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Alkaloids from *Nerine filifolia* ☆

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Abstract

The novel compounds *N*-demethylbelladine, 6α-methoxybuphanidrine and filifoline, in addition to five known alkaloids, and phenol have been isolated from fresh bulbs of *Nerine filifolia* (Amaryllidaceae). The structure and stereochemistry of the compounds were determined by physical and spectroscopic methods, including 1D and 2D NMR and mass spectroscopic techniques. An unusual circular dichroism response from filifoline has required a semi-synthetic derivatisation strategy towards key C-11*endo* analogues of the β-crinane representative ambelline in which the nature of substituents was observed to have a profound effect on molecular ellipticity. Filifoline was not cytotoxic to myoblast (L6) cells and exhibited no anti-protozoal activity in an in vitro screen against four different parasitic protozoa.

Keywords: *Nerine filifolia*; Amaryllidaceae; Bulbs; Alkaloids; Phenol; Belladine; *N*-Demethylbelladine; 6α-Hydroxybuphanidrine; 6α-Methoxybuphanidrine; Filifoline; Ambelline; 11-*O*-Acetylambelline; Undulatine; Circular dichroism

1. Introduction

The genus *Nerine* Herbert (Amaryllidaceae), a member of the tribe Amaryllideae, is one of seven representatives of the subtribe Amaryllidinae which is confined to the temperate regions of southern Africa (Snijman and Linder, 1996). This genus, the second largest within the Amaryllideae with ca. 23 species, is an autumn-flowering perennial bulbous plant group, whose species inhabit areas with summer rainfall and cool, dry winters. Most *Nerine* species have been cultivated for their elegant flowers, presenting a source of innumerable horticultural hybrids (Snijman and Linder, 1996; Dyer, 1976). The exploitation of plants of this genus in the traditional medicinal practices of the indigenous people of southern Africa has been documented (Watt and Breyer-Brandwijk, 1962). In particular, the southern Sotho and Zulu tribes have made use of decoctions of bulbs to treat coughs and colds, in renal and hepatic conditions, for the relief of back pain and as a remedy for infertility. In continuation of phytochemical analyses of the South African Amaryllidaceae (Viladomat et al., 1997), *Nerine filifolia* Baker was examined for alkaloidal constituents. This evergreen plant, with small bulbs and thin thread-like leaves, flowers between September and November and occurs in the Eastern Cape, Transkei, Orange Free State, Swaziland and

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Mpumalanga. Following the usual extractive and chromatographic procedures (see Section 4), eight alkaloids as well as phenol were isolated. The known compounds belladine (**1**), 11-*O*-acetylambelline (**6**) and undulatine (**8**) were isolated as the principal constituents in addition to the minor components ambelline (**5**) and 6α-hydroxybuphanidrine (**3**), while *N*-demethylbelladine (**2**), 6α-methoxybuphanidrine (**4**) and filifoline (**7**), the 11-*O*-nicotinyl analogue of ambelline, are reported here for the first time as natural products. A previous phytochemical analysis of *Nerine filifolia* has led to the detection of small quantities of galanthamine (4×10^{-3} % dry wt) by radioimmunoassay techniques (Tanahashi et al., 1990). Interestingly, the crinane-related compounds reported here all exhibit a β-5,10b-ethano bridge orientation and have in common methoxyl substituents at C-3α and C-7. An unusual circular dichroism (CD) response from filifoline (**7**) has led to the novel discovery that substituent effects at C-11*endo* in crinane alkaloids play a critical role in molecular ellipticity as expressed by the shape of the CD curve, which is indicative of the stereochemistry of the dominant C-10b benzylic ring junction and hence the orientation of the 5,10b-ethano bridge. Synthetic 11-*O*-benzoyl and 11-*O*-methanesulphonyl analogues of ambelline (**5**) (see Section 4) collectively provided further unequivocal evidence that, for crinane alkaloids, functionalization at C-11*endo* is an integral factor in molecular ellipticity.

2. Results and discussion

Compound **1**, $C_{19}H_{25}NO_3$, was characterized as belladine by its UV, IR, EIMS and NMR spectral data (Ghosal et al., 1983, 1988). A complete assignment of the proton signals, hitherto unreported, is afforded here taking into account both homonuclear (COSY) and heteronuclear (HMQC) correlations. The ^{1}H NMR spectrum had seven aromatic signals, three of which were attributable to H-3′, H-6′ and H-7′ of ring A while those of ring B were an A_2B_2 system (H-4/H-8 and H-5/H-7) (Table 1). Both 2H-1′ (δ 3.49, br *s*) and 2H-1 (δ 2.60, *m*) were assignable due to three-bond (HMBC) correlations to the *N*-methyl carbon (δ 42.2, *q*); and 2H-2 (δ 2.77, *m*) by a COSY contour to 2H-1. Similarly, the three methoxyl groups were assigned by HMBC correlations. The ^{13}C NMR data of belladine (Table 1) were in accordance with a previous report (Ghosal et al., 1983). Compound **2**, $C_{18}H_{23}NO_3$, isolated as a light-brown oil, differed in molecular formular and mass from belladine due to the absence of the *N*-methyl group. Its EIMS had the $[M]^+$ signal at *m*/*z* 301 as well as characteristic peaks at *m*/*z* 180 $[C_{10}H_{14}NO_2]^+$, 151 $[C_9H_{11}O_2]^+$, 121 $[C_7H_5O_2]^+$ and 107 $[C_7H_7O]^+$, while IR absorption bands at 3225, 2833 and 1030 cm^{-1} were indicative of NH and C–O groups; and the UV spectrum was characteristic of a *N*-benzylphenethylamine of the belladine type (Ghosal et al., 1983). The ^{1}H and ^{13}C NMR data of belladine and compound **2** (Table 1) were very similar and the only significant differences were due to the effect of *N*-substitution. Thus, in **2**, both 2H-1′ (δ 3.74, br *s*) and 2H-1 (δ 2.87, *m*) were slightly deshielded (Δδ = +0.25 and +0.27, respectively) and C-1′ (δ 53.6, *t*) and C-1 (δ 50.6, *t*) markedly shielded (Δδ = −8.4 and −8.5, respectively), in relation to the corresponding signals in belladine. Three-bond HMBC correlations facilitated assignment of the quartenary centres. Thus, C-6 correlated to H-4, H-8 and the 6-methoxyl group

Table 1
^{1}H NMR and ^{13}C NMR data for compounds **1** and **2** (*J* in Hz within parentheses)

