



## Alkaloids from *Ammocharis tinneana*

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### Abstract

Sixteen alkaloids have been isolated from fresh bulbs of *Ammocharis tinneana* (Amaryllidaceae), seven of which contain a 1,2 $\beta$ -epoxide group. 6 $\alpha$ -Hydroxycrinamidine and 6 $\alpha$ -hydroxyundulatine are reported here for the first time from a natural source. The structures and stereochemistry of these new alkaloids have been determined by physical and spectroscopic methods. <sup>1</sup>H and <sup>13</sup>C NMR spectra of flexinine, 1,2 $\beta$ -epoxyambelline and 11-*O*-acetyl-1,2 $\beta$ -epoxyambelline were completely assigned by means of 2D NMR techniques. © 1999 Elsevier Science Ltd. All rights reserved.

**Keywords:** *Ammocharis tinneana*; Amaryllidaceae; Alkaloids; Lycorine; Sternbergine; 9-*O*-Demethylpluviine; Crinine; Powelline; Buphanisine; Buphanidrine; Ambelline; 11-*O*-Acetylambelline; Flexinine; Crinamidine; 6 $\alpha$ -Hydroxycrinamidine; Undulatine; 6 $\alpha$ -Hydroxyundulatine; 1,2 $\beta$ -Epoxyambelline; 11-*O*-Acetyl-1,2 $\beta$ -epoxyambelline

### 1. Introduction

*Ammocharis tinneana* (Kotschy & Peyr.) Milne-Redh. & Schweick. (Amaryllidaceae) which inhabits seasonal wet places of the eastern Africa region, is one of the five species in the widely distributed sub-Saharan *Ammocharis* Herb. genus (tribe Amaryllideae, subtribe Crininae) (Snijman & Linder, 1996). Bulbs of a closely related species, *Ammocharis coranica* (Ker-Gawl.) Herb. are used by the Zulu people of South Africa to treat any illness believed to be caused by witchcraft (Hutchings, Scott, Lewis, & Cunningham, 1996), while the Southern Sotho use a thick paste of the cooked bulbs to repair cracks in clay pots (Watt & Breyer-Brandwijk, 1962). In this paper we report the isolation and characterization of sixteen alkaloids from *A. tinneana*. Ambelline, the main constituent alkaloid was reported to have inhibitory activity against the

murine P-388 lymphocytic leukemia (Pettit, Gaddamidi, Goswami, & Cragg, 1984). 1,2 $\beta$ -Epoxyambelline (**1**), the other principal constituent was reported as an immunostimulant alkaloid from *Crinum latifolium* L. (Ghosal, Saini, & Arora, 1984). Lycorine, the third principal alkaloid and widely distributed in this family, is known to exhibit several biological and pharmacological activities (Bastida, Viladomat, & Codina, 1998). The alkaloids 6 $\alpha$ -hydroxycrinamidine (**3**) and 6 $\alpha$ -hydroxyundulatine (**4**) are reported here for first time from a natural source.

### 2. Results and discussion

Circular dichroism (CD) curves of all the thirteen crinane type alkaloids reported here included a maximum around 250 nm, which indicated a  $\beta$ -5,10b-ethano bridge (De Angelis & Wildman, 1969; Wagner, Pham, & Döpke, 1996). Ten of these alkaloids also have a methoxyl group at the C-7 position, a common feature in the tribe Amaryllideae (Viladomat, Bastida, Codina, Nair, & Campbell, 1997). The EIMS of **1**, 11-

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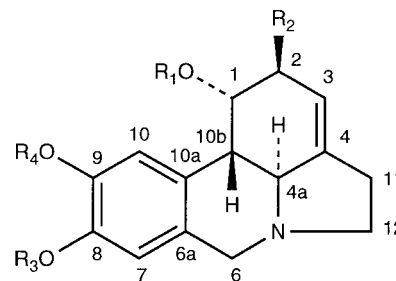
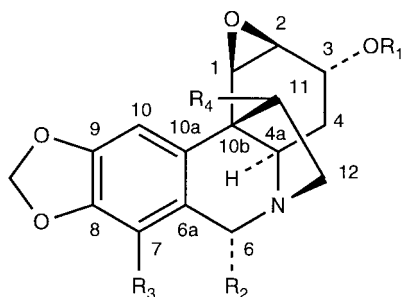
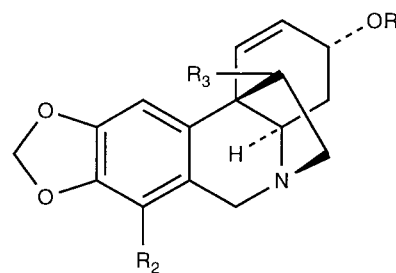
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Table 1

<sup>1</sup>H NMR, HMQC and HMBC data of compound 1. Carbon multiplicities were established by DEPT data

