

## Constituents of *Clausena excavata*. Isolation and Structural Elucidation of Seven New Carbazole Alkaloids and a New Coumarin

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Seven new carbazole alkaloids, named clauszoline-A (1), -B (2), -C (3), -D (4), -E (5), -F (6), and -G (7), and a new coumarin named 5-geranyloxy-7-hydroxycoumarin (8) were isolated from stem bark of *Clausena excavata* (Rutaceae) collected in Singapore, and their structures were elucidated by means of spectral methods.

**Key words** *Clausena excavata*; carbazole alkaloid; clauszoline; coumarin; 5-geranyloxy-7-hydroxycoumarin; Rutaceae

*Clausena excavata* BURM. f. (Rutaceae), widely distributed in southern Asia, are shrubs, and their branchlets are pubescent.<sup>1)</sup> The extracts of the leaves and barks of this tree have been used as a folk medicine for the treatment of snakebite and abdominal pain.<sup>2)</sup> The plants of *Clausena* species are known to be rich sources of carbazole alkaloids and coumarins.<sup>3-6)</sup> We have studied the constituents of *Murraya koenigii* (L.) SPRENG<sup>7)</sup> and *Murraya euchrestifolia* HAYATA,<sup>8-13)</sup> which are closely related to *Clausena* sp., but found only carbazole alkaloids, and no coumarins. Because we were interested in this difference from a phytochemical viewpoint, we have studied the constituents of *Clausena excavata* collected in Singapore. This paper describes the isolation and structural elucidation of fourteen carbazole alkaloids and one coumarin, including seven and one new components, respectively.

### Results and Discussion

The acetone extract of the stem bark of the plant was fractionated by a combination of silica gel column chromatography and preparative TLC to give seven new alkaloids and a new coumarin, along with known carbazoles, as shown in Chart 1.

#### Structure of Clauszoline-A (1) and Clauszoline-B (2)

Clauszoline-A (1) was obtained as a pale yellow powder. The molecular formula was determined as C<sub>23</sub>H<sub>23</sub>NO<sub>3</sub> by high-resolution (HR)-MS. The UV spectrum showed typical absorption of a carbazole nucleus.<sup>3,5)</sup> The IR spectrum showed bands at  $\nu_{\max}$  3450 and 3440 (br) cm<sup>-1</sup> due to an imino group and a hydroxyl group, respectively. The <sup>1</sup>H-NMR spectrum showed two 1H-singlets attributable to a formyl group [ $\delta$  9.89 (s)] and a typically deshielded H-4 [ $\delta$  7.98 (s)]<sup>3,5)</sup> on the carbazole nucleus, as well as an imino [ $\delta$  8.31 (1H, brs)] and a strongly hydrogen-bonded hydroxyl group [ $\delta$  11.64 (1H, s)]. Observations of a singlet (6H) at  $\delta$  1.53 assignable to geminal dimethyls attached to an oxygenated carbon and AB-type doublets at  $\delta$  6.48 and 5.63 (each 1H,  $J=9.9$  Hz), together with those of *ortho*-coupled protons [ $\delta$  7.44 and 6.90 (each 1H,  $J=7.7$  Hz)] including a lower-field H-5 proton at  $\delta$  7.44<sup>3,5)</sup> indicated the presence of a dimethylpyran ring fused with the carbazole nucleus at C-7 and 8, and the orientation of the pyran ring was also supported by the observation of a nuclear Overhauser effect (NOE) enhancement between the H-6 ( $\delta$  6.90) and H-4' ( $\delta$  6.48) signals. Further, in the NOE experiment, irradiation of H-4 at  $\delta$  7.98 showed enhancements both at the CHO ( $\delta$  9.89) and the H-5 ( $\delta$  7.44) signals, indicating