Proton	**1**		**2**	
	^{1}H	^{13}C	^{1}H	^{13}C
2H-1	2.60 *m*	59.1 *t*	2.87 *m*	50.6 *t*
2H-2	2.77 *m*	32.9 *t*	2.77 *m*	35.3 *t*
–	–	132.5 *s* (C-3)	–	132.8 *s* (C-3)
4	7.10 *d* (8.7)	129.6 *d*	7.12 *d* (8.7)	129.6 *d*
5	6.82 *d* (8.7)	113.7 *d*	6.83 *d* (8.7)	113.8 *d*
–	–	157.8 *s* (C-6)	–	157.9 *s* (C-6)
7	6.82 *d* (8.7)	113.7 *d*	6.83 *d* (8.7)	113.8 *d*
8	7.10 *d* (8.7)	129.6 *d*	7.12 *d* (8.7)	129.6 *d*
2H-1′	3.49 br *s*	62.0 *t*	3.74 br *s*	53.6 *t*
–	–	131.6 *s* (C-2′)	–	131.9 *s* (C-2′)
3′	6.85 br *s*	110.6 *d*	6.82 br *s*	110.9 *d*
–	–	148.8 *s* (C-4′)	–	148.9 *s* (C-4′)
–	–	147.9 *s* (C-5′)	–	147.9 *s* (C-5′)
6′	6.79 br *s*	111.9 *d*	6.80 br *s*	111.2 *d*
7′	6.79 br *s*	121.0 *d*	6.80 br *s*	120.2 *d*
6-OCH_3	3.78 *s*	55.2 *q*	3.79 *s*	55.2 *q*
4′-OCH_3	3.86 *s*	55.8 *q*	3.86 *s*	55.8 *q*
5′-OCH_3	3.87 *s*	55.8 *q*	3.87 *s*	55.8 *q*
N-CH_3	2.28 *s*	42.2 *q*	–	–

protons; C-3 to H-5, H-7 and 2H-1, while C-5′ was distinguished from C-4′ due to three-bond correlations to H-3′, H-7′ and the 5′-methoxyl group protons, leading to the structure of compound **2** as *N*-demethylbelladine. Incidentally, such compounds, which are fully *O*-methylated, have no biosynthetic significance since they cannot be further oxidized (by phenolic coupling) into the tetracyclic skeleton of the Amaryllidaceae alkaloids, and occur merely as organic bases of the plant (Ghosal et al., 1983).

The molecular ellipticities of the crinane-type alkaloids (**3**, **4**, **5**, **6**, **8**) discussed herein all showed CD curves which were qualitatively similar to those of the β-5,10b-ethano bridge series, having a maximum around 250 nm (Ali et al., 1984; Wagner et al., 1996), with the exception of filifoline (**7**) whose CD curve (Fig. 1) was reminiscent of a compound with an α-5,10b-bridge since a maximum was detectable in the region of 290 nm. However, as we have chemically proved (see Section 4) the ethano bridge in filifoline is in fact β-orientated.

6α-Hydroxybuphanidrine (**3**) ($C_{18}H_{21}NO_5$), previously isolated from *Nerine bowdenii* Watson (Slabaugh and Wildman, 1971), and 6α-methoxybuphanidrine (**4**) ($C_{19}H_{23}NO_5$), described here for the first time from a natural source, exhibited several similar spectral properties. Their EIMS fragmentations were consistent with compounds of the crinine series with no bridge substituent, and having a double bond in the 1,2-position (Longevialle et al., 1973). The difference in mass between them could be accounted for by the presence of the additional methoxyl group in **4**. IR absorption bands for methoxyl and methylenedioxy groups were detected in both, as well as a hydroxyl group in **3**. We present here the complete assignment of ^{1}H and ^{13}C NMR spectra (Table 2) of both compounds, previously not undertaken. The spectra were similar to that of buphanidrine

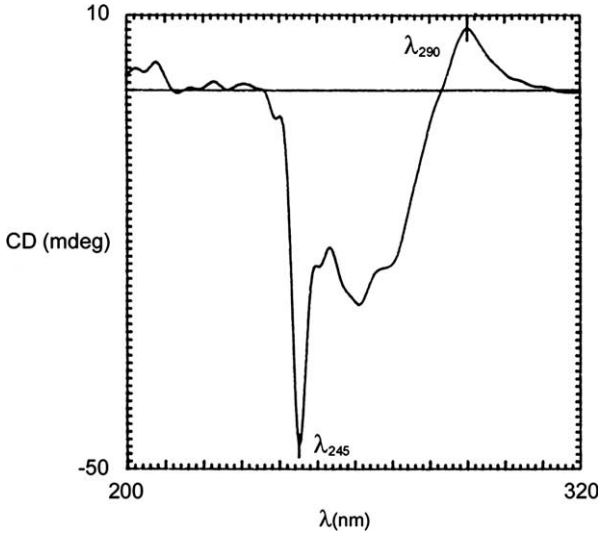


Fig. 1. CD curve of filifoline (**7**).

(Viladomat et al., 1995) and the only noteworthy differences were due to the effect of C-6 substitution in **3** and **4**. The single aromatic proton resonance was ascribable to H-10 due to three-bond HMBC correlations to C-6a, C-8 and C-10b, in addition to a ROESY correlation to H-1, allowing the aromatic methoxyl group to be placed at C-7. The large *trans* diaxial coupling (J = 13.5 Hz) between H-4a and H-4β, as well as the small vicinal coupling of both H-4α (J = 1.5 Hz, indicating a dihedral angle ca. 90°) and H-4β (J = 4.0 Hz) with H-3, vindicated the pseudoaxial disposition for the C-3 methoxyl group. Both H-11*exo* and H-12*exo* were assignable due to their spatial proximity to H-4β, in agreement with the low field assignment of H-12*exo* (relative to H-12*endo*) due to its *syn* disposition with the nitrogen lone pair. ROESY correlation between H-12*endo* and H-6β allowed us to assign the respective hydroxyl and methoxyl groups at C-6 to the α-position. The one-proton doublets at δ 3.95 (H-6β) and δ 4.35 (H-6α) in buphanidrine (Viladomat et al., 1995) were replaced by singlets at δ 5.24 and 4.59, respectively, in compounds **3** and **4**, in accordance with the hydroxyl and methoxyl groups. The ^{13}C NMR spectrum of both compounds were confirmed by HMQC and HMBC techniques (Tables 2 and 3), indicating for each a skeleton with 18 and 19 carbon atoms, respectively. The large deshielding of C-6 in compound **3** (δ 85.6) and **4** (δ 93.4) in relation to that in buphanidrine (δ 58.1, *t*) (Viladomat et al., 1995) was indicative of oxy-substitution at this carbon. The shielding ($\Delta\delta$ = −6.4) of C-4a in both compounds (relative to buphanidrine) can be explained in terms of the 'γ-gauche' effect as a result of the C-3 and C-6 pseudoaxial substituents. Three-bond correlations of H-6 to C-7 and C-10a, in addition to those described above for H-10, allowed assignment of all quartenary centres.

