atm. afforded more zephyranthine (62 mg), kirkine (36 mg), crinine (13.4 mg), hamayne (6 mg) and macowine (38 mg).

3.4. Noraugustamine (*1*)

Found: C, 65.78; H, 6.26; N, 4.76 $C_{16}H_{17}NO_4$ requires: C, 66.87; H, 5.96; N, 4.88%; m.p. 149–151°. $[\alpha]_D^{20} = -50.0°$ (MeOH; *c* 0.87). IR ν_{max} cm^{-1}: 3346, 2933, 1725, 1618 (OCH_2O), 1504, 1484 (OCH_2O), 1445, 1373, 1318, 1246, 1101, 1076, 1038, 994, 939 (OCH_2O), 866, 825, 753. EIMS 70 eV, *m/z* (rel. int.): 287 $[M]^+$ (100), 286 $[M - H]^+$ (49), 243 (15), 230 $[M - C_3H_5O]^+$ (90), 215 $[M - C_3H_6NO]^+$ (17), 214 $[M - C_3H_6NO\text{-}H]^+$ (19), 202 (27), 201 $[M - C_4H_8NO]^+$ (27) 187 (21), 188 (15), 185 (20), 115 (17). ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$), see Table 1.

3.5. 4a,N-dedihydronoraugustamine (*2*)

Found: C, 66.52; H, 5.51; N, 4.79 $C_{16}H_{15}NO_4$ requires: C, 67.36; H, 5.30; N, 4.91%; m.p. 127–130°. $[\alpha]_D^{20} = -242.6°$ (MeOH; *c* 0.27). IR ν_{max} cm^{-1}: 3357, 2953, 1718, 1657 (C=N), 1619 (OCH_2O), 1503, 1484 (OCH_2O), 1443, 1375, 1312, 1258, 1212, 1100, 1090, 1080, 1038, 984, 938 (OCH_2O), 866, 826, 754. EIMS 70 eV, *m/z* (rel. int.): 285$[M]^+$ (60), 284 $[M - H]^+$ (5), 257 $[M - C_2H_4]^+$ (13), 256 $[M - C_2H_4\text{–}H]^+$ (31) 229 (17), 228 $[M - C_3H_5O]^+$ (36), 201 $[M - C_4H_8NO]^+$

Table 4
Scalar and spacial correlations of the protons of 4a,*N*-dedihydronorangustamine (**2**)

H	COSY	ROESY
1	H-2	H-2, H-3*ax*, 11*exo*
2	H-1, H-3*ax*, H-3*eq*	H-1, H-3*ax*, H-4*eq*
3*ax*	H-2, H-3*eq*, H-4*ax*, H-4*eq*	H-1, H-3*eq*, H-4*ax*, H-4*eq* H-6
3*eq*	H-2, H-3*ax*, H-4*ax*, H-4*eq*	H-3*ax*, H-4*ax*, H-4*eq*
4*ax*	H-3*ax*, H-3*eq*, H-4 *eq*	H-3*ax*, H-3*eq*, H-4 *eq*
4*eq*	H-3*ax*, H-3*eq*, H-4 *ax*, H-12*endo*, H-12*exo*	H-3*ax*, H-3*eq*, H-4 *ax*
6	H-10	H-3α, H-7
7		H-6
10		11*endo*, H-12*endo*, H-12*exo*
11*endo*	H-11*exo*, H-12*endo*, H-12*exo*	H-10, H-11*exo*, H-12*endo*, H-12*exo*
11*exo*	H-11*endo*, H-12*endo*, H-12*exo*	H-1, H-12*endo*, H-12*exo*
12*endo*	H-4*eq*, H-11*endo*, H-11*exo*, H-12*exo*	H-10, H-11*endo*, H-11*exo*, H-12*exo*
12*exo*	H-4α, H-11*endo*, H-11*exo*, H-12*endo*	H-10, H-11*endo*, H-11*exo*, H-12*endo*,
OCH_2O		

(100), 173 (26), 172 (31). ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$), see Table 3 and 4.

3.6. 3-*O*-Acetylsanguinine (**3**)

