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Crinane, augustamine, and β-carboline alkaloids from *Crinum latifolium*

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ABSTRACT

Three new crinane-type alkaloids, namely, 6-methoxyundulatine (**1**), 6-methoxycrinamidine (**2**), and undulatine *N*-oxide (**3**), along with the known compounds 6-hydroxyundulatine (**4**), 6-hydroxybuphanidrine (**5**), undulatine (**6**), crinamidine (**7**), ambelline (**8**), filifoline (**9**), augustamine (**10**), and perlolyrine (**11**), were isolated from the leaves of *Crinum latifolium* by using various chromatographic separations. Their structures were established by extensive analysis, including 1D and 2D NMR, HR-QTOF-MS, and CD data. Among the isolated compounds, perlolyrine (**11**) showed significant cytotoxicity against five human cancer cell lines, namely, KB, HepG2, MCF7, SK-Mel2, and LNCaP with the IC_{50} values ranging from 22.12 ± 2.80 to 28.45 ± 3.75 μM.

1. Introduction

Crinum L. is the only pantropical genus of the family Amaryllidaceae, which is mainly distributed in Africa, America, southern Asia, and Australia (Meerow et al., 2003; Snijman and Linder, 1996). This genus includes approximately 65 species with approximately 40 in Africa (Snijman and Linder, 1996). In Vietnam, 6 *Crinum* species have been recorded and described (Anh et al., 2005). Some *Crinum* species are traditionally used (especially in Africa, tropical Asia and South America) as emetics, laxatives, expectorants, tonics, antipyretics, diuretics, diaphoretics, anti-asthmatics, anti-malarial, antiaging, anti-tumor, and lactagogues (Refaat et al., 2013). Previous investigations showed that alkaloids (Ghosal et al., 1983; Ghosal et al., 1985; Ghosal and Singh, 1986; Ghosal et al., 1989; Son et al., 2001a, b; Son et al., 2003; Sung and Lien, 1997; Tram et al., 2001) are the main chemical constituents of this species with some showing immuno-regulant (Ghosal et al., 1985), immuno-stimulant (Ghosal et al., 1984) and cytotoxic (Son et al., 2003; Tram et al., 2001) effects. *Crinum* alkaloids have attracted considerable attention due to their interesting pharmacological effects (Refaat et al., 2012a, b, c, 2013). In line with the investigations on *Crinum* alkaloids, here, we report the isolation, structure elucidation, and cytotoxic evaluation of three new crinane-type alkaloids (**1**–**3**), along with eight known compounds (**4**–**11**) obtained from *C. latifolium*.

2. Results and discussion

The methanol residue of the *C. latifolium* leaves was suspended in water and partitioned successively with *n*-hexane, dichloromethane, and ethyl acetate. The dichloromethane and ethyl acetate extracts were separated by various chromatographic experiments to yield eleven alkaloids including three new compounds (Fig. 1) 6α-methoxyundulatine (**1**), 6α-methoxycrinamidine (**2**), and undulatine *N*-oxide (**3**). Structures of the known compounds 6α-hydroxyundulatine (**4**) (Machocho et al., 1999; Son et al., 2001a), 6α-hydroxybuphanidrine (**5**) (Nair et al., 2005; Son et al., 2001a), undulatine (**6**) (Machocho et al., 1999; Tram et al., 2001), crinamidine (**7**) (Machocho et al., 1999; Son et al., 2001a), ambelline (**8**) (Ghosal et al., 1983; Son et al., 2001a; Viladomat et al., 1995), filifoline (**9**) (Nair et al., 2005), augustamine (**10**) (Ali et al., 1983; Son et al., 2001b), and perlolyrine (**11**) (Bremner et al., 2004) were elucidated by careful analysis of their NMR, CD, and ESI–MS data and compared to the literature data. This work represents the first isolation of compounds **9** and **11** from *C. latifolium*.