Filifoline (**7**), isolated as a white powder, had a weak molecular ion peak (1%) at *m/z* 436, correct for the molecular formula $C_{24}H_{24}N_2O_6$. The IR spectrum had absorption bands for ester carbonyl (1734 cm^{-1}), C–O (1040 cm^{-1}) and methylenedioxy (934 cm^{-1}) groups. Three-bond HMBC correlations (Table 4) to C-6a, C-8 and C-10b, as well as the single ROESY contour to H-1 (Table 5), allowed the aromatic proton singlet at δ 6.48 to be ascribed to H-10, thus allowing placement of the aromatic methoxyl group at C-7 (as in compounds **3** and **4**). The pseudoaxial disposition for the 3-methoxyl group was arrived at by the large coupling observed between H-4a and H-4β (J = 13.5 Hz, *trans* diaxial interaction), H-4α and H-4β (J = 14.0 Hz, geminal) and smaller couplings of both H-4α and H-4β with H-3, confirmed by a ROESY correlation between H-3 and H-4β. H-6α (δ 4.34) was assignable to a lower field than H-6β (δ 3.90) due to its proximity to the nitrogen lone pair, the same reason that H-12*exo* was further downfield-shifted (relative to H-12*endo*), all confirmed

Table 2
^{1}H NMR and ^{13}C NMR data for compounds **3** and **4** (*J* in Hz within parentheses)

Proton	**3**		**4**	
	^{1}H	^{13}C	^{1}H	^{13}C
1	6.52 *d* (10.0)	131.9 *d*	6.53 *d* (10.0)	132.6 *d*
2	5.94 *ddd* (10.0, 5.0, 0.5)	125.8 *d*	5.94 *ddd* (10.0, 5.0, 1.0)	125.5 *d*
3	3.79 *ddd* (5.0, 4.0, 1.5)	72.3 *d*	3.81 *ddd* (5.0, 4.0, 1.5)	72.4 *d*
4α	2.08 *dddd* (14.0, 4.0, 1.5, 0.5)	27.9 *t*	1.94 *dddd* (14.0, 4.0, 1.5, 1.0)	28.9 *t*
4β	1.54 *ddd* (14.0, 13.5, 4.0)	27.9 *t*	1.57 *ddd* (14.0, 13.5, 4.0)	28.9 *t*
4a	3.84 *ddd* (13.5, 4.0, 0.5)	56.4 *d*	3.65 *ddd* (13.5, 4.0, 0.5)	56.4 *d*
6β	5.24 *s*	85.6 *d*	4.59 *s*	93.4 *d*
–	–	119.2 *s* (C-6a)	–	119.2 *s* (C-6a)
–	–	142.5 *s* (C-7)	–	143.0 *s* (C-7)
–	–	134.2 *s* (C-8)	–	134.7 *s* (C-8)
–	–	149.3 *s* (C-9)	–	149.3 *s* (C-9)
10	6.55 *s*	97.0 *d*	6.54 *s*	97.0 *d*
–	–	139.8 *s* (C-10a)	–	140.2 *s* (C-10a)
–	–	44.2 *s* (C-10b)	–	44.2 *s* (C-10b)
11*endo*	1.90 *dddd* (12.5, 9.0, 4.5, 0.5)	40.8 *t*	1.87 *dddd* (12.5, 9.0, 4.5, 0.5)	41.3 *t*
11*exo*	1.84 *ddd* (12.5, 10.5, 6.0)	40.8 *t*	1.81 *ddd* (12.5, 10.0, 6.5)	41.3 *t*
12*endo*	2.79 *ddd* (13.0, 9.0, 6.0)	47.7 *t*	2.67 *ddd* (13.0, 9.0, 6.5)	47.6 *t*
12*exo*	3.31 *ddd* (13.0, 10.5, 4.5)	47.7 *t*	3.27 *ddd* (13.0, 10.0, 4.5)	47.6 *t*
OCH_2O	5.84–5.87 2*d* (1.5)	100.8 *t*	5.83–5.87 2*d* (1.5)	100.8 *t*
3-OCH_3	3.32 *s*	56.3 *q*	3.32 *s*	56.2 *q*
6-OCH_3	–	–	3.56 *s*	56.1 *q*
7-OCH_3	4.01 *s*	59.8 *q*	3.95 *s*	59.8 *q*

Table 3
COSY, ROESY and HMBC correlations for the protons of compound **4**

H	COSY	ROESY	HMBC
1	H-2	H-2, H-10	C-3, C-4a, C-10a
2	H-1, H-3, H-4α	H-1, H-3	C-3, C-4, C-10b
3	H-2, H-4α, H-4β	H-2, H-4α, H-4β, 3-OCH_3	C-1, C-2, 3-OCH_3
4α	H-2, H-3, H-4β, H-4a	H-3, H-4a, H-4β	C-2, C-4a, C-10b
4β	H-3, H-4α, H-4a	H-3, H-4α, H-11*exo*, H-12*exo*	C-4a, C-10b
4a	H-4α, H-4β	H-4α	C-4, C-11, C-12
6	–	H-12*endo*, 6-OCH_3	C-4a, C-6a, C-7, C-10a, C-12, 6-OCH_3
10	–	H-1	C-6a, C-7, C-8, C-9, C-10b
11*endo*	H-11*exo*, H-12*endo*, H-12*exo*	H-11*exo*, H-12*endo*, H-12*exo*	C-4a, C-10a, C-10b
11*exo*	H-11*endo*, H-12*endo*, H-12*exo*	H-4β, H-11*endo*, H-12*endo*, H-12*exo*	C-4a, C-10a, C-10b
12*endo*	H-11*endo*, H-11*exo*, H-12*exo*	H-6β, H-11*endo*, H-11*exo*, H-12exo	C-4a, C-6, C-11
12*exo*	H-11*endo*, H-11*exo*, H-12*endo*	H-4β, H-11*endo*, H-11*exo*, H-12*endo*	C-6
OCH_2 O	–	–	C-8, C-9
3-OCH_3	–	H-3	C-3
6-OCH_3	–	H-6	C-6
7-OCH_3	–	–	C-7