Found: C, 67.62; H, 6.53; N, 4.52 Calc. for $C_{18}H_{21}NO_4$ C, 68.55; H, 6.71; N, 4.44%; m.p. 215–218°. $[\alpha]_D^{20} = -13.5°$ (MeOH; *c* 0.2). IR ν_{max} cm^{-1}: 3300, 2925, 1726 (C=O), 1598, 1508, 1454, 1372, 1301, 1251, 1225, 1198, 1168, 1055, 1035, 1000, 805, 756, 603. EIMS 70 eV, *m/z* (rel. int.): 315$[M]^+$ (37), 314 $[M - H]^+$ (12), 256 $[M - OAc]^+$ (100), 255 $[M - OAc–H]^+$ (48), 254 $[M - OAc–2H]^+$ (37), 226 (14), 225 (12) 212 (28), 211 (19), 202 (31), 197 (29), 195 (23), 194 (20), 166 (31), 165 (42). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.54 (1H, *ddd*, $J = 12.5, 3.5, 1.5$ Hz, H-11β), 1.99 (3H, *s*, $OCOCH_3$), 2.09 (1H, *ddd*, $J = 16.0, 5.5, 3.0$ Hz, H-2α), 2.13 (1H, *ddd*, $J = 12.5, 3.5, 0.5$ Hz, H-11α), 2.37 (3H, *s*, N–CH_3), 2.58 (1H, *dddd*, $J = 16.0, 3.0, 1.5, 1.5$ Hz, H-2β), 3.05 (1H, *br d*, $J = 12.5$ Hz, H-12α), 3.28 (1H, *t*, $J = 12.5$ Hz, H-12 β), 3.65 (1H, *d*, $J = 15.0$ Hz, H-6α), 4.10 (1H, *d*, $J = 15.0$ Hz, H-6β), 4.52 (1H, *dd*, $J = 3.0, 2.0$ Hz, H-1), 5.30 (1H, *t*, $J = 5.5$ Hz, H-3), 5.87 (1H, *ddd*, $J = 10.0, 5.0, 1.0$ Hz, H-4), 6.25 (1H, *d*, $J = 10.0$ Hz, H-4a), 6.48 (1H, *d*, $J = 8.5$ Hz, H-7), 6.58 (1H, *d*, $J = 8.5$ Hz, H-8). ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.4 (*q*, $OCO\underline{C}H_3$), 27.9 (*t*, C-2), 33.8 (*t*, C-11), 41.1 (*q*, N–CH_3), 48.1 (*s*, C-10b), 53.4 (*t*, C-12), 60.0 (*t*, C-6), 63.3 (*d*, C-3), 86.2 (*d*, C-1), 115.6 (*d*, C-8), 122.1 (*d*, C-7), 122.9 (*d*, C-4), 126.8 (*s*, C-6a), 130.3 (*d*, C-4a), 131.7 (*s*, C-10a), 140.7 (*s*, C-9), 145.3 (*s*, C-10), 170.9 (*s*, $O\underline{C}OCH_3$).

Hippadine (Ali et al., 1981b), lycorine and amabiline (Likhitwitayawuid et al., 1993), 1-*O*-acetyllycorine (Evidente, 1986; Kabayashi et al., 1984), 2-*O*-acetyllycorine and 1,2-*O*-diacetyllycorine (Kabayashi et al., 1984; Campbell et al., 1998), augustamine (Ali et al., 1983), sanguinine (Kobayashi et al., 1991; Capo and Saa, 1989), *O*-methylnorbelladine (Nair et al., 2000), flexinine (Ali et al., 1986; Machocho et al., 1999), crinine (Viladomat et al., 1995), hamayne (Viladomat et al., 1994), (Likhitwitayawuid et al., 1993), kirkine (Bastida et al., 1995), zephyranthine and 1,2-*O*-diacetylzephyranthine (Herrera et al., 2000), and macowine (Nair et al., 2000) were identified by comparison of their chromatographic and spectroscopic properties (TLC, $[\alpha]_D$, CD, IR, MS, ^{1}H and ^{13}C NMR) with those of authentic samples obtained from other plant sources.

The biological activity tests against the parasitic protozoa were performed as described by Labraña et al. (2002).

Acknowledgements

The authors thank Mr. Simon Mathenge of the Herbarium of the University of Nairobi for the collection and authentication of the plant material. Thanks are also due to Dr. Reto Brun of Swiss Tropical Institute, Basel, Switzerland for carrying out the parasitic assays. Part of this work was financially supported by CIRIT-CICYT project and Commissionat per a Universitat i Recerca, Generalitat de Catalunya.

References

Abdallah, O.M., Ali, A.A., Itokawa, H., 1989. 2-*O*-Acetylchlidanthine; an alkaloid from *Haemanthus multiflorus*. Phytochemistry 28, 3248–3249.

Ali, A.A., El Sayed, H.M., Abdallah, O.M., Steglich, W., 1986. Oxocrinine and other alkaloids from *Crinum americanum*. Phytochemistry 25, 2399–2401.

Ali, A.A., Hambloch, H., Frahm, A.W., 1983. Relative configuration of the alkaloid augustamine. Phytochemistry 22, 283–287.

Ali, A.A., Kating, H., Frahm, A.W., El-Moghazi, A.M., Ramadan, M.A., 1981a. Two non-hydroxylated alkaloids in *Crinum augustum*. Phytochemistry 20, 1121–1123.

Ali, A.A., Mesbah, M.K., Frahm, A.W., 1981b. Phytochemical investigation of *Hippeastrum vittatum* growing in Egypt. Planta Medica 43, 407–409.

Ali, A.A., Ramadan, M.A., Frahm, A.W., 1984. Alkaloidal constituents of *Crinum bulbispermum* III: bulbispermine, a new alkaloid of *Crinum bulbispermum*. Planta Medica 50, 424–427.

Bastida, J., Codina, C., Peeters, P., Rubiralta, M., Orozco, M., Luque, F.J., Chhabra, S.C., 1995. Alkaloids from *Crinum kirkii*. Phytochemistry 40, 1291–1293.