Compound **1** was isolated as a white amorphous powder with the

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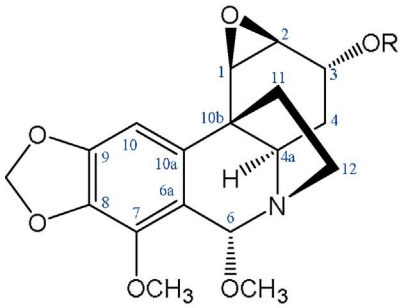

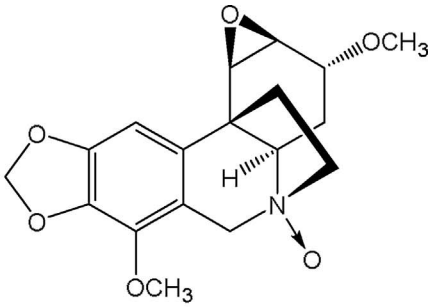

Fig. 1. The structures of new compounds **1**–**3**.

Table 1
NMR spectroscopic data of **1**–**3**.

No	**1**		**2**		**3**	
	δ_C[a,b]	δ_H[a,c] (mult., *J* in Hz)	δ_C[b,d]	δ_H[c,d] (mult., *J* in Hz)	δ_C[a,b]	δ_H[a,c] (mult., *J* in Hz)
1	54.3	3.82 d (3.0)	54.3	3.83 d (3.5)	53.0	3.83 d (3.5)
2	55.8	3.33[e]	56.9	3.25 dd (2.5, 3.5)	55.7	3.46 dd (2.5, 3.5)
3	76.2	4.00[e]	65.9	4.39 br d (2.5)	74.8	4.16 dd (2.5, 5.0)
4	26.0	1.40 dt (3.0, 14.0) 1.65 br d (14.0)	30.0	1.51 m	21.1	1.58 dt (3.0, 14.0) 2.68 dt (14.0, 3.0)
4a	56.5	3.40 dd (3.5, 13.5)	56.1	3.55 dd (5.0, 12.5)	73.6	3.48 dd (3.5, 13.0)
6	94.9	4.59 s	94.8	4.59 s	73.8	4.53 dd (2.5, 16.0) 4.71 d (16.0)
6a	119.9	–	119.8	–	114.5	–
7	144.5	–	144.4	–	141.4	–
8	136.0	–	135.8	–	135.6	–
9	151.3	–	151.2	–	151.4	–
10	97.6	6.76 s	97.6	6.76 s	97.6	6.84 s
10a	140.8	–	140.8	–	136.4	–
10b	43.0	–	43.0	–	45.4	–
11	36.7	1.71 m/ 2.37 m	36.6	1.71 m/ 2.37 m	36.4	2.02 m/2.80 m
12	47.3	2.65 m/ 3.13 m	47.2	2.66 m/ 3.13 m	68.2	3.63 m/3.90 m
OCH_2O	102.4	5.93 br s	120.3	5.93 s	102.9	5.97 d (1.0)/ 5.99 d (1.0)
3-OMe	57.8	3.45 s			58.1	3.49 s
6-OMe	56.9	3.58 s	57.3	3.57 s		
7-OMe	60.1	3.99 s	60.1	4.00 s	60.0	4.08 s

All assignments were done by HSQC, COSY, HMBC, and NOESY experiments.
[a] Measured in CD_3OD.
[b] 125 MHz.
[c] 500 MHz.
[d] Measured in CD_3OD + $CDCl_3$.
[e] Overlapped narrow signals.