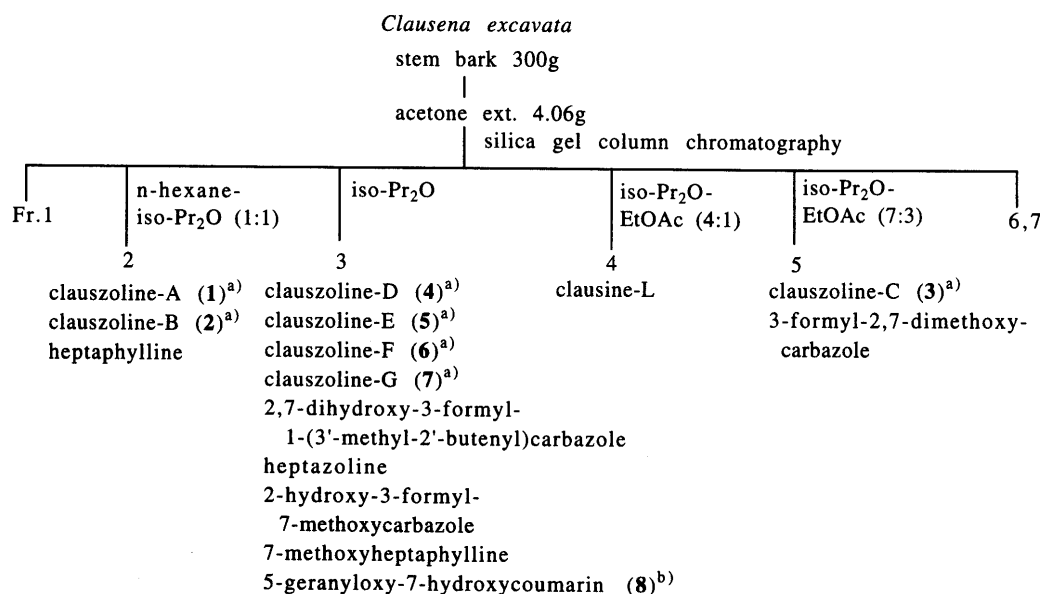


Chart 1. Isolation of Carbazole Alkaloids and Coumarin from *Clausena excavata*

a) New carbazole alkaloids. b) New coumarin.

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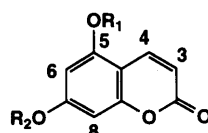
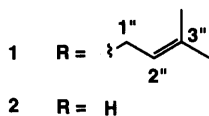
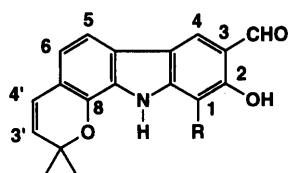
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the location of the formyl group to be at C-3, and thus suggesting the location of a hydroxyl group ( $\delta$  11.64) at C-2. The presence of a prenyl group was revealed by the appearance of  $^1\text{H-NMR}$  signals at  $\delta$  5.36 (1H, t,  $J=7.3$  Hz), 3.65 (2H, d,  $J=7.3$  Hz), 1.94 (3H, s), and 1.79 (3H, s), and a fragment peak at  $m/z$  306 [ $M^+ - \cdot\text{CH}=\text{C}(\text{CH}_3)_2$ ] in the electron impact (EI)-MS. These spectral data led us to propose the structure **1** for clauszoline-A.

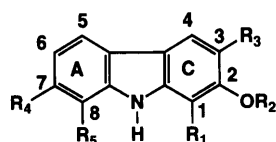
Clauszoline-B (**2**) was isolated as a yellow oil, and its molecular formula was found to be  $\text{C}_{18}\text{H}_{15}\text{NO}_3$  by HR-MS. The UV spectrum showed a close resemblance to that of **1**, suggesting the pyranocarbazole structure for this compound, as in the case of **1**. The  $^1\text{H-NMR}$  spectrum also showed a similar signal pattern to that of **1**, except for the appearance of a higher-field sharp 1H singlet at  $\delta$  6.83 in the aromatic proton region, instead of signals due to the prenyl side chain [ $-\text{CH}_2\text{CH}=\text{C}(\text{CH}_3)_2$ ]. In the NOE experiments, irradiation of a lower-field 1H singlet at  $\delta$  8.07, a typically deshielded

H-4 on the carbazole nucleus,<sup>3,5</sup> caused 4 and 16% enhancements of one of the doublets at  $\delta$  7.43 (H-5) and a singlet at  $\delta$  9.89 (3-CHO), respectively. Irradiation of the doublet at  $\delta$  6.45 (H-4') showed 5% enhancement of the doublet at  $\delta$  6.90 (H-6), in the case of **1**. Based on these results, we assigned the structure **2**, corresponding to the deprenylated derivative of **1**, to clauszoline-B. Clauszoline-A (**1**) and -B (**2**) are the first reported examples of naturally occurring 8-oxygenated carbazole alkaloids having a dimethylpyran ring fused with the carbazole nucleus at C-7 and 8.

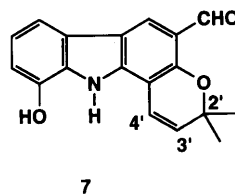
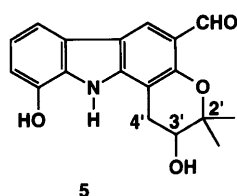
**Structure of Clauszoline-C (3)** This compound was obtained as a pale yellow powder. The molecular formula  $\text{C}_{16}\text{H}_{15}\text{NO}_4$  was confirmed by HR-MS. The UV spectrum suggested the presence of a carbazole nucleus.<sup>3,5</sup> The IR spectrum showed an absorption band due to an NH group at  $\nu_{\text{max}}$   $3467\text{cm}^{-1}$ . In the  $^1\text{H-NMR}$  spectrum, ABC-type signals at  $\delta$  7.94 (1H, d,  $J=7.7$  Hz), 6.82 (1H, dd,  $J=7.7, 2.4$  Hz), and 7.02 (1H, d,  $J=2.4$  Hz), two