H		<sup>1</sup> H NMR	Correlated C-atom	
			HMQC	HMBC
1	3.69	d (3.5)	53.1 d	C-4a, C-10a, C-10b
2	3.29	ddd (3.5, 2.5, <1.0)	54.4 d	—
3	3.95	dt (3.5, 2.5)	74.5 d	—
4α	1.76	dddd (14.0, 3.5, 3.0, <1.0)	24.9 t	—
4β	1.48	ddd (14.0, 13.5, 3.5)	24.9 t	C-4a
4a	3.12	dd (13.5, 3.0)	61.2 d	—
6α	4.23	d (17.5)	59.1 t	C-4a, C-6a, C-7, C-10a
6β	3.76	d (17.5)	59.1 t	C-4a, C-6a, C-7, C-10a
10	6.69	s	99.7 d	C-6a, C-7, C-8, C-9, C-10b
11	4.86	br dd (7.5, 4.0)	81.0 d	—
12endo	2.38	ddd (14.0, 4.0, 1.5)	60.7 t	C-4a, C-6, C-11
12exo	3.50	dd (14.0, 7.5)	60.7 t	C-6
OCH <sub>2</sub> O	5.91	s	100.8 t	C-8, C-9
3-OMe	3.39	s	57.6 q	C-3
7-OMe	3.98	s	59.1 q	C-7

*O*-acetyl-1,2β-epoxyambelline (**2**), **3**, **4**, flexinine (**5**), crinamidine (**6**) and undulatine (**7**) exhibited the typical fragmentation pattern for structures with an epoxide group at the 1,2 position (Frahm, Ali, & Kating, 1981; Samuel, 1975). Compound **2** showed an additional ion at *m/z* 330, associated to the loss of the acetoxy group. The IR spectra of **1–7** displayed absorption bands at around 1280 and 930 cm<sup>-1</sup> characteristic of epoxide and methylenedioxy groups, respectively. Additionally, **1** and **3–6** showed intense absorption bands at 3200–3350 cm<sup>-1</sup> associated to hydroxyl groups. On the other hand, **2** displayed a band at 1740 cm<sup>-1</sup> which is in agreement with an ester carbonyl group.

lycorine: R<sub>1</sub> = H, R<sub>2</sub> = OH, R<sub>3</sub> + R<sub>4</sub> = CH<sub>2</sub>sternbergine: R<sub>1</sub> = Ac, R<sub>2</sub> = OH, R<sub>3</sub> = H, R<sub>4</sub> = Me9-*O*-demethylpluviine: R<sub>1</sub> = H, R<sub>2</sub> = H, R<sub>3</sub> = Me, R<sub>4</sub> = H**1**: R<sub>1</sub> = Me, R<sub>2</sub> = H, R<sub>3</sub> = OMe, R<sub>4</sub> = OH**2**: R<sub>1</sub> = Me, R<sub>2</sub> = H, R<sub>3</sub> = OMe, R<sub>4</sub> = OAc**3**: R<sub>1</sub> = H, R<sub>2</sub> = OH, R<sub>3</sub> = OMe, R<sub>4</sub> = H**4**: R<sub>1</sub> = Me, R<sub>2</sub> = OH, R<sub>3</sub> = OMe, R<sub>4</sub> = H**5**: R<sub>1</sub> = H, R<sub>2</sub> = H, R<sub>3</sub> = H, R<sub>4</sub> = H**6**: R<sub>1</sub> = H, R<sub>2</sub> = H, R<sub>3</sub> = OMe, R<sub>4</sub> = H**7**: R<sub>1</sub> = Me, R<sub>2</sub> = H, R<sub>3</sub> = OMe, R<sub>4</sub> = Hambelline: R<sub>1</sub> = Me, R<sub>2</sub> = OMe, R<sub>3</sub> = OH11-*O*-acetylambelline: R<sub>1</sub> = Me, R<sub>2</sub> = OMe, R<sub>3</sub> = OAccrinine: R<sub>1</sub> = H, R<sub>2</sub> = H, R<sub>3</sub> = Hpowelline: R<sub>1</sub> = H, R<sub>2</sub> = OMe, R<sub>3</sub> = Hbuphanidrine: R<sub>1</sub> = Me, R<sub>2</sub> = OMe, R<sub>3</sub> = Hbuphanisine: R<sub>1</sub> = Me, R<sub>2</sub> = H, R<sub>3</sub> = H

1,2β-Epoxyambelline (**1**), C<sub>18</sub>H<sub>21</sub>NO<sub>6</sub>, and 11-*O*-acetyl-1,2β-epoxyambelline (**2**), C<sub>20</sub>H<sub>23</sub>NO<sub>7</sub>, were first isolated from *Crinum latifolium* (Ghosal et al., 1984)