molecular formula of $C_{19}H_{23}NO_6$, determined by the HR-QTOF-MS quasi-molecular ion peak at *m/z* 362.1599 $[M+H]^+$ (calcd. for $C_{19}H_{24}NO_6^+$, 362.1598). This compound is sensitive to the Dragendorff reagent, indicating an alkaloid that is a main constituent of the *Crinum* species (Refaat et al., 2012a, b, c). The ^{13}C NMR spectrum exhibited 19 signals assigned to one epoxy [δ_C 54.3 (CH, C-1) and 55.8 (CH, C-2)/δ_H 3.82 (1H, d, *J* = 3.0 Hz, H-1) and 3.33 (1H, H-2)], two oxymethine [δ_C 76.2 (C-3) and 94.9 (C-6)/δ_H 4.00 (1H, H-3) and 4.59 (1H, s, H-6)], one dioxymethylene [δ_C 102.4/δ_H 5.93 (2H, br s)], three methoxy [δ_C 57.8 (3-OMe), 56.9 (6-OMe), and 60.1 (7-OMe)/δ_H 3.45 (3-OMe), 3.58 (6-OMe), and 3.99 (7-OMe), each 3H, s] groups and one pentasubstituted aromatic ring [δ_C 119.9 (C, C-6a), 144.5 (C, C-7), 136.0 (C, C-8), 151.3 (C, C-9), 97.6 (CH, C-10), and 140.8 (C, C-10a)/δ_H 6.76 (1H, s, H-10)] using HSQC experiments. The other signals belong to one methine, two methylene, and one quaternary carbon (Table 1). The ^{13}C NMR data of **1** were similar to those of 6α-hydroxyundulatine (**4**) (Machocho et al., 1999), except for the presence of an additional signal for a methoxy group in **1**. The carbon signal at C-6 of **1** was strongly shifted downfield at δ_C 94.9 relative to that of 6α-hydroxyundulatine (**4**) (Machocho et al., 1999) at δ_C 85.2 (C-6), suggesting the attached position of the additional methoxy group at C-6 of **1**. This position was confirmed by HMBC cross-peak of the additional methoxy proton (δ_H 3.58) with C-6 (δ_C 94.9). Detailed analysis of other COSY and HMBC correlations (Fig. 2) clearly confirmed the planar structure of **1**. The CD spectrum (Fig. 3) showed a positive Cotton effect at 250 nm, similar to those of the β-5,10b-ethano bridge series (Machocho et al., 1999; Viladomat et al., 1995; Wagner et al., 1996), indicating the (–)-crinane-type skeleton of **1** versus a negative Cotton effect approximately 245 nm of the α-5,10b-ethano bridge series (Ali et al., 1984). The β-orientation of H-6 was determined by a NOESY spectrum with the spatial proximity between H-6 (δ_H 4.59) and H-12 (δ_H 2.65) as shown in Fig. 2. In addition, the small coupling constants (*J* ~ 3 Hz) between H-1 and H-2, H-2 and H-3, and between H-3 and H-4 confirmed the β-orientation of the epoxy ring and H-3 (Machocho et al., 1999). Consequently, compound **1** was identified as 6α-methoxyundulatine.

The ^1H and ^{13}C NMR data of **2** were similar to those of **1**, except for the absence of the signals for a methoxy group. This finding was further

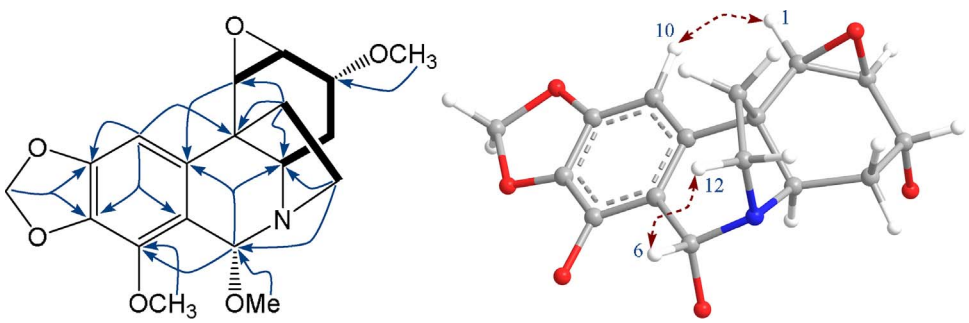

Fig. 2. Key COSY (▬), HMBC (↷), and NOESY (⇢) correlations of **1**.