	R <sub>1</sub>	R <sub>2</sub>
8		H
9		CH <sub>3</sub>
10	H	H



	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>
3	H	CH <sub>3</sub>	COOCH <sub>3</sub>	OCH <sub>3</sub>	H
4		H	CHO	H	OH
6		H	CHO	H	OH



singlet signals at  $\delta$  8.40 (1H, s) and 7.10 (1H, s) in the aromatic proton region, and three OMe signals [ $\delta$  3.89, 3.86, and 3.82] were observed. Among these aromatic proton signals, the lower signals at  $\delta$  7.94 and 8.40 were assignable to H-5 and H-4, respectively.<sup>3,5</sup> In NOE experiments, a 1H singlet at  $\delta$  7.10 (H-1) showed a 16% NOE enhancement on irradiation of the methoxy signal at  $\delta$  3.89 and irradiation of another methoxy signal at  $\delta$  3.86 gave 5 and 11% enhancements of the signals at  $\delta$  6.82 (H-6) and  $\delta$  7.02 (H-8). Further irradiation of the NH signal at  $\delta$  10.36 gave 5 and 5% increases of the signals at  $\delta$  7.10 (H-1) and  $\delta$  7.02 (H-8). These results suggested the locations of OMe ( $\delta$  3.89) at C-2 on ring-C and OMe ( $\delta$  3.86) at C-7 on ring-A. Further, an IR band at  $\nu_{\max}$  1710  $\text{cm}^{-1}$ , two significant mass fragments at  $m/z$  254 [ $\text{M}^+ - \cdot\text{OCH}_3$ ] and 223 [ $\text{M}^+ - \cdot\text{COOCH}_3 - \cdot\text{H}$ ] in the EI-MS, and  $^{13}\text{C}$ -NMR signals at  $\delta$  167.41 and  $\delta$  51.62 suggested the presence of a carbomethoxy group on the carbazole nucleus. Based on these results, we assigned the structure **3** to clauszoline-C.

**Structures of Clauszoline-D (4), -E (5), and -F (6)**  
 Clauszoline-D (**4**), -E (**5**), and -F (**6**) showed analogous UV absorptions, having two sharp high-intensity and a broad low-intensity bands at  $\lambda_{\max}$  242 and 273–276 nm and 354–358 nm, respectively. These features are characteristic of the 2,8-oxygenated 3-formylcarbazole chromophore.<sup>14</sup> Further, in the  $^1\text{H}$ -NMR spectra of these compounds, as common features, a deshielded singlet at  $\delta$  8.02–8.34 (1H) assignable to H-4, a three-spin system including a lower-field H-5 and *ortho*-coupled signals at  $\delta$  7.57–7.58 (1H, d,  $J=7.7$  Hz), 7.03–7.10 (1H, t,  $J=7.7$  Hz), and 6.83–6.88 (1H, d,  $J=7.7$  Hz), and a formyl proton signal at  $\delta$  9.90–10.47 (1H, s), together with NH and OH signals suggested the presence of two additional substituents at C-1 and C-3 in the 2,8-oxygenated carbazole skeleton. The formyl substituent at C-3 was revealed by the observation of NOE enhancement

between a deshielded H-4 and formyl proton signals. These results indicated these alkaloids to be 2,8-oxygenated 3-formylcarbazoles having a substituent at C-1. We will discuss the structure of the substituent at C-1 in each alkaloid below.

Clauszoline-D (**4**) was isolated as a yellow oil,  $[\alpha]_{\text{D}}^{20}$ , and the molecular formula was determined as  $\text{C}_{18}\text{H}_{19}\text{NO}_5$  by HR-MS. The structure of the substituent at C-1 was suggested by the following  $^1\text{H}$ -NMR and EI-MS results. In the  $^1\text{H}$ -NMR spectrum, two methyl signals at  $\delta$  1.33 and 1.31 attached to an oxygenated quaternary carbon and ABC-type signals having geminal and vicinal couplings at  $\delta$  3.46 (1H, dd,  $J=14.3, 1.8$  Hz), 2.89 (1H, dd,  $J=9.5, 14.3$  Hz) and 3.73 (1H, brd,  $J=9.5$  Hz) were seen, along with two hydroxy signals at  $\delta$  4.28 and 3.72. The EI-MS showed two characteristic ions at  $m/z$  270 and 240 assignable to fragments corresponding to loss of the side chain from the molecular ion, [ $\text{M}^+ - \cdot\text{C}(\text{OH})(\text{CH}_3)_2$ ] and [ $\text{M}^+ - \cdot\text{CH}(\text{OH})\text{C}(\text{OH})(\text{CH}_3)_2$ ], respectively. This let us to conclude that clauszoline-D has the structure **4**.

