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Alkaloids from *Ammocharis tinneana*

A. Machocho[a], S.C. Chhabra[a], F. Viladomat[b], C. Codina[b], J. Bastida[b,*]

[a]*Chemistry Department, Kenyatta University, P.O. Box 43844, Nairobi, Kenya*
[b]*Departament de Productes Naturals, Facultat de Farmàcia, Universitat de Barcelona, Avda. Diagonal 643, 08028Barcelona, Spain*

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Abstract

Sixteen alkaloids have been isolated from fresh bulbs of *Ammocharis tinneana* (Amaryllidaceae), seven of which contain a 1,2β-epoxide group. 6α-Hydroxycrinamidine and 6α-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. ^{1}H and ^{13}C NMR spectra of flexinine, 1,2β-epoxyambelline and 11-*O*-acetyl-1,2β-epoxyambelline were completely assigned by means of 2D NMR techniques.

Keywords: *Ammocharis tinneana*; Amaryllidaceae; Alkaloids; Lycorine; Sternbergine; 9-*O*-*D*emethylpluviine; Crinine; Powelline; Buphanisine; Buphanidrine; Ambelline; 11-*O*-Acetylambelline; Flexinine; Crinamidine; 6α-Hydroxycrinamidine; Undulatine; 6α-Hydroxyundulatine; 1,2β-Epoxyambelline; 11-*O*-Acetyl-1,2β-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). 1,2β-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 6α-hydroxycrinamidine (**3**) and 6α-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 β-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-

* Corresponding author. Tel.: +34-93-402-4493; fax: +34-93-402-9043.

E-mail address: bastida@farmacia.far.ub.es (J. Bastida)

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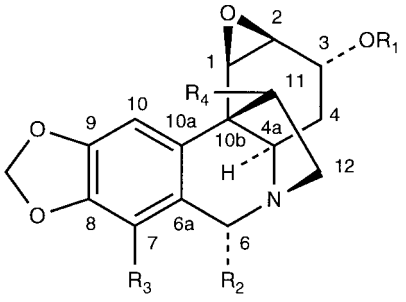
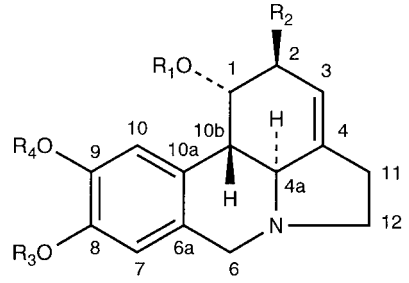
Table 1
^{1}H NMR, HMQC and HMBC data of compound **1**. Carbon multiplicities were established by DEPT data

H		^{1}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_2O	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

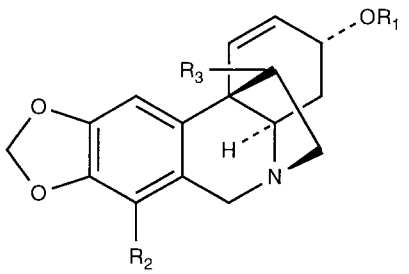
O-acetyl-1,2β-epoxyambelline (**2**), **3**, **4**, flexinine (**5**), crinamidine (**6**) and undulatine (**7**) exhibited the typical fragmentation pattern for structures with an epoxide group at the 1,2 position (Frahm, Ali, & Kating, 1981; Samuel, 1975). Compound **2** showed an additional ion at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



1: R_1 = Me, R_2 = H, R_3 = OMe, R_4 = OH
2: R_1 = Me, R_2 = H, R_3 = OMe, R_4 = OAc
3: R_1 = H, R_2 = OH, R_3 = OMe, R_4 = H
4: R_1 = Me, R_2 = OH, R_3 = OMe, R_4 = H
5: R_1 = H, R_2 = H, R_3 = H, R_4 = H
6: R_1 = H, R_2 = H, R_3 = OMe, R_4 = H
7: R_1 = Me, R_2 = H, R_3 = OMe, R_4 = H



lycorine: R_1 = H, R_2 = OH, $R_3 + R_4 = CH_2$
sternbergine: R_1 = Ac, R_2 = OH, R_3 = H, R_4 = Me
9-*O*-demethylpluviine: R_1 = H, R_2 = H, R_3 = Me, R_4 = H



ambelline: R_1 = Me, R_2 = OMe, R_3 = OH
11-*O*-acetylambelline: R_1 = Me, R_2 = OMe, R_3 = OAc
crinine: R_1 = H, R_2 = H, R_3 = H
powelline: R_1 = H, R_2 = OMe, R_3 = H
buphanidrine: R_1 = Me, R_2 = OMe, R_3 = H
buphanisine: R_1 = Me, R_2 = H, R_3 = H

