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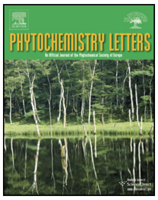

Carbazole alkaloids and coumarins from the roots of *Clausena guillauminii*

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ABSTRACT

Two novel carbazole alkaloids, guillauminines A and B (**1** and **2**), and sixteen known compounds were isolated and identified from the acetone extract of *Clausena guillauminii* roots. Their structures were elucidated by spectroscopic methods. The cytotoxic, antimalarial and antimycobacterial activities of the isolated compounds were evaluated.

1. Introduction

Clausena is an abundant source of secondary metabolites, especially carbazole alkaloids (Maneerat et al., 2011, 2012, 2013; Songsiang et al., 2011, 2012; Thongthoom et al., 2010), coumarins (Maneerat et al., 2012; Songsiang et al., 2011, 2012; Thongthoom et al., 2010) and amide derivitives (Riemer et al., 1997; Wang et al., 2013). Many of the isolated compounds have a wide range of pharmacological activities including anticancer (Maneerat et al., 2012, 2013; Songsiang et al., 2011; Thongthoom et al., 2010), anti-HIV (Kongkathip et al., 2005, 2010; Krahl et al., 2006), anti-malarial (Maneerat et al., 2011; Thongthoom et al., 2010) and anti-TB (Maneerat et al., 2011; Thongthoom et al., 2010). In this paper, we investigated the phytochemicals from the roots of *Clausena guillauminii* (Rutaceae family), which led to the isolation and characterization of two new carbazole alkaloids (**1** and **2**) along with sixteen known compounds (**3**–**18**). The structures of the isolated compound were elucidated using spectroscopic methods especially 1D and 2D NMR spectroscopy. The structures of the known compounds were determined and confirmed by comparison of their ^{1}H and/or ^{13}C spectroscopic data with those reported in the literature. The cytotoxic, antimalarial and antimycobacterial activities of the isolated compounds were examined.

2. Results and discussion

Investigation of the acetone extract from the air-dired roots *C. guillauminii* led to the isolation of two new carbazole alkaloids, guillauminines A and B (**1** and **2**); fourteen known carbazole alkaloids, heptahylline (**3**) (Songsiang et al., 2012), 7-methoxyheptaphylline (**4**) (Songsiang et al., 2012), 3-formyl-7-hydroxy-9*H*-carbazole (**5**) (Ito et al., 1992; Krahl et al., 2006), fluroclausine A (**6**) (Wu et al., 1997), mukonal (**7**) (Thongthoom et al., 2010), 7-methoxymukomal (**8**) (Songsiang et al., 2012), clauszoline K (**9**) (Thongthoom et al., 2010), 7-methoxymurrayacine (**10**) (Songsiang et al., 2012), clauraila D (**11**) (Songsiang et al., 2011), heptazoline (**12**) (Songsiang et al., 2012), glycosinine (**13**) (Thongthoom et al., 2010), 7-hydroxyheptaphylline (**14**) (Songsiang et al., 2012), 3-formyl-2,7-dimethoxycarbazole (**15**) (Ruangrungsi and Ariyaprayoon, 1990) and clauszoline C (**16**) (Krahl et al., 2013); and two known coumarins, osthol (**17**) (Thongthoom et al., 2010) and xanthoxyletin (**18**) (Songsiang et al., 2012) (Fig. 1).

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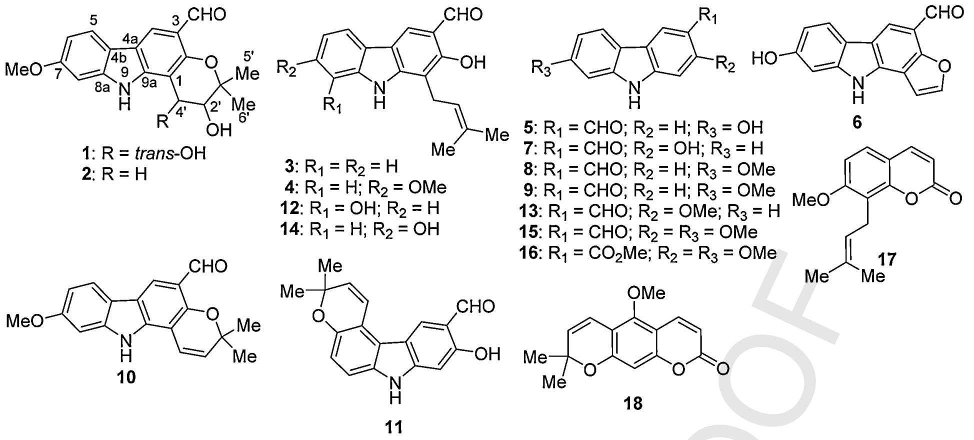

Fig. 1. Structures of the isolated compounds **1**–**18** from *C. guillauminii* roots.