Clauszoline-E (**5**) was isolated as a yellow powder,  $[\alpha]_{\text{D}}^{20}$ , having the molecular formula  $\text{C}_{18}\text{H}_{17}\text{NO}_4$  by HR-MS. In the  $^1\text{H}$ -NMR spectrum (DMSO- $d_6$ , see Experimental), the appearance of two 3H singlets assignable to geminal dimethyl attached to the oxygenated carbon at  $\delta$  1.38 and 1.30 and a multiplet at  $\delta$  3.83 coupled with a hydroxy proton at  $\delta$  5.32 (1H, d,  $J=5.5$  Hz, disappeared with  $\text{D}_2\text{O}$ ) and double doublets due to benzylic methylene protons at  $\delta$  3.17 (1H,  $J=17.1, 5.1$  Hz), 2.83 (1H,  $J=17.1, 7.0$  Hz) indicated the presence of a 2,2-dimethyl-3-hydroxydihydropyran ring system. Based on these spectral data, coupled with the results of the NOE (Experimental) and  $^1\text{H}$ -detected heteronuclear multiple bond connectivity (HMBC) experiments, shown by arrows in Fig. 1, the structure of clauszoline-E was concluded to be **5**.

Clauszoline-F (**6**) was isolated as a yellow powder. The molecular formula,  $\text{C}_{23}\text{H}_{25}\text{NO}_3$ , was determined by

Table 1.  $^1\text{H}$ -NMR Data (in  $\text{CDCl}_3$ ) of New Carbazole Alkaloids

	1	2	3 <sup>a)</sup>	4 <sup>a)</sup>	5 <sup>a)</sup>	6	7 <sup>a)</sup>
H-1	—	6.83 (s)	7.10 (s)	—	—	—	—
2-R	11.64 (s)	11.42 (s)	3.89 (3H, s)	11.77 (s)	—	11.62 (s)	—
2-OH	—	2-OH	2-OMe	2-OH	—	2-OH	—
3-CHO	9.89 (s)	9.89 (s)	—	9.97 (s)	10.47 (s)	9.90 (s)	10.48 (s)
H-4	7.98 (s)	8.07 (s)	8.40 (s)	8.30 (s)	8.34 (s)	8.02 (s)	8.33 (s)
H-5	7.44 (d, 7.7)	7.43 (d, 7.7)	7.94 (d, 7.7)	7.57 (d, 7.7)	7.58 (d, 7.7)	7.57 (d, 7.7)	7.61 (d, 7.7)
H-6	6.90 (d, 7.7)	6.90 (d, 7.7)	6.82 (dd, 7.7, 2.4)	7.05 (t, 7.7)	7.03 (t, 7.7)	7.10 (t, 7.7)	7.04 (t, 7.7)
H-7	—	—	—	6.88 (d, 7.7)	6.85 (d, 7.7)	6.83 (d, 7.7)	6.88 (d, 7.7)
H-8	—	—	7.02 (d, 2.4)	—	—	—	—
NH	8.31 (brs)	8.34 (brs)	10.36 (brs)	10.43 (brs)	10.43 (brs)	8.45 (brs)	10.58 (brs)
Others	5.36 (t, 7.3, H-2'')	6.45 (d, 9.9, H-4')	3.86 (3H, s, 7-OMe)	3.73 (brd, 9.5, H-2')	4.01 (m, H-3')	5.35 (t, 7.0, H-2')	8.90 (br, 8-OH)
	3.65 (2H, d, 7.3, H-1'')	5.61 (d, 9.9, H-3')	3.82 (3H, s, 3-COOMe)	3.46 (dd, 14.3, 1.8, H-1')	3.34 (dd, 16.9, 5.5, H-4')	5.07 (m, H-6')	7.18 (d, 9.9, H-4')
	1.94 (3H, s, 3'-Me)	1.49 (6H, s, 2'-Me)	—	2.89 (dd, 9.5, 14.3, H-1')	2.99 (dd, 7.3, 16.9, H-4')	3.67 (2H, d, 7.0, H-1')	5.90 (d, 9.9, H-3')
	1.79 (3H, s, 3'-Me)	—	—	1.33 (3H, s, 3'-Me)	1.49 (3H, s, 2'-Me)	2.10 (4H, m, H-4', 5')	1.56 (6H, s, 2'-Me)
	6.48 (d, 9.9, H-4')	—	—	1.31 (3H, s, 3'-Me)	1.40 (3H, s, 2'-Me)	1.92 (3H, s, 3'-Me)	—
	5.63 (d, 9.9, H-3')	—	—	8.95 (br, 8-OH)	8.91 (br, 8-OH)	1.60 (3H, s, 7'-Me)	—
	1.53 (6H, s, 2'-Me)	—	—	4.28 (br, OH)	4.53 (br, 3'-OH)	1.56 (3H, s, 7'-Me)	—
	—	—	—	3.72 (br, OH)	—	5.44 (br, 8-OH)	—

Values in ( $\delta$ ) ppm. The coupling constants ( $J$ ) in parentheses are in Hz. All signals correspond to 1H, unless otherwise stated. a) Spectra were taken in acetone- $d_6$ .