by ROESY contours of H-6β with H-12*endo*; H-6α with H-4a, as well as H-12*exo* with H-4β. Spatial proximity was also established between H-4β and H-11*exo* (δ 5.36), which suggested substitution at C-11*endo*. The presence of the nicotinate ester at C-11*endo* was indicated by the chemical shift, splitting pattern and coupling constants of the four distinct protons arising from the *m*-substituted pyridine ring: H-3′ (δ 8.74) and H-5′ (δ 8.69), which are markedly deshielded by the vicinal nitrogen atom, as well as H-6′ (δ 7.27) and H-7′ (δ 7.93). The H-3′, H-7′ and H-11*exo* protons had three-bond correlations to the ester carbonyl (δ 165.0). The use of HMQC and HMBC techniques facilitated assignment of all carbon signals. The 24-carbon skeleton of filifoline was characterized by the presence of additional aromatic signals (in comparison to the usual crinane skeleton): two singlets (for C-2′ and the carbonyl group) and four doublets (attributable to C-3′, C-5′, C-6′ and C-7′), all arising from the nicotinate ester. All quaternary centres were adequately revealed by three-bond correlations of 2H-6 to C-10a and C-7, which was shifted downfield to δ 140.7 by the presence of the methoxyl group, the methylenedioxy protons to C-8 and C-9, those mentioned above for H-10, as well as H-6′ to C-2′, in addition to the H-3′/C-2′ two-bond correlation. Filifoline (**7**) represents the first member of the crinane series

Table 4
^{1}H NMR, HMQC and HMBC data for filifoline (**7**)

H	δ	HMQC	HMBC
1	6.58 *d* (10.0)	131.3 *d*	C-3, C-4a, C-10a, C-10b
2	6.07 *ddd* (10.0, 5.0, 1.0)	126.8 *d*	C-3, C-4, C-10b
3	3.87 *ddd* (5.0, 4.0, 2.0)	72.2 *d*	C-1, C-2, C-4a, 3-OCH_3
4α	2.15 *dddd* (14.0, 4.5, 2.0, 1.0)	28.8 *t*	C-4a, C-10b
4β	1.76 *ddd* (14.0, 13.5, 4.0)	28.8 *t*	C-2, C-3, C-4a, C-10b
4a	3.48 *ddd* (13.5, 4.5, 1.0)	63.3 *d*	C-6, C-11, C-12
6α	4.34 *d* (17.5)	58.8 *t*	C-6a, C-7, C-10a, C-12
6β	3.90 *d* (17.5)	58.8 *t*	C-4a, C-6a, C-7, C-10a, C-12
–	–	117.3 *s* (C-6a)	–
–	–	140.7 *s* (C-7)	–
–	–	133.9 *s* (C-8)	–
–	–	147.9 *s* (C-9)	–
10	6.48 *s*	99.2 *d*	C-6a, C-8, C-9, C-10b
–	–	133.5 *s* (C-10a)	–
–	–	47.5 *s* (C-10b)	–
11*exo*	5.36 *dd* (8.0, 4.0)	88.4 *d*	C-1, C-10a, C-1′
12*endo*	2.83 *ddd* (14.0, 4.0, 1.0)	59.8 *t*	C-4a, C-6, C-11
12*exo*	3.88 *dd* (14.0, 8.0)	59.8 *t*	C-4a, C-6, C-11
OCH_2 O	5.77–5.81 2*d* (1.5)	100.5 *t*	C-8, C-9
3-OCH_3	3.35 *s*	56.6 *q*	C-3
7-OCH_3	3.97 *s*	59.2 *q*	C-7
–	–	165.0 *s* (C-1′)	–
–	–	125.8 *s* (C-2′)	–
3′	8.74 *ddd* (2.0, 1.0, 0.5)	150.7 *d*	C-1′, C-2′, C-5′, C-7′
5′	8.69 *ddd* (5.0, 2.0, 0.5)	153.4 *d*	C-3′, C-6′, C-7′
6′	7.27 *ddd* (8.0, 5.0, 1.0)	123.2 *d*	C-2′, C-5′
7′	7.93 *ddd* (8.0, 2.0, 2.0)	136.9 *d*	C-1′, C-3′, C-5′

Table 5
COSY and ROESY data for filifoline (**7**)

H	COSY	ROESY
1	H-2	H-2, H-10
2	H-1, H-3, H-4α	H-1, H-3, 3-OCH_3
3	H-2, H-4α, H-4β	H-2, H-4α, H-4β, 3-OCH_3
4α	H-2, H-3, H-4β, H-4a	H-3, 3-OCH_3, H-4β, H-4a
4β	H-3, H-4a, H-4α	H-3, H-4α, H-11*exo*, H-12*exo*
4a	H-4α, H-4β, H-12*endo*	H-4α, H-6α
6α	H-6β	H-4a, H-6β, 7-OCH_3
6β	H-6α	H-6α, 7-OCH_3, H-12*endo*
10	–	H-1
11*exo*	H-12*endo*, H-12*exo*	H-4β, H-12*endo*, H-12*exo*
12*endo*	H-4a, H-11*exo*, H-12*exo*	H-6β, H-11*exo*, H-12*exo*
12*exo*	H-11*exo*, H-12*endo*	H-4β, H-11*exo*, H-12*endo*
OCH_2 O	–	–
3-OCH_3	–	H-2, H-3, H-4α
7-OCH_3	–	H-6α, H-6β
3′	H-5′, H-7′	–
5′	H-3′, H-6′	H-6′
6′	H-5′, H-7′	H-5′, H-7′
7′	H-3′, H-6′	H-6′

to possess a nicotinate ester. Precedence for this heteroaromatic ester exists for the homolycorine analogues cliviamartine, miniatine and clivimine as well as the unusual degraded compound clivialine, all extractives of the southern African Amaryllidaceae representative *Clivia miniata* (Lindl.) Regel as reviewed by Viladomat et al. (1997).

The β-orientation of the 5,10b-ethano bridge in filifoline (**7**) was unequivocally ascertained by cleavage of the nicotinate ester (see Section 4) affording a synthetic product which was unambiguously identified from its chromatographic and spectroscopic properties as ambelline (Viladomat et al., 1994, 1995), the molecular ellipticity of which had a shape in the CD curve typical

of a β-5,10b-ethano bridge compound (maximum around 250 nm). Subsequent semi-synthetic derivatisation of ambelline (**5**) (see Section 4) provided unequivocal evidence that bulky substituents at C-11*endo* in crinane alkaloids are responsible for reversing molecular ellipticity as expressed in the shape of CD curves. Thus, both 11-*O*-acetylambelline (**6**) and synthetic 11-*O*-methanesulphonylambelline (**10**), with relatively small respective acyl and sulphonyl groups at C-11*endo*, had CD curves (Figs. 2 and 4) with maxima around 250 nm, as is expected of β-crinane structures, antipodal to that of filifoline (**7**) and synthetic 11-*O*-benzoylambelline (**9**) in which maxima were detectable near 290 nm (Figs. 1 and 3). The shape of CD curves of crinane alkaloids is determined by the configuration of the benzylic B:C ring junction in conjunction with

