Natural Occurrence of 11-*O*-Acetylambelline and 11-*O*-Acetyl-1,2-β-epoxyambelline in *Crinum latifolium*: Immuno-regulant Alkaloids[3]

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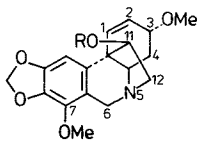
The occurrence of two new bioactive 5,10b-ethanophenanthridine alkaloids, 11-*O*-acetylambelline (**1**) and 11-*O*-acetyl-1,2-β-epoxyambelline (**2**), in the resting bulbs of *Crinum latifolium* Linn. (Amaryllidaceae) is reported. The structures of the two alkaloids have been established on the basis of chemical and spectral evidence. Immunobiological screening data would seem to suggest a combination (1 : 1) of **1** and **2** to act as a potential immunoregulant.

This investigation was aimed at delving into the catabolic sequence of ambelline and 1,2-β-epoxyambelline, occurring in a ratio of approximately 3 : 1 (1) in several *Crinum* species (2) during pre- and post-flowering stages covering a span of approximately 8 weeks. In the resting bulbs of *C. latifolium*, the two alkaloids are vicariously represented by their respective 11-*O*-acetyl derivatives.

The mixture of alkaloids (0.12 g) from the silica gel column chromatography of 'fraction B_3' (3), obtained from the resting bulbs of the title species (collected during January 1980, '81, '82) (1 kg wet weight, each), was taken up in MeOH-H_2O (90 : 10, 5 ml). The solution was subjected to semi-preparative H.P.L.C. [Spectra Physics model 8000, UV detector (254 nm); RP-8 column (25 × 0.8 cm, i. d.); MeOH-H_2O (90 : 10) as the mobile phase, flow rate 8 ml/min]. Compounds **2** and **1** were obtained in succession (t_R 406 and 488 s, respectively). Analytical HPLC [Waters Associates model 440, 6000 A/U 6K; 254 nm/0.1 aufs; RP-18 column; MeOH-H_2O (80 : 20) mobile phase, flow rate 1 ml/min] of the mixture ('fraction B_3' from the resting bulbs) showed only traces of ambelline (t_R 390 s) and 1,2-β-epoxyambelline (t_R 300 s).

11-O-Acetylambelline (**1**). This compound crystallized from MeOH-AcMe as colourless prisms (36 mg, yield ca. 0.003 %), mp 232-235 °C, λ_{max}^{MeOH} nm (log ε) 240 sh (3.0), 290 (3.08); ν_{max}^{KBr} cm^{-1} 1730, 1612, 1208, 1070, 938; δ_H ($CDCl_3$) 6.55 (1H, d, J = 10 Hz, H-1), 6.40 (1H, s, H-10), 5.92 (1H, dd, J = 10, 5 Hz, H-2), 5.81 (2H, s, OCH_2O), 5.18 (1H, dd, J = 4, 8 Hz, H-11), 4.28 (1H, d, J = 17 Hz, H_β-6), 4.02 (1H, m, H-3), 3.96 (3H, s, C_7-OMe), 3.75 (1H, d, J = 17 Hz, H_α-6), 3.33 (3H, s, C_3-OMe),



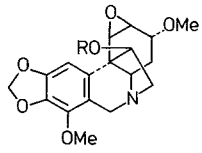


Table I. Effects of **1** and **2** on Mouse Splenic Lymphocytes[a], Peritoneal Macrophage[b], and Spleen Weight.

Compound	Stimulation Index[c,d]			Macrophage[e]			Spleen weight[e]	
	qs	4 days of tumor growth	12 days of tumor growth	normal × 10^6	RN (elicited) × 10^6 48 h	72 h	normal in mg	treated in mg
Concanavalin A	7.95 ±0.43	7.87 ±0.50	2.53 ±0.66					
1	3.11 ±0.72	2.94 ±0.64	2.38 ±0.58		2.55	4.62		191.6
2	5.05 ±1.75	4.98 ±0.77	3.38 ±0.28		2.60	4.71		248.4
1 + **2** (1 : 1)	6.46 ±0.86	6.74 ±0.24	4.55 ±0.21		2.93	14.97		348.95
				2.42			198.7	

[a, b] protocols as described before (8, 9). [c] n = 6. [d] 5 µg/ml in phosphate buffered saline (PBS, pH 7.2); mean ± s. d. [e] n = 14 [Swiss mice (adult male) inoculated intraperitoneally (i. p.) with **1** (50 µg) + **2** (50 µg), in PBS; peritoneal cells were counted after 48 and 72 h; spleen weighth were recorded]. [f] Sarcoma 180 ascites tumor cells; qs, quiescent state (normal mice).

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obtained in succession (t_R 406 and 488 s, respectively). Analytical HPLC [Waters Associates model 440, 6000 A/U 6K; 254 nm/0.1 aufs; RP-18 column; MeOH-H_2O (80 : 20) mobile phase, flow rate 1 ml/min] of the mixture ('fraction B_3'

11-O-Acetyl-1,2-β-epoxyambelline (**2**). This compound crystallized from AcMe as colourless micro-crystals (14 mg, yield ca. 0.001 %), mp 201–204 °C; λ_{max}^{MeOH} 240 sh (2.99), 286 (3.04); ν_{max}^{KBr} 1728, 1622, 1209, 940; δ_H

3.2–2.8 (2H, m, H,H-12), 1.81 (3H, s, strongly shielded 11-*O*-Ac), 1.7–1.4 (2H, m, H,H-4); m/z 373 (M^+, 100%), 299 (22), 247 (14), 211 (12) (Found: C, 64.1; H, 6.32; N, 3.33. $C_{20}H_{23}NO_6$ requires C, 64.34; H, 6.16; N, 3.75%). Deacetylation of **1** with NaOMe in MeOH (4) gave ambelline **3** (mp, mmp, co-TLC, IR).

11-O-Acetyl-1,2-β-epoxyambelline (**2**). This compound crystallized from AcMe as colourless micro-crystals (14 mg, yield ca. 0.001%), mp 201–204 °C; λ_{max}^{MeOH} 240 sh (2.99), 286 (3.04); ν_{max}^{KBr} 1728, 1622, 1209, 940; δ_H ($CDCl_3$) 6.59 (1H, s, H-10), 5.90 (2H, s, OCH_2O), 5.16 (1H, dd, J = 4, 8 Hz, H-11), 4.30 (1H, d, J = 17 Hz, H_β-6), 4.04 (3H, s, C_7-OMe), 4.0 (1H, br. m, H-3), 3.74 (1H, d, J = 17 Hz, H_α-6), 3.39 (3H, s, C_3-OMe), 3.3–2.85 (2H, m, H,H-12), 1.79 (3H, s, OAc), 1.7–1.4 (2H, m, H,H-4) [Found (by accurate mass measurement) M, 389.147. $C_{20}H_{23}NO_7$ requires M, 389.147]. On deacylation, as before, **2** gave 1,2-β-epoxyambelline (**4**) (1) (mp, mmp, co-TLC, MS).

To our knowledge, this is the first demonstration of the natural occurrence of **1** and **2**. *O*-Acetylation of *Amaryllidaceae* alkaloids, seems to be a common biochemical process of transformation in the *Crinum* species. Thus, a number of *O*-acetyl derivatives, viz. 1-*O*-acetyl and 1,2-*O*-diacetyllycorine, 3-*O*-acetylcrinine, 3-*O*-acetylhamayane, were previously reported from this genus (5, 6, 7).

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