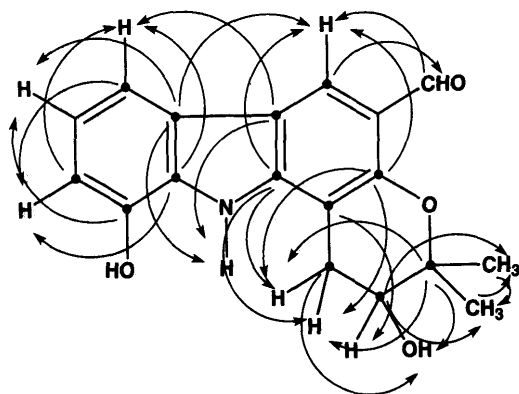


Fig. 1. C-H Three-Bond Long-Range Correlations in the HMBC Spectrum of Clauszoline-E (**5**) in DMSO- $d_6$ .

HR-MS. The  $^1\text{H-NMR}$  spectrum differs from that of **4** only in the presence of signals [ $\delta$  1.56 (3H, s), 1.60 (3H, s), 1.92 (3H, s), 2.10 (4H, m), 3.67 (2H, d,  $J=7.0$  Hz), 5.07 (1H, m), and 5.35 (1H, t,  $J=7.0$  Hz)] assignable to the side chain  $[-\text{CH}_2\text{CH}=\text{C}(\text{CH}_3)-\text{CH}_2\text{CH}_2\text{CH}=\text{C}(\text{CH}_3)_2]$  instead of the signals due to the side chain  $[-\text{CH}_2\text{CH}(\text{OH})-\text{C}(\text{OH})(\text{CH}_3)_2]$  in the spectrum of **4**. The appearance of mass fragment ions at  $m/z$  294 and 240 arising from loss of  $[\cdot\text{C}_5\text{H}_9]$  and  $[\cdot\text{C}_9\text{H}_{15}]$  from the molecular ion, respectively, together with the observation of NOE enhancement between the H-1' ( $\delta$  3.67) and 3'-Me ( $\delta$  1.92) signals in the  $^1\text{H-NMR}$  spectrum suggested the presence of the geranyl side chain in the molecule. Further, in the NOE experiment, irradiation of NH ( $\delta$  8.45) gave 3% and 4% enhancements of the H-1' ( $\delta$  3.67) and H-2' ( $\delta$  5.35) signals, respectively, also supporting the location of the geranyl side chain at C-1. These results led us to conclude that clauszoline-F has the structure **6**.

**Structure of Clauszoline-G (7)** Clauszoline-G (**7**) was obtained as a yellow powder. The HR-MS analysis indicated the molecular formula to be  $\text{C}_{18}\text{H}_{15}\text{NO}_3$ , a difference of  $\text{H}_2\text{O}$  compared with **5**. The UV spectrum and IR bands (see Experimental) suggested the presence of a carbazole skeleton.<sup>3,5</sup> The  $^1\text{H-NMR}$  spectrum showed a similar signal pattern to that of **5**, except for the appearance of signals due to a 2,2-dimethylpyran ring [ $\delta$  5.90 (1H, d,  $J=9.9$  Hz), 7.18 (1H, d,  $J=9.9$  Hz), 1.56 (6H, s)], instead of signals due to the 2,2-dimethyl-3-hydroxydihydropyran ring. On the basis of these spectral data, we propose the structure **7** for clauszoline-G.

**Structure of 5-Geranyloxy-7-hydroxycoumarin (8)** This compound (**8**) was isolated as a colorless powder. The molecular formula was determined as  $\text{C}_{19}\text{H}_{22}\text{O}_4$  by HR-MS. The UV spectrum ( $\lambda_{\text{max}}$  nm: 211, 226 (sh), 250, 257, 331) was similar to that of 5,7-dihydroxycoumarin (**10**),<sup>15</sup> and IR bands appeared at  $\nu_{\text{max}}$  3247 and 1718  $\text{cm}^{-1}$  (a hydroxy group and an  $\alpha,\beta$ -unsaturated lactone). The  $^1\text{H-NMR}$  spectrum showed AB-type doublets at  $\delta$  6.15 (H-3) and 8.06 (H-4) (each 1H,  $J=9.5$  Hz), and *meta*-coupled doublets at  $\delta$  6.62 (H-6) and 6.32 (H-8) (each 1H,  $J=2.2$  Hz). These results, coupled with the observation of the H-4 proton signal at  $\delta$  8.06, at lower field compared with that of coumarin lacking a C-5 oxygen function,<sup>6</sup> indicated the presence of a 5,7-oxygenated coumarin nucleus in the molecule. The remaining