1,2β-Epoxyambelline (**1**), $C_{18}H_{21}NO_6$, and 11-*O*-acetyl-1,2β-epoxyambelline (**2**), $C_{20}H_{23}NO_7$, 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-4α	H-1, H-3
3	H-2, H-4α, H-4β	H-2, 3-OMe, H-4α, H-4β
4α	H-2, H-3, H-4β, H-4a	H-3, H-4β, H-4a
4β	H-3, H-4α, H-4a	H-3, H-4α, H-11
4α	H-4α H-4β	H-4α, H-6α
6α	H-6β	H-4a, H-6β
6β	H-6α	H-6α, H-12endo
10	–	H-1
11	H-12endo, H-12exo	H-4β, H-12exo
12endo	H-11, H-12exo	H-6β, H-12exo
12exo	H-11, H-12endo	H-11, H-12endo
OCH_2O	–	–
3-OMe	–	H-3
7-OMe	–	–

but the published data on ^1H and ^{13}C NMR were incomplete. Complete assignment is reported here using 2D NMR techniques. Their ^1H 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 δ 3.69 (d) and δ 3.29 (ddd), were assigned to H-1 and H-2, respectively. The configuration of the epoxide ring and the α-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-4β ($J = 3.5$ Hz), together with the long range coupling in a W-mechanism between H-2 and H-4α ($J < 1.0$ Hz) (Frahm et al., 1981; Viladomat et al., 1995). The signal at δ 4.86 (br dd) was assigned to H-11*exo* for it showed spatial proximity to H-4β (δ 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 δ 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-6α (δ 4.23) to H-4a, and H-6β (δ 3.76) to H-12*endo*. The ^{13}C NMR (Table 7) was also closely related to the one of ambelline (Viladomat et al., 1995), except for the signals at δ 53.1 and δ 54.4 assigned to C-1 and C-2, respectively, by means of HMQC (Bax & Subramanian, 1986) and HMBC spectra (Table 1).

The ^1H 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 δ 1.76 and its pronounced deshielding effect ($\Delta\delta \sim 0.6$ ppm) on H-11*exo* (δ 5.45). Long range W coupling between H-12*endo* (δ 2.65) and H-4a (δ 3.15) of $J = 1.5$ Hz was another significant feature observed. In the ^{13}C NMR spectrum of **2** (Table 7), the signals at δ 20.8 (q) and at δ 170.4 (s) further confirmed the presence of the acetoxy group. Another notable feature in the ^{13}C NMR spectrum is the deshielding effect of the acetoxy group to α and γ carbon atoms (C-11, C-1, C-4a and C-10a) and its shielding effect to the β carbon atoms (C-10b and C-12) as compared to their counterparts in **1** (Delmond, Taran, Valade, Petraud, & Barbe, 1981).

Compounds **3**, $C_{17}H_{19}NO_6$, and **4**, $C_{18}H_{21}NO_6$, are

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

H	^1H 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
4α	1.72 m	28.3 t	C-4a, C-10b
4β	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
6β	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_2O	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-4α, H-4β	H-2, H-4α, H-4β
4α	H-3, H-4β, H-4a	H-3, H-4β, H-4a
4β	H-3, H-4α, H-4a	H-3, H-4α, H-11exo
4a	H-4α, H-4β	H-4α
6β	–	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-4β, H-11endo, H-12exo
12endo	H-11endo, H-11exo, 12exo	H-6β, H-11endo, H-12exo
12exo	H-11endo, H-11exo, H-12endo	H-11exo, H-12endo
OCH_2O	–	–
7-OMe	–	H-6β

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-4α, H-4β	H-2, H-4α, H-4β, 3-OMe
4α	H-3, H-4β, H-4a	H-3, H-4β, H-4a
4β	H-3, H-4α, H-4a	H-3, H-4α, H-11exo
4a	H-4β, H-4α	H-4β
6β	–	H-12endo
10	–	H-1, H-11endo
11 endo	H-11exo, H-12endo, H-12exo	H-11exo, H-12endo
11 exo	H-11endo, H-12endo, H-12exo	H-4β, H-11endo, H-12exo
12 endo	H-11endo, H-11exo, H-12exo	H-6β, H-11endo, H-12exo
12 exo	H-11endo, H-11exo, H-12endo	H-11exo, H-12endo
OCH_2O	–	–
3-OMe	–	H-3
7-OMe	–	–