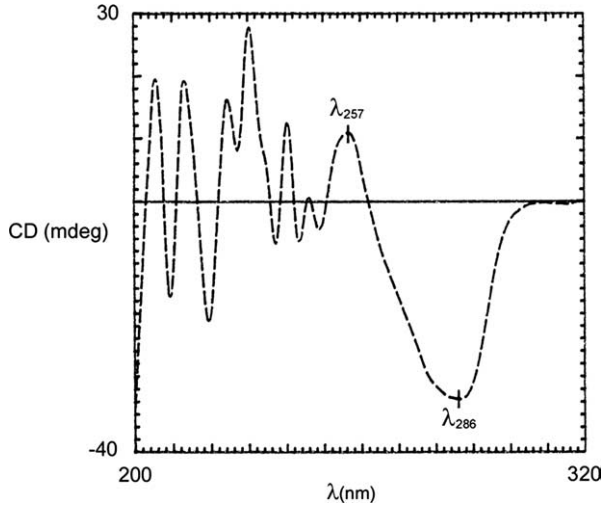


Fig. 2. CD curve 11-*O*-acetylambelline (**6**).

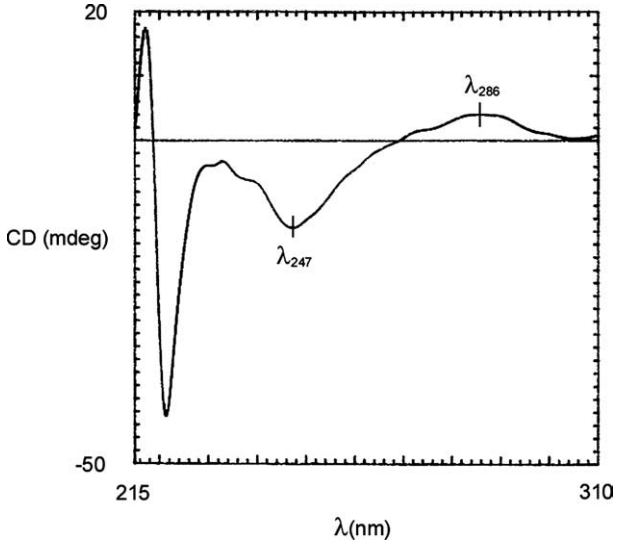


Fig. 3. CD curve 11-*O*-benzoylambelline (**9**).

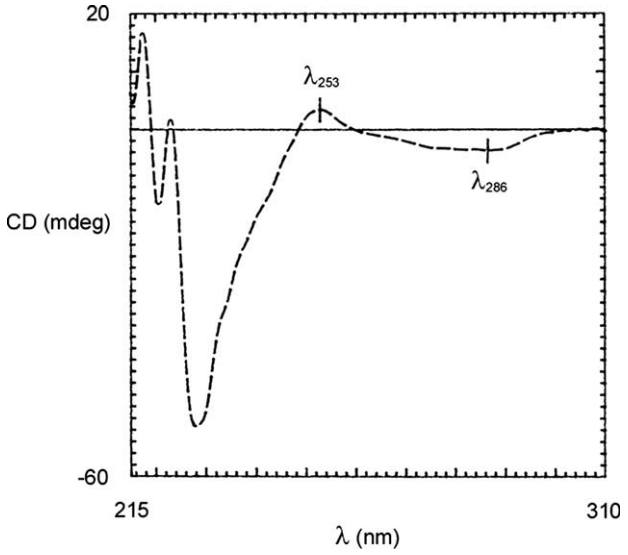
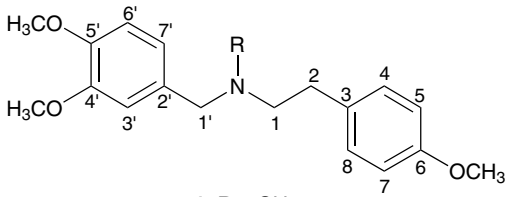


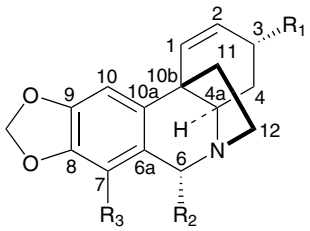
Fig. 4. CD curve 11-*O*-methanesulphonylambelline (**10**).

the dominant methylenedioxyphenyl (MDP) chromophore (Wagner et al., 1996). Since the C-10b terminus of the ethano bridge is part of this junction, its absolute stereochemistry is therefore qualitatively determined from the CD bands at ca. 290 and 250 nm which, respectively, also correspond to the $Ar_{(A1g \rightarrow B2u)}$ and $Ar_{(A1g \rightarrow B1u)}$ UV bands of the MDP chromophore (DeAngelis, 1968). This has been proven theoretically by sector rules, such as the quadrant rule for predicting the absolute configuration of molecules possessing an asymmetric centre adjacent to an aromatic moiety, as in crinane compounds (DeAngelis and Wildman, 1969). While the effect of substituents on CD in crinane derivatives remains mainly empirical (DeAngelis, 1968) and unexamined, phenomena such as C-3 epimerisation (Wagner et al., 1996) have been observed to have a marked (but not drastic) effect on the amplitude and magnitude of CD. However, no precedence exists for the effect on CD by bulky, electron-rich substituents which supersede the MDP chromophore. The nicotinate ester at C-11*endo* in filifoline, as well as the benzoate ester in compound **9**, are both geometrically and electronically predisposed so as to perturb the λ_{290} and λ_{250} CD bands emanating from the MDP chromophore responsible for molecular ellipticity i.e., the aryl esters are now the dominant chromophores. We suggest that a combination of regio, stereo and electronic factors introduced by the bulky aryl esters are responsible for the anomalous reverse effect on molecular ellipticity as expressed in the shape of the CD curves.

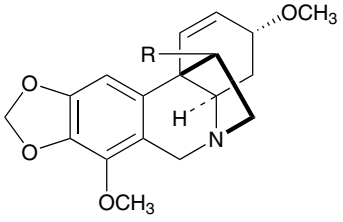
Filifoline was not cytotoxic to rat myoblast cells and exhibited no activity in the in vitro screens against the four different protozoan parasites *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani* and *Plasmodium falciparum*.