Bastida, J., Viladomat, F., Codina, C., 1998. Narcissus Alkaloids. In: Atta-ur-Rahman (Ed.), Studies in Natural Product Chemistry, vol. 20. Elsevier Science Publishers, Amsterdam, p. 323.

Bax, A., Subramanian, S., 1986. Sensitivity-enhanced two-dimentional heteronuclear shift correlation NMR spectroscopy. Journal of Magnetic Resonance 67, 565–569.

Bax, A., Summers, M.F., 1986. ^{1}H and ^{13}C Assignments from sensitivity-enhanced detection of heteronuclear multiple-bond connectivity by multiple quantum NMR. Journal of the American Chemical Society 108, 2093–2094.

Campbell, W.E., Nair, J.J., Gammon, D.W., Bastida, J., Codina, C., Viladomat, F., Smith, P.J., Albrecht, C.F., 1998. Cytotoxic and antimalarial alkaloids from *Brunsvigia littoralis*. Planta Medica 64, 91–93.

Capo, M., Saa, J.M., 1989. Alkaloids from *Leucojum aestivum* Sub, Pulchellum Amaryllidaceae. Anales de Quimica 85, 119–121.

Çitoglu, G., Tanker, M., Gümüsel, B., 1998. Antiinflammatory effects of lycorine and haemanthidine. Phytotherapy Research 12, 205–206.

Chattopadhyay, S., Chattopadhyay, U., Mathur, P.P., Saini, K.S., Ghosal, S., 1983. Effects of hippadine; an Amaryllidaceae alkaloid, on testicular function in rats. Planta Medica 49, 252–254.

Evidente, A., 1986. Isolation and structural characterization of lutessine, a new alkaloid from bulbs of *Sternbergia lutea*. Journal of Natural Products 49, 90–94.

Herrera, M.R., Machocho, A.K., Brun, R., Viladomat, F., Codina, C., Bastida, J., 2000. Alkaloids from *Zephyranthes citrina* Baker. Planta Medica 67, 191–193.

Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A.B., 1996. Zulu Medicinal Plants. An Inventory. University of Natal Press, Pietermaritzburg.

Kokwaro, J.O., 1993. Medicinal plants of East Africa, second ed. Kenya Literature Bureau, Nairobi.

Kobayashi, S., Satoh, K., Numata, A., Shingu, T., Kihara, M., 1991. Alkaloid *N*-oxides from *Lycoris sanguinea*. Phytochemistry 30, 675–677.

Kabayashi, S., Tokumoto, T., Kihara, M., Imakura, Y., Shingu, T., Taira, Z., 1984. Alkaloidal constituents of *Crinum latifolum* and *Crinum bulbispermum* (Amaryllidaceae). Chemical and Pharmaceutical Bulletin 32, 3015–3022.

Labraña, J., Machocho, A.K., Kricsfalusy, V., Brun, R., Codina, C., Viladomat, F., Bastida, J., 2002. Alkaloids from *Narcissus angustifolius* subsp. *Transcarpathicus* (Amaryllidaceae). Phytochemistry 60, 847–852.

Likhitwitayawuid, K., Angerhofer, C.K., Chai, H., Pezzuto, J.M., Cordell, G.A., Ruangrungsi, N., 1993. Cytotoxic and antimalarial from the bulbs of *Crinum amabile*. Journal of Natural Products 56, 1331–1338.

Machocho, A., Chhabra, S.C., Viladomat, F., Codina, C., Bastida, J., 1999. Alkaloids from *Ammocharia tinneana*. Phytochemistry 51, 1185–1191.

Nair, J.J., Machocho, A.K., Campbell, W.E., Viladomat, F., Codina, C., Bastida, J., 2000. Alkaloids from *Crinum macowanii*. Phytochemistry 54, 945–950.

Snijman, D.A., Linder, H.P., 1996. Phylogenetic relationship, seed character and dispersal system evolution in Amaryllideae (Amaryllidaceae). Annals of the Missouri Botanical Gardens 83, 362–386.

Viladomat, F., Bastida, J., Codina, C., Campbell, W.E., Mathee, S., 1994. Alkaloids from *Brunsvigia josephinae*. Phytochemistry 35, 809–812.

Viladomat, F., Codina, C., Bastida, J., Mathee, S., Campbell, W.E., 1995. Further alkaloids from *Brunsvigia josephinae*. Phytochemistry 40, 961–965.

Wagner, J., Pham, H.L., Döpke, W., 1996. Alkaloids from *Hippeastrum equestre* Herb.—5. Circular dichroism studies. Tetrahedron 52, 6591–6600.

Watt, J.M., Breyer-Brandwijk, M.G., 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa. E. and S. Livingston, Edinburgh, London.

Yui, S., Mikami, M., Kitahara, M., Yamazaki, M., 1998. The inhibitory effects of lycorine and tumor cell apoptosis enduced by polymorphonuclear leukocyte-derived calprotectin. Immunopharmacology 40, 151–162.