Table 2  
Scalar and spatial correlations of the protons of compound **1**

H	COSY	ROESY
1	H-2	H-2, H-10
2	H-1, H-3, H-4 $\alpha$	H-1, H-3
3	H-2, H-4 $\alpha$ , H-4 $\beta$	H-2, 3-OMe, H-4 $\alpha$ , H-4 $\beta$
4 $\alpha$	H-2, H-3, H-4 $\beta$ , H-4a	H-3, H-4 $\beta$ , H-4a
4 $\beta$	H-3, H-4 $\alpha$ , H-4a	H-3, H-4 $\alpha$ , H-11
4 $\alpha$	H-4 $\alpha$ H-4 $\beta$	H-4 $\alpha$ , H-6 $\alpha$
6 $\alpha$	H-6 $\beta$	H-4a, H-6 $\beta$
6 $\beta$	H-6 $\alpha$	H-6 $\alpha$ , H-12endo
10	—	H-1
11	H-12endo, H-12exo	H-4 $\beta$ , H-12exo
12endo	H-11, H-12exo	H-6 $\beta$ , H-12exo
12exo	H-11, H-12endo	H-11, H-12endo
OCH <sub>2</sub> O	—	—
3-OMe	—	H-3
7-OMe	—	—

but the published data on <sup>1</sup>H and <sup>13</sup>C NMR were incomplete. Complete assignment is reported here using 2D NMR techniques. Their <sup>1</sup>H NMR spectra closely compared to the one for ambelline (Viladomat, Codina, Bastida, Mathee, & Campbell, 1995), but the 1,2-olefinic unsaturation was lacking. The spectra on the other hand showed some similarities with the spectrum of compound **7** (Viladomat et al., 1995), suggesting presence of an epoxide group. In the spectrum of compound **1** (Table 1), the chemical shifts at  $\delta$  3.69 (d) and  $\delta$  3.29 (ddd), were assigned to H-1 and H-2, respectively. The configuration of the epoxide ring and the  $\alpha$ -substituent in position 3 were assigned from the small coupling constant between H-1 and H-2 ( $J=3.5$  Hz), H-2 and H-3 ( $J=2.5$  Hz), and between H-3 and H-4 $\beta$  ( $J=3.5$  Hz), together with the long range coupling in a W-mechanism between H-2 and H-4 $\alpha$  ( $J < 1.0$  Hz) (Frahm et al., 1981; Viladomat et

al., 1995). The signal at  $\delta$  4.86 (br dd) was assigned to H-11<sub>exo</sub> for it showed spatial proximity to H-4 $\beta$  ( $\delta$  1.48) from the ROESY contour correlation (Bax & Davis, 1985) (Table 2), and thus implying that the hydroxyl substituent is in the *endo*-position. The ROESY spectrum was also used in assigning the aromatic signal at  $\delta$  6.69 to H-10 for its proximity to H-1. The three bond correlation of H-10 with C-10b, C-8 and C-6a in the HMBC experiment (Bax & Summers, 1986) was also observed, corroborating the assignment of this aromatic proton. The assignment of the H-6 protons was based on the ROESY spectrum which showed spatial proximity of H-6 $\alpha$  ( $\delta$  4.23) to H-4a, and H-6 $\beta$  ( $\delta$  3.76) to H-12endo. The <sup>13</sup>C NMR (Table 7) was also closely related to the one of ambelline (Viladomat et al., 1995), except for the signals at  $\delta$  53.1 and  $\delta$  54.4 assigned to C-1 and C-2, respectively, by means of HMQC (Bax & Subramanian, 1986) and HMBC spectra (Table 1).

The <sup>1</sup>H NMR spectrum of **2** was similar to that of **1**, and the assignment was based on the above alkaloid. However, the presence of an acetoxy group was indicated by the signal at  $\delta$  1.76 and its pronounced deshielding effect ( $\Delta\delta \sim 0.6$  ppm) on H-11<sub>exo</sub> ( $\delta$  5.45). Long range W coupling between H-12endo ( $\delta$  2.65) and H-4a ( $\delta$  3.15) of  $J=1.5$  Hz was another significant feature observed. In the <sup>13</sup>C NMR spectrum of **2** (Table 7), the signals at  $\delta$  20.8 (q) and at  $\delta$  170.4 (s) further confirmed the presence of the acetoxy group. Another notable feature in the <sup>13</sup>C NMR spectrum is the deshielding effect of the acetoxy group to  $\alpha$  and  $\gamma$  carbon atoms (C-11, C-1, C-4a and C-10a) and its shielding effect to the  $\beta$  carbon atoms (C-10b and C-12) as compared to their counterparts in **1** (Delmond, Taran, Valade, Petraud, & Barbe, 1981).