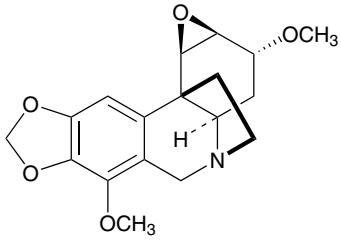
1 R = CH_3
2 R = H



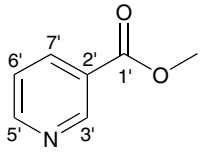
3 $R_1 = R_3 = OCH_3$, $R_2 = OH$
4 $R_1 = R_2 = R_3 = OCH_3$



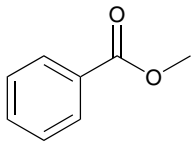
5 R = OH
6 R = OAc
7 R = ONic
9 R = OBz
10 R = OMes



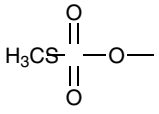
8



ONic (Nicotinate)



OBz (Benzoate)



OMes
(Methanesulphonate)

3. Concluding remarks

The present work contributes significantly to the understanding of CD as related to crinane alkaloids; we will report elsewhere in a more comprehensive manner the implications of this unique phenomenon as observed in filifoline.

4. Experimental

4.1. General

Mps are uncorr. and were measured on a Gallenkamp Melting Point apparatus. Optical rotations were determined on a Perkin–Elmer 241 Polarimeter installed with a λ_{589} sodium lamp. A Jasco J-700 Spectropolarimeter was utilized to run CD spectra, all recorded in MeOH. UV absorption spectra were determined on a Varian Cary IE UV–Vis spectrophotometer. IR spectra were measured on a Perkin–Elmer 1600 FTIR series spectrometer in dry film. EIMS were run on a Hewlett–Packard 5989A Mass Spectrometer fitted with a direct injection probe (DIP) with ionization energy set at 70 eV. Elemental analysis was performed on a Carlo Erba-Fisons EA1108 apparatus. ^1H, ^{13}C NMR, DEPT, ^1H COSY, HMQC, HMBC and ROESY spectra were recorded on a Varian VXR 500 or Gemini 300 spectrometer in $CDCl_3$ with TMS as internal standard. Chemical shifts are reported in units of δ (ppm) and coupling constants (J) are expressed in Hz. Silica gel SDS chromagel 60 A CC (230–400 mesh) and silica gel Merck (70–230 mesh) were used for flash CC and CC, respectively. Sephadex LH-20 (Pharmacia) was used for gel filtration and silica gel SIL G/UV_{254} for analyt. and SIL G-25 UV_{254} for prep. TLC (both Macherey–Nagel). Spots on chromatograms were detected under UV light (254 nm) and by Dragendorff's reagent stain.

4.2. Plant material

Bulbs of *Nerine filifolia* Baker were purchased at the van den Berg Nurseries in Durbanville, Cape Province

(South Africa) and were authenticated by the proprietor Mrs. J. van den Berg.

4.3. Extraction and isolation of alkaloids

Fresh bulbs (2.08 kg) were crushed and macerated with EtOH for 48 h after which the solvent was removed under reduced pressure, the residue (179 g) dissolved in H_2O (200 ml) and acidified to pH 4 with dilute HCl. After removing neutral material with Et_2O, the acidic soln. was extracted with $CHCl_3$ to provide extract A (1.2 g). Basifying the soln. to pH 8–9 with aq. NH_4OH and extracting again with $CHCl_3$ afforded extract B (3.9 g). Finally, a $CHCl_3$–MeOH (3:2) extraction of the basic soln. gave extract C (0.65 g). Extracts A, B and C were combined (5.75 g) after TLC [EtOAc–MeOH (1:1)] indicated that they contained the same alkaloids, and subjected to flash CC on silica gel by gradient elution with *n*-hexane, *n*-hexane-EtOAc, EtOAc, EtOAc–MeOH and finally MeOH. Four fractions were generated in this manner; PhOH (15 mg) was obtained directly from fr. I by recrystallization with MeOH. Fr. II was rechromatographed by CC using an EtOAc–MeOH step gradient, followed by prep. TLC (in Me_2CO) from which belladine (70 mg), 11-*O*-acetylambelline (10 mg) and 6α-hydroxybuphanidrine (5 mg) were obtained. Similarly, fr. III yielded further quantities of belladine (5 mg) and 11-*O*-acetylambelline (11 mg) and fr. IV, belladine (8 mg), 6α-hydroxybuphanidrine (16 mg), 6α-methoxybuphanidrine (10 mg), undulatine (27 mg) and ambelline (9 mg), together with *N*-demethylbelladine (7 mg) and filifoline (17 mg) by gel filtration on Sephadex LH-20.

4.4. Belladine (**1**)

Found: C, 71.83; H, 8.02; N, 4.38. Calc. for $C_{19}H_{25}NO_3$: C, 72.35; H, 7.99; N, 4.44%. UV, IR and EIMS data were in agreement with those reported previously (Ghosal et al., 1983, 1988). For ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) spectral data see Table 1.

4.5. N-Demethylbelladine (**2**)

Found: C, 71.25; H, 7.76; N, 4.79. $C_{18}H_{23}NO_3$ requires: C, 71.73; H, 7.69; N, 4.65%. UV λ_{max}^{MeOH} nm ($\log\epsilon$): 220 (14.63), 240 (5.95), 260 (3.82), 280 (5.11). IR ν_{max} cm^{-1}: 3225 (NH), 2926, 2833 (C–O), 1733, 1611, 1512, 1462, 1243, 1157, 1030 (C–O), 811. EIMS 70 eV, *m/z* (rel. int.): 301 $[M]^+$ (2), 299 (4), 273 (2), 195 (5), 180 $[C_{10}H_{14}NO_2]^+$ (16), 151 $[C_9H_{11}O_2]^+$ (100), 138 (5), 128 (1), 121 $[C_7H_5O_2]^+$ (9), 107 $C_7H_7O^+$ (7), 91 (6), 77 (7). ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) spectral data are tabulated in Table 1.

4.6. 6α-Hydroxybuphanidrine (**3**)

Found: C, 64.54; H, 6.44; N, 4.11. Calc. for $C_{18}H_{21}NO_5$: C, 65.24; H, 6.39; N, 4.23%. $[\alpha]_D^{20}$ +19 (MeOH; *c* 0.80); CD$[\theta]_\lambda^{20}$: $[\theta]_{250}$ +2141, $[\theta]_{288}$ −1337 (MeOH; *c* 0.163). IR ν_{max} cm^{-1}: 3500–3300, 2933, 2893, 1733, 1618, 1479, 1286, 1241, 1209, 1084, 1043 (C–O), 937 (OCH_2O), 754. EIMS 70 eV, *m/z* (rel. int.): 331 $[M]^+$ (54), 299 (22), 287 (49), 276 (100), 261 (37), 255 (37), 243 (20), 217 (34), 203 (20), 128 (23), 115 (38), 91 (21), 77 (36), 63 (22), 56 (75). For ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) spectral data see Table 2.