signals at  $\delta$  5.48 (1H, t,  $J=6.6$  Hz), 5.09 (1H, m), 4.61 (2H, d,  $J=6.6$  Hz), 2.11 (4H, m), 1.75, 1.68, 1.61 (each 3H, s) in the  $^1\text{H-NMR}$  spectrum, coupled with two characteristic ions at  $m/z$  245 and 191 arising from loss of  $[\cdot\text{C}_5\text{H}_9]$  and  $[\cdot\text{C}_9\text{H}_{15}]$  from the molecular ion in EI-MS, respectively, and the appearance of NOE enhancement between the H-1' ( $\delta$  4.61) and 3'-Me ( $\delta$  1.75) signals suggested that this new coumarin (**8**) contains a geranyloxy moiety  $[-\text{OCH}_2\text{CH}=\text{C}(\text{CH}_3)-\text{CH}_2\text{CH}_2\text{CH}=\text{C}(\text{CH}_3)_2]$  in the molecule. The location of the geranyloxy moiety at C-5 (not at C-7) was based in the following NOE enhancements. a) In the original coumarin (**8**), irradiation of the methylene signal at  $\delta$  4.61 (H-1') enhanced the signal at 6.32 (H-6). b) In the methyl ether (**9**), irradiation of the methoxy group at  $\delta$  3.83 (7-OCH<sub>3</sub>) enhanced the signals at  $\delta$  6.26 (H-8) and 6.39 (H-6), respectively. On the basis of these spectral data, the structure of 5-geranyloxy-7-hydroxycoumarin was proposed to be represented by the formula **8**.

Other carbazole alkaloids isolated from the plant material were characterized as 2,7-dihydroxy-3-formyl-1-(3'-methyl-2'-butenyl)carbazole,<sup>16</sup> clausine-L,<sup>17</sup> heptazoline,<sup>14</sup> 2-hydroxy-3-formyl-7-methoxycarbazole,<sup>18</sup> 3-formyl-2,7-dimethoxycarbazole,<sup>19</sup> heptaphylline,<sup>20</sup> and 7-methoxyheptaphylline<sup>18</sup> by comparisons of the  $^1\text{H-NMR}$  and IR data with those reported in the literature.<sup>14,16-20</sup>

#### Experimental

Melting points were measured on a micromelting point hot-stage apparatus (Yanagimoto).  $^1\text{H-NMR}$  spectra were recorded on an A-400 (JEOL) spectrometer in  $\text{CDCl}_3$ , unless otherwise stated. Chemical shifts are shown in  $\delta$  values (ppm) with tetramethylsilane (TMS) as an internal reference. All MS were taken under electron impact (EI) conditions using an M-80 (Hitachi) having a direct inlet system. UV spectra were recorded on a UVIDEC-610C double-beam spectrophotometer (JASCO) in methanol, IR spectra on an IR-230 (JASCO) in  $\text{CHCl}_3$ , and optical rotations on a DIP-370 (JASCO) in  $\text{CHCl}_3$  at 25 °C. Preparative TLC was done on Kieselgel 60 F<sub>254</sub> (Merck).

**Extraction and Isolation** The dried stem bark (300 g) of *Clausena excavata* BURM. f. collected in Singapore was extracted with acetone. The acetone extract was subjected to silica gel column chromatography eluted with *n*-hexane, *n*-hexane-*iso*-Pr<sub>2</sub>O (1:1), *iso*-Pr<sub>2</sub>O, *iso*-Pr<sub>2</sub>O-EtOAc (4:1), *iso*-Pr<sub>2</sub>O-EtOAc (7:3), *iso*-Pr<sub>2</sub>O-EtOAc (1:1), and acetone, successively, to give 7 fractions. Each fraction was further subjected to silica gel column and preparative thin layer chromatographies with appropriate combinations of hexane,  $\text{CH}_2\text{Cl}_2$ , *iso*-Pr<sub>2</sub>O, benzene,  $\text{CHCl}_3$ , and acetone as developing solvents to give seven new carbazoles and a new coumarin, as well as seven known carbazoles, as stated below. From the *n*-hexane-*iso*-Pr<sub>2</sub>O (1:1) eluate: clauszoline-A (**1**) (1.5 mg), clauszoline-B (**2**) (6.0 mg), and heptaphylline (1.0 mg). From the *iso*-Pr<sub>2</sub>O eluate: clauszoline-D (**4**) (1.2 mg), clauszoline-E (**5**) (23 mg), clauszoline-F (**6**) (3.6 mg), clauszoline-G (**7**) (3.0 mg), 2,7-dihydroxy-3-formyl-1-(3'-methyl-2'-butenyl)carbazole (1.2 mg), heptazoline (89 mg), 2-hydroxy-3-formyl-7-methoxycarbazole (2.2 mg), 7-methoxyheptaphylline (1.5 mg), and 5-geranyloxy-7-hydroxycoumarin (**8**) (1.5 mg). From the *iso*-Pr<sub>2</sub>O-EtOAc (4:1) eluate: clausine-L (1.0 mg). From the *iso*-Pr<sub>2</sub>O-EtOAc (7:3) eluate: clauszoline-C (**3**) (1.0 mg) and 3-formyl-2,7-dimethoxycarbazole (1.0 mg). Known components were fully characterized by comparisons of the  $^1\text{H-NMR}$  and IR data with those reported in the literature.<sup>14,16-20</sup>