Compounds **3**, C<sub>17</sub>H<sub>19</sub>NO<sub>6</sub>, and **4**, C<sub>18</sub>H<sub>21</sub>NO<sub>6</sub>, are

Table 3  
<sup>1</sup>H NMR, HMQC and HMBC data of compound **3**. Carbon multiplicities were established by DEPT data

H	<sup>1</sup> H NMR	Correlated C-atom	
		HMQC	HMBC
1	3.65 d (3.5)	53.5 d	C-2, C-4a, C-10a, C-10b
2	3.20 br s	56.1 d	C-3, C-4
3	4.44 br s	65.2 d	C-1, C-4a
4 $\alpha$	1.72 m	28.3 t	C-4a, C-10b
4 $\beta$	1.45 ddd (14.0, 13.5, 3.0)	28.3 t	C-4a, C-10b
4a	3.93 dd (13.5, 3.0)	54.1 d	C-1, C-4, C-6, C-10a, C-11
6 $\beta$	5.33 s	86.3 d	C-4a, C-6a, C-7, C-8, C-10a, C-12
10	6.61 s	96.7 d	C-6a, C-8, C-9, C-10b
11 endo	1.75 ddd (12.0, 9.0, 4.0)	35.3 t	C-4a, C-10a, C-10b
11 exo	2.33 ddd (12.0, 11.5, 6.5)	35.3 t	C-1, C-10a, C-10b, C-12
12 endo	2.70 ddd (13.0, 9.0, 6.5)	46.0 t	C-4a, C-6, C-11
12 exo	3.18 ddd (13.0, 11.5, 4.0)	46.0 t	C-4a, C-6
OCH <sub>2</sub> O	5.88 d–5.89 d (1.5)	100.9 t	C-8, C-9
7-OMe	4.01 s	59.8 q	C-7

Table 4

Scalar and spatial correlations of the protons of compound of **3**

H	COSY	ROESY
1	H-2	H-2, H-10
2	H-1, H-3	H-1, H-3
3	H-2, H-4 $\alpha$ , H-4 $\beta$	H-2, H-4 $\alpha$ , H-4 $\beta$
4 $\alpha$	H-3, H-4 $\beta$ , H-4a	H-3, H-4 $\beta$ , H-4a
4 $\beta$	H-3, H-4 $\alpha$ , H-4a	H-3, H-4 $\alpha$ , H-11exo
4a	H-4 $\alpha$ , H-4 $\beta$	H-4 $\alpha$
6 $\beta$	—	H-12endo, 7-OMe
10	—	H-1, H-11endo
11endo	H-11exo, H-12endo, H-12exo	H-10, H-11exo, H-12endo
11exo	H-11endo, H-12endo, H-12exo	H-4 $\beta$ , H-11endo, H-12exo
12endo	H-11endo, H-11exo, 12exo	H-6 $\beta$ , H-11endo, H-12exo
12exo	H-11endo, H-11exo, H-12endo	H-11exo, H-12endo
OCH <sub>2</sub> O	—	—
7-OMe	—	H-6 $\beta$

the new Amaryllidaceae alkaloids reported here for the first time from a natural source and were identified as 6 $\alpha$ -hydroxycrinamidine and 6 $\alpha$ -hydroxyundulatine, respectively. Alkaloid **4** was first reported having been synthesised from undulatine (Slabaugh & Wildman, 1971). The <sup>1</sup>H NMR spectra of **3** and **4** (Tables 3 and 5) were closely related to those of **6** Viladomat et al., 1996) and **7** (Viladomat et al., 1995), respectively, however, the usual AB system of benzylic (H-6) protons was lacking. The notable difference between the <sup>1</sup>H NMR spectra of **3** and **4** was the presence of an aliphatic methoxyl signal at  $\delta$  3.39 in the latter. The significant chemical shifts at  $\delta$  5.33 and 5.21 for **3** and **4**, respectively, suggested a benzylic proton geminal to a hydroxyl group, for the relatively low field shift of the signal ruled out the typical hydroxylation at C-3 or C-11. No evidence of the presence of epimers in both **3** and **4** as observed in 6-hydroxycrinine or 6-hydroxybu-

Table 6

Scalar and spatial correlations of the protons of compound **4**

H	COSY	ROESY
1	H-2	H-2, H-10
2	H-1, H-3	H-1, H-3
3	H-2, H-4 $\alpha$ , H-4 $\beta$	H-2, H-4 $\alpha$ , H-4 $\beta$ , 3-OMe
4 $\alpha$	H-3, H-4 $\beta$ , H-4a	H-3, H-4 $\beta$ , H-4a
4 $\beta$	H-3, H-4 $\alpha$ , H-4a	H-3, H-4 $\alpha$ , H-11exo
4a	H-4 $\beta$ , H-4 $\alpha$	H-4 $\beta$
6 $\beta$	—	H-12endo
10	—	H-1, H-11endo
11endo	H-11exo, H-12endo, H-12exo	H-11exo, H-12endo
11exo	H-11endo, H-12endo, H-12exo	H-4 $\beta$ , H-11endo, H-12exo
12endo	H-11endo, H-11exo, H-12exo	H-6 $\beta$ , H-11endo, H-12exo
12exo	H-11endo, H-11exo, H-12endo	H-11exo, H-12endo
OCH <sub>2</sub> O	—	—
3-OMe	—	H-3
7-OMe	—	—