4.7. 6α-Methoxybuphanidrine (**4**)

Found: C, 65.48; H, 6.87; N, 3.99. $C_{19}H_{23}NO_5$ requires: C, 66.07; H, 6.71; N, 4.06%. $[\alpha]_D^{20}$ +34.6 ($CHCl_3$; *c* 0.13); CD$[\theta]_\lambda^{20}$: $[\theta]_{250}$ +2883, $[\theta]_{285}$ −1863 (MeOH; *c* 0.1). IR ν_{max} cm^{-1}: 2924, 2853, 1736, 1618, 1477, 1403, 1366, 1329, 1286, 1241, 1206, 1080, 1045 (C–O), 976, 935 (OCH_2O), 829, 757. EIMS 70 eV, *m/z* (rel. int.): 345 (65), 330 $[M - CH_3]^+$ (32), 315 $[M - 2CH_3]^+$ (27), 314 (100), 290 (33), 287 (41), 259 (88), 227 (30), 129 (24), 97 (26), 81 (44), 69 (97). ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) spectral data are tabulated in Table 2.

4.8. Filifoline (**7**)

Found: C, 65.55; H, 5.62; N, 6.48. $C_{24}H_{24}N_2O_6$ requires: C, 66.05; H, 5.54; N, 6.42%. Mp 191–193 °C. UV λ_{max}^{MeOH} nm ($\log\epsilon$): 280 (5.93). $[\alpha]_D^{20}$ +12 (MeOH; *c* 0.85); CD$[\theta]_\lambda^{20}$: $[\theta]_{245}$ −2391, $[\theta]_{290}$ +419 (MeOH; *c* 0.171). IR ν_{max} cm^{-1}: 2930, 1734 (>C=O), 1617, 1477, 1401, 1317, 1279, 1216, 1131, 1095, 1073, 1040 (C–O), 979, 934 (OCH_2O), 755. EIMS 70 eV, *m/z* (rel. int.): 436 $[M]^+$ (1), 296 (1), 185 (2), 165 (3), 145 (2), 122 $[C_6H_4NO_2]^+$ (3), 110 (3), 106 $[C_6H_4NO]^+$ (45), 99 (7), 91 (26), 79 $[C_5H_5N]^+$ (100), 69 (25), 55 (63). For ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) spectral data see Table 4.

4.9. Hydrolysis of filifoline (**7**)

Filifoline (8 mg) was dissolved in 2 ml of an aqueous methanolic solution of K_2CO_3 and the mixture warmed to gentle reflux. After TLC [MeOH/EtOAc (2:3)] indicated the reaction to be complete (6 h), the solution was neutralized with 1 M HCl, diluted with water (5

ml) and extracted with EtOAc (3 × 5 ml). The combined organic fractions were then dried over anhydrous $MgSO_4$, filtered and the solvent removed under reduced pressure to yield a crude gum (15 mg) which was purified by prep. TLC [MeOH/EtOAc (2:3)] to give ambelline (5.8 mg, 95%) as white crystals from Me_2CO (Viladomat et al., 1994, 1995).

4.10. Synthesis of 11-*O*-benzoylambelline (**9**)

Sodium hydride (60% suspension in mineral oil) (4.8 mg, 120.8 μmol) was added to a solution of ambelline (20 mg, 60.4 μmol) in 1 ml DMF and the mixture stirred at rt for 20 min, then cooled to 0 °C and benzoyl chloride (17.0 mg, 120.8 μmol) introduced and the solution allowed to warm to rt. After TLC [MeOH/EtOAc (2:3)] indicated the reaction to be complete (6 h) the mixture was diluted with water (5 ml) and extracted with CH_2Cl_2 (3 × 5 ml). The combined organic fractions were then dried over anhydrous $MgSO_4$, filtered and the solvent removed under reduced pressure to yield a crude gum (34 mg) which was purified by prep. TLC [MeOH/EtOAc (2:3)] to give 11-*O*-benzoylambelline (**9**) (25.5 mg, 97%) as white crystals from Me_2CO. Found: C, 68.36; H, 5.58; N, 2.99. $C_{25}H_{25}NO_6$ requires: C, 68.95; H, 5.79; N, 3.22%. Mp 118–120 °C. $[\alpha]_D^{20}$ +25.8 ($CHCl_3$; *c* 0.295); CD $[\theta]_\lambda^{20}$: $[\theta]_{247}$ −6746, $[\theta]_{286}$ +2024 (MeOH; *c* 0.02). IR ν_{max} cm^{-1}: 2925, 1716 (>C═O), 1617, 1477, 1401, 1315, 1277, 1217, 1086, 1072, 1046 (C–O), 978, 933 (OCH_2O), 756. EIMS 70 eV, *m/z* (rel. int.): 435 $[M]^+$ (23), 420 $[M - CH_3]^+$ (2), 330 $[M - PhCO]^+$ (4), 313 (54), 298 (21), 282 (21), 270 (8), 254 (30), 240 (22), 227 (6), 211 (11), 105 $[PhCO]^+$ (100), 77 $[C_6H_5]^+$ (57). ^1H NMR spectral data (500 MHz, $CDCl_3$): δ 1.82 (1H, *ddd*, *J* = 14.0, 13.6, 4.0 Hz, H-4β), 2.42 (1H, *ddd*, *J* = 14.0, 4.0, 2.0 Hz, H-4α), 2.92 (1H, *dd*, *J* = 14.0, 4.0 Hz, H-12*endo*), 3.38 (3H, *s*, 3-O*CH*$_3$), 3.65 (1H, *dd*, *J* = 13.6, 4.0 Hz, H-4a), 3.90 (1H, *ddd*, *J* = 5.0, 4.0, 2.0 Hz, H-3), 4.01 (3H, *s*, 7-O*CH*$_3$), 4.03 (1H, *d*, *J* = 17.2 Hz, H-6β), 4.08 (1H, *dd*, *J* = 14.0, 8.0 Hz, H-12*exo*), 4.47 (1H, *d*, *J* = 17.2 Hz, H-6α), 5.37 (1H, *dd*, *J* = 8.0, 4.0 Hz, H-11*exo*), 5.81–5.86 (2H, 2*d*, *J* = 1.2 Hz, O*CH*$_2$O), 6.11 (1H, *dd*, *J* = 10.0, 5.0 Hz, H-2), 6.55 (1H, *s*, H-10), 6.58 (1H, *d*, *J* = 10.0 Hz, H-1), 7.35 (2H, *d*, *J* = 7.6 Hz, H-4′/H-6′), 7.51 (1H, *t*, *J* = 7.6, 7.2 Hz, H-5′), 7.66 (2H, *d*, *J* = 7.6 Hz, H-3′/H-7′). ^{13}C NMR spectral data (75 MHz, $CDCl_3$): δ 28.3 (*t*, C-4), 47.8 (*s*, C-10b), 57.0 (*q*, 3-O*C*H$_3$), 58.5 (*t*, C-6), 59.4 (*t*, C-12), 59.5 (*q*, 7-O*C*H$_3$), 63.8 (*d*, C-4a), 72.0 (*d*, C-3), 86.5 (*d*, C-11), 99.9 (*d*, C-10), 101.0 (*t*, O*C*H$_2$O), 115.7 (*s*, C-6a), 124.8 (*s*, C-2′), 127.3 (*d*, C-2), 128.5 (2*d*, C-4′/C-6′), 129.7 (2*d*, C-3′/C-7′), 130.5 (*d*, C-1), 133.1 (*s*, C-10a), 133.4 (*d*, C-5′), 134.3 (*s*, C-8), 141.0 (*s*, C-7), 148.6 (*s*, C-9), 166.4 (*s*, C-1′).