**Clauszoline-A (1)** Pale yellow powder. UV  $\lambda_{\text{max}}$  nm: 204, 240, 265, 306, 375. IR  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 3450, 3440 (br), 1630. EI-MS  $m/z$  (%): 361 ( $\text{M}^+$ , 50), 346 (100), 318 (12), 306(9), 290 (37), 262 (9), 246 (9), 234 (14), 229 (12), 204 (13), 186 (11), 167 (14). NOE: irradiation of H-4 ( $\delta$  7.98) gave 5% NOE at H-5 ( $\delta$  7.44) and 22% NOE at 3-CHO ( $\delta$  9.89); irradiation of NH ( $\delta$  8.31) gave 3% NOE at H-1' ( $\delta$  3.65) and 2% NOE at H-2' ( $\delta$  5.36); irradiation of H-4' ( $\delta$  6.48) gave 6% NOE at H-6 ( $\delta$  6.90). HR-MS Calcd for  $\text{C}_{23}\text{H}_{23}\text{NO}_3$ : 361.1676. Found: 361.1651.

**Clauszoline-B (2)** Yellow oil. UV  $\lambda_{\max}$  nm: 204, 242, 252 (sh), 264, 291 (sh), 304, 376. IR  $\nu_{\max}$   $\text{cm}^{-1}$ : 3450, 3300 (br), 1650, 1630. EI-MS  $m/z$  (%): 293 ( $M^+$ , 83), 278 (100), 264 (5), 249 (9), 232 (8), 220 (16), 209 (7), 204 (6), 167 (6), 165 (7). NOE: irradiation of H-4 ( $\delta$  8.07) gave 4% NOE at H-5 ( $\delta$  7.43) and 16% NOE at 3-CHO ( $\delta$  9.89); irradiation of H-4' ( $\delta$  6.45) gave 5% NOE at H-6 ( $\delta$  6.90). HR-MS Calcd for  $C_{18}H_{15}NO_3$ : 293.1050. Found: 293.1050.

**Clauszoline-C (3)** Pale yellow powder. UV  $\lambda_{\max}$  nm: 205, 222, 246, 282, 309, 320, 336 (sh). IR  $\nu_{\max}$   $\text{cm}^{-1}$ : 3467, 1710, 1618, 1577. EI-MS  $m/z$  (%): 285 ( $M^+$ , 100), 270 (91), 254 (51), 242 (14), 240 (23), 226 (9), 223 (8), 211 (10), 196 (24), 183 (18), 169 (13).  $^{13}\text{C}$ -NMR (100 MHz, acetone- $d_6$ )  $\delta_c$ : 167.41 (s), 159.71 (s), 158.93 (s), 144.65 (s), 142.81 (s), 123.85 (d), 121.09 (d), 117.73 (s), 117.21 (s), 113.50 (s), 109.18 (d), 95.96 (d), 94.93 (d), 56.39 (q), 55.73 (q), 51.62 (q). NOE: irradiation of NH ( $\delta$  10.36) gave 5% NOE at H-1 ( $\delta$  7.10) and 5% NOE at H-8 ( $\delta$  7.02); irradiation of 2-OMe ( $\delta$  3.89) gave 16% NOE at H-1 ( $\delta$  7.10); irradiation of 3-COOMe ( $\delta$  3.82) gave 1% NOE at H-4 ( $\delta$  8.40); irradiation of 7-OMe ( $\delta$  3.86) gave 5% NOE at H-6 ( $\delta$  6.82) and 11% NOE at H-8 ( $\delta$  7.02). HR-MS Calcd for  $C_{16}H_{15}NO_4$ : 285.1000. Found: 285.0973.

**Clauszoline-D (4)** Yellow oil,  $[\alpha]_D^{20}$  ( $c=0.1$ ). UV  $\lambda_{\max}$  nm: 206, 217, 242, 276, 292, 301 (sh), 356. IR  $\nu_{\max}$   $\text{cm}^{-1}$ : 3330 (br), 1631, 1600. EI-MS  $m/z$  (%): 329 ( $M^+$ , 77), 311 (9), 293 (9), 270 (40), 240 (100), 226 (8), 223 (13), 211 (13), 199 (8), 196 (12), 183 (16), 166 (10). HR-MS Calcd for  $C_{18}H_{19}NO_5$ : 329.1261. Found: 329.1239.