phanisine, which is attributed by the presence of the aromatic methoxyl group at C-7 (Slabaugh & Wildman, 1971; Ali, Kating, & Frahm, 1981). The signals were assigned to H-6 $\beta$  because of their spatial proximity to H-12endo as was observed from the ROESY experiment (Tables 4 and 6). The presence of the hydroxyl group at C-6 in the  $\alpha$  orientation in **3** was further supported by the pronounced deshielding of the H-4a ( $\delta$  3.93), which is sandwiched between two hydroxyl groups as compared to its counterpart in **6** ( $\delta$  3.17) (Viladomat et al., 1996). Similarly, the signal of H-4a in **4** ( $\delta$  3.65) was more deshielded than its counterpart in **7** ( $\delta$  3.06) (Viladomat et al., 1995). Additionally, the H-6 of both **3** and **4** showed three bond connectivities with C-4a, C-7, C-10a and C-12 in the HMBC spectra (Tables 3 and 5). The <sup>13</sup>C NMR spectra of **3** and **4** were assigned taking into account

Table 5

<sup>1</sup>H NMR, HMQC and HMBC data of compound **4**. Carbon multiplicities were established by DEPT data

H	<sup>1</sup> H NMR	Correlated C-atom	
		HMQC	HMBC
1	3.73 d (3.4)	53.8 d	C-2, C-10a, C-10b
2	3.32 ddd (3.4, 2.5, 1.0)	55.0 d	C-3, C-4
3	3.96 dd (3.0, 2.5)	74.4 d	C-1, C-4a
4 $\alpha$	1.84 m	24.3 t	C-10b
4 $\beta$	1.35 ddd (14.0, 13.5, 3.0)	24.3 t	C-2, C-4a, C-10b
4a	3.65 dd (13.5, 3.5)	54.6 d	C-10a, C-10b
6 $\beta$	5.21 s	85.2 d	C-4a, C-7, C-10a, C-12
10	6.61 s	96.6 d	C-6a, C-8, C-9, C-10b
11endo	1.78 ddd (12.0, 9.5, 4.5)	35.5 t	C-4a, C-10a, H-10b
11exo	2.39 ddd (12.0, 10.5, 6.0)	35.5 t	C-1, C-10a, C-10b
12endo	2.75 ddd (13.5, 9.5, 6.0)	46.1 t	C-4a, C-6
12exo	3.17 ddd (13.5, 10.5, 4.5)	46.1 t	C-4a, C-6
OCH <sub>2</sub> O	5.88 d–5.90 d (1.5)	100.8 t	C-8, C-9
3-OMe	3.39 s	57.2 q	C-3
7-OMe	4.03 s	59.7 q	C-6, C-7

Table 7

<sup>13</sup>CMR chemical shifts assignments of compounds 1–7. Carbon multiplicities were established by DEPT data

C	1	2	3	4	5	6	7
1	53.1 d	53.7 d	53.5 d	53.8 d	53.7 d	53.8 d	53.8 d
2	54.4 d	54.7 d	56.1 d	55.0 d	56.3 d	56.4 d	55.0 d
3	74.5 d	74.5 d	65.2 d	74.4 d	65.2 d	65.5 d	74.8 d
4	24.9 t	24.9 t	28.3 t	24.3 t	29.8 t	29.7 t	25.1 t
4a	61.2 d	62.0 d	54.1 d	54.6 d	61.2 d	61.0 d	61.1 d
6	59.1 t	58.5 t	86.3 d	85.2 d	62.2 t	58.6 t	58.5 t
6a	118.5 s	117.0 s	119.5 s	119.2 s	126.5 s	117.6 s	117.6 s
7	140.8 s	140.8 s	142.7 s	142.8 s	107.0 d	141.1 s	141.2 s
8	134.4 s	133.6 s	134.3 s	134.4 s	145.6 s	133.4 s	133.2 s
9	148.0 s	148.0 s	149.4 s	149.2 s	146.4 s	148.1 s	150.0 s
10	99.7 d	98.6 d	96.7 d	96.6 d	102.5 d	96.4 d	96.3 d
10a	131.6 s	132.8 s	138.8 s	138.8 s	137.6 s	138.7 s	138.8 s
10b	45.7 s	45.2 s	42.0 s	41.7 s	41.6 s	41.6 s	41.4 s
11	81.0 d	84.0 d	35.3 t	35.5 t	39.1 t	39.2 t	39.2 t
12	60.7 t	58.6 t	46.0 t	46.1 t	52.2 t	52.5 t	52.5 t
OCH <sub>2</sub> O	100.8 t	100.5 t	100.9 t	100.8 t	100.8 t	100.7 t	100.5 t
3-OMe	57.6 q	57.6 q	—	57.2 q	—	—	57.5 q
7-OMe	59.1 q	59.0 q	59.8 q	59.7 q	—	59.1 q	59.0 q
OCOMe	—	170.4 s	—	—	—	—	—
OCOMe	—	20.8 q	—	—	—	—	—

the HMQC and HMBC connectivities (Tables 3 and 5), and also closely compared to those of **6** and **7** (Table 5). The significant differences between the <sup>13</sup>C NMR spectra of **3** and **4** and the other epoxide-containing compounds reported here are the pronounced deshielding effect of the hydroxyl group to C-6 and the “γ-gauche” effect on C-4a (Table 7). The <sup>13</sup>C NMR spectrum of **4** was closely related to the one of **3** except for the deshielded C-3 (δ 74.7) and shielded C-4 (δ 24.3) attributed by the presence of the methoxyl group at C-3 (Table 7).