the new Amaryllidaceae alkaloids reported here for the first time from a natural source and were identified as 6α-hydroxycrinamidine and 6α-hydroxyundulatine, respectively. Alkaloid **4** was first reported having been synthesised from undulatine (Slabaugh & Wildman, 1971). The ^{1}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 ^{1}H NMR spectra of **3** and **4** was the presence of an aliphatic methoxyl signal at δ 3.39 in the latter. The significant chemical shifts at δ 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-hydroxybuphanisine, 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-6β because of their spatial proximity to H-12*endo* as was observed from the ROESY experiment (Tables 4 and 6). The presence of the hydroxyl group at C-6 in the α orientation in **3** was further supported by the pronounced deshielding of the H-4a (δ 3.93), which is sandwiched between two hydroxyl groups as compared to its counterpart in **6** (δ 3.17) (Viladomat et al., 1996). Similarly, the signal of H-4a in **4** (δ 3.65) was more deshielded than its counterpart in **7** (δ 3.06) (Viladomat et al., 1995). Additionally, the H-6 of both **3** and **4** showed three bond connectivies with C-4a, C-7, C-10a and C-12 in the HMBC spectra (Tables 3 and 5). The ^{13}C NMR spectra of **3** and **4** were assigned taking into account

Table 5
^{1}H NMR, HMQC and HMBC data of compound **4**. Carbon multiplicities were established by DEPT data

H	^{1}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
4α	1.84 m	24.3 t	C-10b
4β	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
6β	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
11 endo	1.78 ddd (12.0, 9.5, 4.5)	35.5 t	C-4a, C-10a, H-10b
11 exo	2.39 ddd (12.0, 10.5, 6.0)	35.5 t	C-1, C-10a, C-10b
12 endo	2.75 ddd (13.5, 9.5, 6.0)	46.1 t	C-4a, C-6
12 exo	3.17 ddd (13.5, 10.5, 4.5)	46.1 t	C-4a, C-6
OCH_2O	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
^{13}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_2O	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
O*CO*Me	–	170.4 s	–	–	–	–	–
OCO*Me*	–	20.8 q	–	–	–	–	–

the HMQC and HMBC connectivies (Tables 3 and 5), and also closely compared to those of **6** and **7** (Table 5). The significant differences between the ^{13}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 ^{13}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**), $C_{16}H_{17}NO_4$. Both ^{1}H and ^{13}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 ^{1}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-12*endo,* respectively. In the ^{13}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. ^{1}H, ^{13}C NMR, DEPT, ^{1}H COSY, HMQC, HMBC (60 and 110 ms) and ROESY (300 ms) specta were recorded in a Varian VXR 500, using $CDCl_3$ (except for lycorine where CD_3OD 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_{254} (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_2O and acidified with 5% H_2SO_4 to pH 3–4. After removing the neutral material with Et_2O, the acidic soln was basified with 10% NH_3 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_3 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_2Cl_2–MeOH (24:1) in NH_3 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_3 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_2CO (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 $C_{18}H_{21}NO_6$: 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 ν_{max} cm^{-1}: 3200 (–OH), 2919, 1617, 1482, 1388, 1330, 1276 (epoxide), 1211, 1134, 1070, 1041, 938 (–OCH_2O–), 850. EIMS 70 eV, *m/z* (rel. int.): 347 $[M]^+$ (33), 318 $[M–CHO]^+$ (100), 274 $[M–C_3H_5O_2]^+$ (25), 244 (24), 231 (27), 205 $[C_{12}H_{13}O_3]^+$ (47), 189 (14), 173 (11), 115 (18).). ^1H NMR (500 MHz, $CDCl_3$): Table 1; ^{13}C NMR (50 MHz, $CDCl_3$): 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 $C_{20}H_{23}NO_7$: C, 61.7; H, 6.0; N, 3.6% M.p. 195-197°. $[\alpha]_D^{20}$ −49.9° (MeOH; *c* 0.38). CD $[\Theta]_{253}$ +970, $[\Theta]_{282}$ −464. IR ν_{max} cm^{-1}: 2936, 1740 (>C=O), 1672, 1617, 1481, 1376, 1315, 1280 (epoxide), 1250, 1118, 980, 931 (–OCH_2O–), 853, 751. EIMS 70 eV, *m/z* (rel. int.): 389 $[M]^+$ (66), 330 $[M–OAc]^+$ (75), 316 $[M–C_3H_5O_2]^+$ (100), 274 (28), 256 (60), 231 (42), 228 (12), 205 $[C_{12}H_{13}O_3]^+$ (39), 203 (33), 190 (25), 173 (15), 159 (10), 115 (31). ^1H NMR (500 MHz, $CDCl_3$): 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*, OCO*Me*), 2.65 (1H, ddd, *J* = 14.5, 3.5, 1.5 Hz, H-12*endo*), 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-12*exo*), 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-11*exo*), 5.85 and 5.86 (2H, 2d, *J* = 1.5 Hz, OCH_2O), 6.55 (1H, *s*, H-10). ^{13}C NMR (50 MHz, $CDCl_3$): Table 7.