4.11. Synthesis of 11-*O*-methanesulphonylambelline (**10**)

Ambelline (20 mg, 60.4 μmol) and 4-*N*,*N*-dimethylaminopyridine (DMAP) (0.74 mg, 6.04 μmol) were dissolved at rt in CH_2Cl_2 (1 ml) to which pyridine (9.8 μl, 120.8 μmol) was added. After 15 min methanesulphonyl chloride (9.8 μl, 120.8 μmol) was introduced and the solution stirred at 40 °C. After TLC [MeOH/EtOAc (2:3)] indicated the reaction to be complete (4 h) the mixture was diluted with water (5 ml) and extracted with CH_2Cl_2 (3 × 5 ml). The combined organic fractions were then dried over anhydrous $MgSO_4$, filtered and the solvent removed under reduced pressure to yield a crude gum (30 mg) which was purified by prep. TLC [MeOH/EtOAc (2:3)] to give 11-*O*-methanesulphonylambelline (**10**) (23.7 mg, 96%) as white crystals from Me_2CO. Found: C, 55.47; H, 5.41; N, 3.32. $C_{19}H_{23}NSO_7$ requires: C, 55.73; H, 5.66; N, 3.42%. Mp 98–100 °C. $[\alpha]_D^{20}$ +4.1 ($CHCl_3$; *c* 0.565); CD $[\theta]_\lambda^{20}$: $[\theta]_{253}$ +603, $[\theta]_{286}$ −631 (MeOH; *c* 0.05). IR ν_{max} cm^{-1}: 2925, 1618, 1500, 1479, 1402, 1356 (O═S═O), 1282, 1215, 1176, 1130, 1086, 1045 (C–O), 953, 934 (OCH_2O), 757. EIMS 70 eV, *m/z* (rel. int.): 409 $[M]^+$ (34), 394 $[M - CH_3]^+$ (1), 378 $[M - CH_3–O]^+$ (2), 330 $[M - CH_3–SO_2]^+$ (41), 313 (100), 298 (41), 282 (48), 270 (80), 254 (51), 240 (53), 215 (24). ^1H NMR spectral data (500 MHz, $CDCl_3$): δ 1.67 (1H, *ddd*, *J* = 14.0, 13.6, 4.0 Hz, H-4β), 2.21 (1H, *ddd*, *J* = 14.0, 4.0, 2.0 Hz, H-4α), 2.88 (3H, *s*, S-C*H*$_3$), 2.91 (1H, *dd*, *J* = 14.0, 4.0 Hz, H-12*endo*), 3.35 (3H, *s*, 3-OC*H*$_3$), 3.49 (1H, *dd*, *J* = 13.6, 4.0 Hz, H-4a), 3.85 (1H, *ddd*, *J* = 5.6, 4.0, 2.0 Hz, H-3), 3.86 (1H, *dd*, *J* = 14.0, 8.4 Hz, H-12*exo*), 3.93 (1H, *d*, *J* = 17.6 Hz, H-6β), 3.99 (3H, *s*, 7-OC*H*$_3$), 4.36 (1H, *d*, *J* = 17.6 Hz, H-6α), 5.14 (1H, *dd*, *J* = 8.4, 4.0 Hz, H-11*exo*), 5.87-5.90 (2H, 2*d*, *J* = 1.4 Hz, OC*H*$_2$O), 6.10 (1H, *dd*, *J* = 10.4, 5.6 Hz, H-2), 6.57 (1H, *s*, H-10), 6.58 (1H, *d*, *J* = 10.4 Hz, H-1). ^{13}C NMR spectral data (75 MHz, $CDCl_3$): δ 28.8 (*t*, C-4), 38.4 (*q*, S-*C*H$_3$), 47.0 (*s*, C-10b), 56.9 (*q*, 3-O*C*H$_3$), 59.3 (*q*, 7-O*C*H$_3$), 59.4 (*t*, C-6), 59.5 (*t*, C-12), 63.5 (*d*, C-4a), 72.1 (*d*, C-3), 91.5 (*d*, C-11), 100.2 (*d*, C-10), 101.0 (*t*, O*C*H$_2$O), 117.0 (*s*, C-6a), 127.8 (*d*, C-2), 130.3 (*d*, C-1), 132.3 (*s*, C-10a), 134.4 (*s*, C-8), 140.8 (*s*, C-7), 148.3 (*s*, C-9).

4.12. Biological activity

The anti-protozoal activity screen of filifoline against the four different parasitic protozoa was carried out in vitro in parallel with the respective standards: *Trypanosoma cruzi* (strain Tulahuen C4, stage trypomastigotes)/benznidazole std. IC_{50} = 0.31 μg/ml; *Leishmania donovani* (strain MHOM-ET-67/L82, stage amastigotes)/miltefosine std. IC_{50} = 0.19 μg/ml; *Plasmodium falciparum* (strain K1, stage IEF)/artemisinin std. IC_{50} = 0.0018

μg/ml; *Trypanosoma brucei rhodesiense* (strain STIB 900, stage trypomastigotes)/melarsoprol std. IC_{50} = 0.00245 μg/ml. Filifoline was tested for cytotoxicity towards myoblast (L6) cells (podophyllotoxin std. IC_{50} = 0.002 μg/ml).

Ambelline, 11-*O*-acetylambelline and undulatine were identified by comparison of their chromatographic and spectroscopic properties (TLC, IR, CD, MS, ^{1}H and ^{13}C NMR) with those of authentic samples obtained from other plant sources (Viladomat et al., 1994, 1995).

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