Flexinine (**5**), C<sub>16</sub>H<sub>17</sub>NO<sub>4</sub>. Both <sup>1</sup>H and <sup>13</sup>C NMR spectra were reported (Ali, El Sayed, Abdallah, & Steglich, 1986), but complete and additional information with respect to previously published data is provided. In the <sup>1</sup>H NMR, the signals assigned to the H-6 protons were exchanged. In this respect, the low field signal at δ 4.31 was assigned to H-6α and the high field one at δ 3.66 to H-6β as was confirmed by their ROESY contour correlations with H-4a and H-12endo, respectively. In the <sup>13</sup>C NMR spectrum the C-8 and C-9 were unambiguously assigned from their three bond correlations (HMBC spectra) with H-10 and H-7, respectively.

### 3. Experimental

#### 3.1. General

M.p.'s are uncorr. IR spectra were measured in dry film. EIMS at 70 eV. <sup>1</sup>H, <sup>13</sup>C NMR, DEPT, <sup>1</sup>H

COSY, HMQC, HMBC (60 and 110 ms) and ROESY (300 ms) spectra were recorded in a Varian VXR 500, using CDCl<sub>3</sub> (except for lycorine where CD<sub>3</sub>OD was used) and TMS as internal standard. Chemical shifts were reported in δ units (ppm) and coupling constants (*J*) in Hz. Silica gel SDS silice 60 A CC (6–35 microns) and chromagel silice 60 A CC (70–200 microns) were used for VLC and CC respectively. Silica gel 60 F<sub>254</sub> (Macherey-Nagel) for analyt. (0.25 mm) and prep. TLC (1 mm). Spots on chromatograms were detected under UV light (254 nm) and by Dragendorff's reagent.

#### 3.2. Plant material

Bulbs of *Ammocharis tinneana* were collected in May 1997 during the flowering period in the Athi River, Kenya. A voucher specimen (SM/450/97) has been deposited at the Herbarium of the Botany Department of University of Nairobi, Kenya.

#### 3.3. Extraction and isolation of alkaloids

Fresh bulbs (10.8 kg) were crushed and macerated with MeOH for 48 h and the process repeated twice. The extracts were evapd under red. pres., the residue dissolved in H<sub>2</sub>O and acidified with 5% H<sub>2</sub>SO<sub>4</sub> to pH 3–4. After removing the neutral material with Et<sub>2</sub>O, the acidic soln was basified with 10% NH<sub>3</sub> to pH 8–9. The soln was extracted with EtOAc several times and, finally, with EtOAc–MeOH (9:1). After combining the extracts and drying *in vacuo*, the brown gummy residue (20.3 g) was subjected to VLC on silica gel eluting with *n*-hexane, increasing the polarity with EtOAc and later up to EtOAc–MeOH (8:2), where six fractions containing alkaloids were obtained. Fr. I was subjected to CC on silica gel eluting with *n*-hexane–EtOAc and increasing the polarity to EtOAc, and thereafter cleaned by prep. TLC using *n*-hexane–EtOAc (1:1) to afford 11-*O*-acetylambelline (22 mg) and **2** (25 mg). Fr. II was rechromatographed by VLC in a smaller scale using *n*-hexane–EtOAc and increasing the polarity with EtOAc where lycorine (190 mg) crystallized in MeOH. Prep. TLC was carried out on the soln using EtOAc in NH<sub>3</sub> atm. as the eluent where more lycorine (21 mg), sternbergine (11 mg), **4** (45 mg) and **7** (36 mg) were obtained. Fr. III was subjected to VLC as fr. II where **1** (109 mg) crystallized in MeOH. Prep. TLC of the soln using CH<sub>2</sub>Cl<sub>2</sub>–MeOH (24:1) in NH<sub>3</sub> atm. as eluent yielded more of **1** (30 mg), **4** (9 mg), lycorine (15 mg) and buphanidrine (19 mg). Fr. IV was treated as fr. III above and **1** (27 mg), **3** (24 mg), **6** (21 mg) and ambelline (25 mg) were isolated. Fr. V was initially cleaned by VLC eluting with EtOAc where ambelline (480 mg) crystallized in MeOH. The soln was subjected to prep. TLC eluting with EtOAc in

NH<sub>3</sub> atm. where more ambelline (46 mg), 9-*O*-demethylpluviine (6 mg), **5** (5 mg), **3** (11 mg) and buphanisine (5 mg) were obtained. Fr. VI was purified twice by VLC eluting with EtOAc and increasing the polarity up to EtOAc–MeOH (8:2). Crinine (143 mg) crystallized in EtOAc–Me<sub>2</sub>CO (1:1). Prep. TLC on the soln eluting with EtOAc–MeOH (1:1) afforded more crinine (34 mg), ambelline (16 mg) and powelline (13 mg).