Guillauminine A (**1**) was obtained as a yellow solid with $[\alpha]_{\mathrm{D}}^{24}$-0.06° (*c* 0.04, MeOH) and mp 234.0–235.0 °C. Its molecular formula, $C_{19}H_{19}NO_5$, was deduced from HRESI-TOFMS. The IR spectrum indicated the presence of a hydroxyl, amine and aldehyde groups at 3366, 3212 and 1653 cm^{-1}, respectively (Wu et al., 1997; Thongthoom et al., 2010). The UV spectrum revealed the maxima absorption bands at λ_{max} 241, 279, 301 and 347 nm indicating that **1** had a carbazole alkaloid chromophore (Maneerat et al., 2011, 2012, 2013; Songsiang et al., 2011, 2012; Thongthoom et al., 2010). The ^{1}H NMR spectrum (Table 1) displayed characteristic signals for an aldehyde proton (δ 10.42, s, 1H), an amino proton (δ 10.32, br s, 1H), three aromatic protons of a 1,2,4-trisubstituted benzene ring [δ 7.96 (d, *J* = 8.5 Hz, 1H, H-5), 7.14 (d, *J* = 2.1 Hz, 1H, H-8) and 6.83 (dd, *J* = 8.5, 2.1 Hz, 1H, H-6)], a singlet aromatic proton (δ 8.30, s, 1H, H-4), two oxymethine protons [δ 4.96 (d, *J* = 7.9 Hz, 1H, H-4′) and δ 3.82 (d, *J* = 7.9 Hz, 1H, H-3′)], a methoxy group (δ 3.84, s, 3H) and two methyl groups (δ 1.56 and 1.32, each s, 3H). Compound **1** showed nine quaternary (δ 160.0, 154.2, 144.9, 143.3, 119.0, 118.7, 117.9, 107.6 and 80.9), seven methine (δ 188.9, 121.4, 119.4, 109.5, 96.7, 76.8 and 69.4) and three methyl (δ 55.8, 27.0 and 19.5) carbons in the ^{13}C NMR and DEPT 135 spectra (Table 1). The aromatic protons at δ 7.96, 7.14 and 6.83 were assigned to be H-5, H-8 and H-6, respectively, due to their multiplicity and HMBC correlations (Table 1), H-5/C-4a (δ 118.7), C-4b (δ 119.0), C-7 (δ 160.0) and C-8a (δ 143.3), H-8/C-4b, C-6 (δ 109.5), C-7 and C-8a and H-6/C-4b, C-7 and C-8 (δ 96.7). The methoxy group resonating at δ 3.84 was located at C-7 (δ 160.0) on the basis of the HMBC cross peak between the methoxy protons with C-7 and the chemical shifts of C-6 and C-8. The aldehyde proton, (δ 10.42), showed HMBC cross peaks with C-3 (δ 117.9) and C-4 (δ 119.4), suggesting the location of the formyl group at C-3. The singlet aromatic proton at δ 8.30 was assigned to H-4 according to its HMQC cross peak with C-4 (δ 119.4) as well as the HMBC correlations with C-2 (δ 154.2), C-3 (δ 117.9), C-9a (δ 144.9) and the aldehyde carbon (δ 188.9). In the ^{1}H–^{1}H COSY spectrum, the oxymethine proton, H-4′ (δ 4.96) coupled with the other oxymethine proton, H-3′ (δ 3.82), which correlated with C-2′ (δ 80.9), C-4′ (δ 69.4), C-5′ (δ 27.0) and C-6′ (δ 19.5) in the HMBC spectrum. These data together with the chemical shift of C-2 and the HMBC cross peaks H-4′/C-1 (δ 107.6), C-2 (δ 154.2) and C-9a (δ 144.9) indicated that a dimethylchromane ring with dihydroxy groups at C-3′ and C-4′ was fused at C-1 and C-2 of the carbazole nucleus with an ether linkage at C-2. The oxymethine protons, H-3′ and H-4′, were located in pseudoaxial positions on the basis of the

Table 1
NMR spectral data of guillauminines A and B (**1** and **2**) in acetone-d_6 at 400 MHz.

Position	**1**					**2**			
	δ_H (mult., *J* in Hz)	δ_C		HMBC		δ_H (mult., *J* in Hz)	δ_C		HMBC
1	–	107.6		–		–	103.7		–
2	–	154.2		–		–	154.6		–
3	–	117.9		–		–	120.6		–
4	8.30 (s)	119.4	CH	C-2, C-3, C-9a, CHO		8.24 (s)	117.9	CH	C-2, C-9a, CHO
4a	–	118.7		–		–	120.9		–
4b	–	119.0		–		–	119.3		–
5	7.96 (d, 8.5)	121.4	CH	C-4a, C-4b, C-7, C-8a		7.97 (d, 8.5)	121.4	CH	C-4a, C-7, C-8a
6	6.83 (dd, 8.5, 2.1)	109.5	CH	C-4b, C-7, C-8		6.84 (dd, 8.5, 2.1)	109.3	CH	C-4b, C-8
7	–	160.0		–		–	159.9		–
8	7.14 (d, 2.1)	96.7	CH	C-4b, C-6, C-7, C-8a		7.00 (d, 2.1)	96.4	CH	C-4b, C-6, C-7, C-8a
8a	–	143.3		–		–	143.2		–
9a	–	144.9		–		–	145.1		–
2′	–	80.9		–		–	79.0		–
3′	3.82 (d, 7.9)	76.8	CH	C-2′, C-4′, C-5′, C-6′		4.01 (m)	69.1	CH	C-2′, C-5′, C-6′
4′	4.96 (d, 7.9)	69.4	CH	C-1, C-2, C-9a, C-3′	a	3.23 (dd, 16.5, 5.7)	28.1	CH_2	C-1, C-2, C-9a
	–	–			b	2.90 (dd, 16.5, 7.2)			C-2′, C-3′
5′	1.56 (s)	27.0	CH_3	C-2′, C-3′, C-6′		1.48 (s)	20.7	CH_3	C-2′, C-3′, C-6′
6′	1.32 (s)	19.5	CH_3	C-2′, C-3′, C-5′		1.38 (s)	25.9	CH_3	C-2′, C-3′, C-5′
CHO	10.42 (s)	188.9	CH	C-3, C-4		10.46 (s)	189.9	CH	C-3, C-4
7-OMe	3.84 (s)	55.8	CH_3	C-7		3.85 (s)	55.8	CH_3	C-7
3′-OH	–	–	–		4.53 (d, 5.4)	–	–		
9-NH	10.32 (br s)	–	–		10.44 (br s)	–	–		

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