3.3.3. 6α-Hydroxycrinamidine (*3*)

Found: C, 62.0; H, 5.8; N, 4.0. $C_{17}H_{19}NO_6$ requires: C, 61.2; H, 5.7; N, 4.2%. M.p. 254-256°. $[\alpha]_D^{20}$ +26° (MeOH; *c* 0.45). CD $[\Theta]_{250}$ + 3093, $[\Theta]_{289}$ −1140. IR ν_{max} cm^{-1}: 3342 (–OH), 2931, 1730, 1618, 1481, 1283 (epoxide), 1239, 1210, 1109, 1084, 1043, 996, 945 (–OCH_2O–), 825, 757. EIMS 70 eV, *m/z* (rel. int.): 333 $[M]^+$ (38), 304 $[M–CHO]^+$ (15), 286 $[M–CHO–H_2O]^+$ (34), 274 $[M–C_2H_3O_2]^+$ (40), 256 (26), 244 (13), 231 (35), 219 (67), 204 (30), 173 (12), 159 (8), 115 (17), 56 (100). ^1H NMR (500 MHz, $CDCl_3$): Table 3; ^{13}C NMR (50 MHz, $CDCl_3$): Tables 3 and 7.

3.3.4. 6α-Hydroxyundulatine (*4*)

Found: C, 61.6; H, 6.2; N, 4.2. $C_{18}H_{21}NO_6$ requires: C, 62.2; H, 6.1; N, 4.0%. M.p. 113–116°. $[\alpha]_D^{20}$ +8.4° (MeOH; *c* 0.53). CD $[\Theta]_{255}$ +1841, $[\Theta]_{278}$ −2168. IR ν_{max} cm^{-1}: 3200 (–OH), 2934, 1676, 1616, 1481, 1392, 1282 (epox.), 1242, 1213, 1114, 1045, 926 (–OCH_2O–), 823, 752. EIMS 70 eV, *m/z* (rel. int.): 347 $[M]^+$ (47), 318 $[M–CHO]^+$ (3), 276 (44), 274 $[M–C_3H_5O_2]^+$ (22), 256 (39), 246 (21), 231 (27), 219 (96). 204 (38), 189 (18), 173 (14), 159 (9), 115 (27), 56 (100). ^1H NMR (500 MHz, $CDCl_3$): Table 5; ^{13}C NMR (50 MHz, $CDCl_3$): Tables 5 and 7.

3.3.5. Flexinine (*5*)

Found: C, 68.0; H, 6.2; N, 5.0. Calc for $C_{16}H_{17}NO_4$: C,66.9; H, 6.0; N, 4.9%. M.p. 225–226°. $[\alpha]_D^{20}$ −24° (MeOH; *c* 0.35). CD $[\Theta]_{250}$ +6349, $[\Theta]_{285}$ −1982. IR ν_{max} cm^{-1}: 3084 (–OH), 2884, 1505, 1487, 1341, 1315, 1288 (epox.), 1239, 1096, 1038, 988, 934 (–OCH_2O–), 916, 847. EIMS 70 eV, *m/z* (rel. int.): 287 $[M]^+$ (54), 258 $[M–CHO]^+$ (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). ^1H NMR (500 MHz, $CDCl_3$): 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-11*endo*), 2.38 (1H, ddd, *J* = 12.5, 10.5, 5.5 Hz, H-11*exo*), 2.79 (1H, ddd, *J* = 12.5, 9.0, 5.5 Hz, H-12*endo*), 3.15 (1H, ddd, *J* = 12.5, 10.0, 5.0 Hz, H-12*exo*), 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_2O), 6.47 (1H, *s*, H-7), 6.82 (1H, *s*, H-10). ^{13}C NMR (50 MHz, $CDCl_3$): 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}H and ^{13}C NMR) with those of authentic samples obtained from other plant sources.

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