### 3.3.1. 1,2β-Epoxyambelline (**1**)

Found: C, 61.8; H, 6.1; N, 4.2. Calc. for C<sub>18</sub>H<sub>21</sub>NO<sub>6</sub>: C, 62.2; H, 6.1; N, 4.0%. M.p. 252–254°C.  $[\alpha]_D^{20}$  –14.6° (MeOH; *c* 1.15). CD  $[\Theta]_{253} + 794$ ,  $[\Theta]_{276} - 174$ . IR  $\nu_{\max}$  cm<sup>-1</sup>: 3200 (–OH), 2919, 1617, 1482, 1388, 1330, 1276 (epoxide), 1211, 1134, 1070, 1041, 938 (–OCH<sub>2</sub>O–), 850. EIMS 70 eV, *m/z* (rel. int.): 347 [M]<sup>+</sup> (33), 318 [M–CHO]<sup>+</sup> (100), 274 [M–C<sub>3</sub>H<sub>5</sub>O<sub>2</sub>]<sup>+</sup> (25), 244 (24), 231 (27), 205 [C<sub>12</sub>H<sub>13</sub>O<sub>3</sub>]<sup>+</sup> (47), 189 (14), 173 (11), 115 (18). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): Table 1; <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>): Tables 1 and 7.

### 3.3.2. 11-*O*-Acetyl-1,2β-epoxyambelline (**2**)

Found: C, 62.1; H, 5.9; N, 3.5. Calc. for C<sub>20</sub>H<sub>23</sub>NO<sub>7</sub>: C, 61.7; H, 6.0; N, 3.6%. M.p. 195–197°C.  $[\alpha]_D^{20}$  –49.9° (MeOH; *c* 0.38). CD  $[\Theta]_{253} + 970$ ,  $[\Theta]_{282} - 464$ . IR  $\nu_{\max}$  cm<sup>-1</sup>: 2936, 1740 (>C=O), 1672, 1617, 1481, 1376, 1315, 1280 (epoxide), 1250, 1118, 980, 931 (–OCH<sub>2</sub>O–), 853, 751. EIMS 70 eV, *m/z* (rel. int.): 389 [M]<sup>+</sup> (66), 330 [M–OAc]<sup>+</sup> (75), 316 [M–C<sub>3</sub>H<sub>5</sub>O<sub>2</sub>]<sup>+</sup> (100), 274 (28), 256 (60), 231 (42), 228 (12), 205 [C<sub>12</sub>H<sub>13</sub>O<sub>3</sub>]<sup>+</sup> (39), 203 (33), 190 (25), 173 (15), 159 (10), 115 (31). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 1.53 (1H, ddd, *J* = 14.0, 13.5, 3.0 Hz, H-4β), 1.74 (1H, dddd, *J* = 14.0, 3.5, 3.5, 1.0 Hz, H-4α), 1.76 (3H, *s*, OCOMe), 2.65 (1H, ddd, *J* = 14.5, 3.5, 1.5 Hz, H-12endo), 3.15 (1H, ddd, *J* = 13.5, 3.5, 1.5 Hz, H-4a), 3.29 (1H, ddd, *J* = 3.5, 2.5, 1.0 Hz, H-2), 3.39 (3H, *s*, 3-OMe), 3.54 (1H, dd, *J* = 14.5, 8.0 Hz, H-12exo), 3.75 (1H, d, *J* = 3.5 Hz, H-1), 3.75 (1H, d, *J* = 17.5 Hz, H-6β), 3.97 (1H, overlapped, H-3), 3.97 (3H, *s*, 7-OMe), 4.27 (1H, d, *J* = 17.5 Hz, H-6α), 5.45 (1H, dd, *J* = 8.0, 3.5 Hz, H-11exo), 5.85 and 5.86 (2H, 2d, *J* = 1.5 Hz, OCH<sub>2</sub>O), 6.55 (1H, *s*, H-10). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>): Table 7.

### 3.3.3. 6α-Hydroxycrinamidine (**3**)

Found: C, 62.0; H, 5.8; N, 4.0. C<sub>17</sub>H<sub>19</sub>NO<sub>6</sub> requires: C, 61.2; H, 5.7; N, 4.2%. M.p. 254–256°C.  $[\alpha]_D^{20} + 26^\circ$  (MeOH; *c* 0.45). CD  $[\Theta]_{250} + 3093$ ,  $[\Theta]_{289} - 1140$ . IR  $\nu_{\max}$  cm<sup>-1</sup>: 3342 (–OH), 2931, 1730, 1618, 1481, 1283 (epoxide), 1239, 1210, 1109, 1084, 1043, 996, 945 (–

OCH<sub>2</sub>O–), 825, 757. EIMS 70 eV, *m/z* (rel. int.): 333 [M]<sup>+</sup> (38), 304 [M–CHO]<sup>+</sup> (15), 286 [M–CHO–H<sub>2</sub>O]<sup>+</sup> (34), 274 [M–C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>]<sup>+</sup> (40), 256 (26), 244 (13), 231 (35), 219 (67), 204 (30), 173 (12), 159 (8), 115 (17), 56 (100). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): Table 3; <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>): Tables 3 and 7.

### 3.3.4. 6α-Hydroxyundulatine (**4**)

Found: C, 61.6; H, 6.2; N, 4.2. C<sub>18</sub>H<sub>21</sub>NO<sub>6</sub> requires: C, 62.2; H, 6.1; N, 4.0%. M.p. 113–116°C.  $[\alpha]_D^{20} + 8.4^\circ$  (MeOH; *c* 0.53). CD  $[\Theta]_{255} + 1841$ ,  $[\Theta]_{278} - 2168$ . IR  $\nu_{\max}$  cm<sup>-1</sup>: 3200 (–OH), 2934, 1676, 1616, 1481, 1392, 1282 (epox.), 1242, 1213, 1114, 1045, 926 (–OCH<sub>2</sub>O–), 823, 752. EIMS 70 eV, *m/z* (rel. int.): 347 [M]<sup>+</sup> (47), 318 [M–CHO]<sup>+</sup> (3), 276 (44), 274 [M–C<sub>3</sub>H<sub>5</sub>O<sub>2</sub>]<sup>+</sup> (22), 256 (39), 246 (21), 231 (27), 219 (96), 204 (38), 189 (18), 173 (14), 159 (9), 115 (27), 56 (100). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): Table 5; <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>): Tables 5 and 7.

### 3.3.5. Flexinine (**5**)

Found: C, 68.0; H, 6.2; N, 5.0. Calc for C<sub>16</sub>H<sub>17</sub>NO<sub>4</sub>: C, 66.9; H, 6.0; N, 4.9%. M.p. 225–226°C.  $[\alpha]_D^{20} - 24^\circ$  (MeOH; *c* 0.35). CD  $[\Theta]_{250} + 6349$ ,  $[\Theta]_{285} - 1982$ . IR  $\nu_{\max}$  cm<sup>-1</sup>: 3084 (–OH), 2884, 1505, 1487, 1341, 1315, 1288 (epox.), 1239, 1096, 1038, 988, 934 (–OCH<sub>2</sub>O–), 916, 847. EIMS 70 eV, *m/z* (rel. int.): 287 [M]<sup>+</sup> (54), 258 [M–CHO]<sup>+</sup> (100), 229 (14), 228 (17), 215 (31), 214 (23), 187 (40), 186 (17), 175 (54), 173 (29), 159 (26), 145 (27), 143 (67), 115 (82). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 1.56 (1H, *m*, H-4β), 1.58 (1H, *m*, H-4α), 2.00 (1H, ddd, *J* = 12.5, 9.0, 5.0 Hz, H-11endo), 2.38 (1H, ddd, *J* = 12.5, 10.5, 5.5 Hz, H-11exo), 2.79 (1H, ddd, *J* = 12.5, 9.0, 5.5 Hz, H-12endo), 3.15 (1H, ddd, *J* = 12.5, 10.0, 5.0 Hz, H-12exo), 3.19 (1H, *m*, H-4a), 3.27 (1H, dd, *J* = 3.5, 2.2 Hz, H-2), 3.66 (1H, *d*, *J* = 17.0 Hz, H-6β), 3.78 (1H, *d*, *J* = 3.5, H-1), 4.31 (1H, *d*, *J* = 17.0 Hz, H-6α), 4.48 (1H, dd, *J* = 3.0, 2.2, Hz, H-3), 5.88–5.89 (2H, 2d, *J* = 1.5 Hz, OCH<sub>2</sub>O), 6.47 (1H, *s*, H-7), 6.82 (1H, *s*, H-10). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>): Table 7.

Lycorine (Likhitwitayawuid et al., 1993; Spohn, Brecht, & Frahm, 1994), sternbergine (Evidente, Iasiello, & Randazzo, 1984), 9-*O*-demethylpluviine (Kreh, Matusch, & Witte, 1995), crinine (Viladomat et al., 1995), powelline (Kobayashi et al., 1984; Frahm, Ali, & Ramadan, 1985), buphanisine (Viladomat et al., 1995), buphanidrine (Viladomat et al., 1995), ambelline (Viladomat et al., 1995; Viladomat Bastida, Codina, Campbell, & Mathee, 1994), 11-*O*-acetylambelline (Viladomat et al., 1995), crinamidine (Viladomat et al., 1996) and undulatine (Viladomat et al., 1995) were identified by a comparison of their chromatographic and spectroscopic properties (TLC,  $[\alpha]_D$ , CD, IR, MS,

$^1\text{H}$  and  $^{13}\text{C}$  NMR) with those of authentic samples obtained from other plant sources.

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