**Clauszoline-E (5)** Yellow powder,  $[\alpha]_D^{20}$  ( $c=0.1$ ). UV  $\lambda_{\max}$  nm: 204, 213, 242, 273, 292, 358. IR  $\nu_{\max}^{\text{KBr}}$   $\text{cm}^{-1}$ : 3479, 3287 (br), 1650, 1582. EI-MS  $m/z$  (%): 311 ( $M^+$ , 72), 299 (10), 278 (5), 268 (10), 252 (10), 240 (100), 238 (18), 226 (18), 223 (8), 210 (32), 199 (5), 196 (8), 183 (28).  $^1\text{H}$ -NMR (400 MHz, DMSO- $d_6$ )  $\delta$ : 11.30 (1H, br, NH), 10.35 (1H, s, 3-CHO), 9.85 (1H, br, OH), 8.24 (1H, s, H-4), 7.52 (1H, d,  $J=7.7$  Hz, H-5), 6.97 (1H, t,  $J=7.7$  Hz, H-6), 6.80 (1H, d,  $J=7.7$  Hz, H-7), 5.32 (1H, d,  $J=5.5$  Hz, 3'-OH), 3.83 (1H, m, H-3'), 3.17 (1H, dd,  $J=17.1, 5.1$  Hz, H-4'), 2.83 (1H, dd,  $J=17.1, 7.0$  Hz, H-4'), 1.38, 1.30 (each 3H, s, 2'-Me).  $^{13}\text{C}$ -NMR (100 MHz, DMSO- $d_6$ )  $\delta_c$ : 188.39 (d, CHO), 153.71 (s, C-2), 143.75 (s, C-9a), 143.26 (s, C-8), 129.83 (s, C-8a), 125.16 (s, C-4b), 120.73 (d, C-6), 118.21 (d, C-4), 117.48 (s, C-3), 116.67 (s, C-4a), 110.81 (d, C-5), 110.67 (d, C-7), 103.23 (s, C-1), 77.99 (s, C-2'), 67.08 (d, C-3'), 27.24 (t, C-4'), 25.29 (q, 2'-Me), 20.79 (q, 2'-Me). NOE: irradiation of H-4 ( $\delta$  8.24) gave 2% NOE at H-5 ( $\delta$  7.52) and 2% NOE at 3-CHO ( $\delta$  10.35). HR-MS Calcd for  $C_{18}H_{17}NO_4$ : 311.1157. Found: 311.1170.

**Clauszoline-F (6)** Yellow powder. UV  $\lambda_{\max}$  nm: 204, 216, 242, 276, 291, 300 (sh), 354. IR  $\nu_{\max}$   $\text{cm}^{-1}$ : 3590, 3450, 3300 (br), 1630, 1585. EI-MS  $m/z$  (%): 363 ( $M^+$ , 90), 320 (6), 294 (48), 280 (28), 278 (25), 266 (7), 252 (10), 250 (9), 240 (100), 233 (6), 227 (18), 224 (7), 211 (6), 196 (10), 183 (14).  $^{13}\text{C}$ -NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta_c$ : 195.44 (d), 157.78 (s), 144.99 (s), 141.11 (s), 137.84 (s), 131.75 (s), 129.37 (s), 125.84 (d), 125.56 (s), 123.94 (d), 121.29 (d), 121.05 (d), 117.72 (s), 115.52 (s), 112.57 (d), 111.15 (d), 109.27 (s), 39.68 (t), 26.62 (t), 25.57 (q), 22.85 (t), 17.67 (q), 16.42 (q). NOE: irradiation of H-4 ( $\delta$  8.02) gave 5% NOE at H-5 ( $\delta$  7.57) and 17% NOE at 3-CHO ( $\delta$  9.90); irradiation of NH ( $\delta$  8.45) gave 3% NOE at H-1' ( $\delta$  3.67) and 4% NOE at H-2' ( $\delta$  5.35); irradiation of H-1' ( $\delta$  3.67) gave 10, 8, 6, and 2% NOE at 3'-Me ( $\delta$  1.92), H-2' ( $\delta$  5.35), NH ( $\delta$  8.45), and 2-OH ( $\delta$  11.62), respectively. HR-MS Calcd for  $C_{23}H_{25}NO_3$ : 363.1833. Found: 363.1848.

**Clauszoline-G (7)** Yellow powder. UV  $\lambda_{\max}$  nm: 204, 234, 245 (sh), 278, 300, 361. IR  $\nu_{\max}^{\text{KBr}}$   $\text{cm}^{-1}$ : 3263 (br), 1658, 1631, 1578. EI-MS  $m/z$  (%): 293 ( $M^+$ , 44), 278 (100), 266 (8), 250 (21), 226 (7), 218 (10), 211 (16), 196 (4), 183 (6), 167 (6). NOE: irradiation of H-4 ( $\delta$  8.33) gave 5% NOE at H-5 ( $\delta$  7.61) and 2% NOE at 3-CHO ( $\delta$  10.48); irradiation of NH ( $\delta$  10.58) gave 5% NOE at H-4' ( $\delta$  7.18). HR-MS Calcd for  $C_{18}H_{15}NO_3$ : 293.1050. Found: 293.1035.

**5-Geranyloxy-7-hydroxycoumarin (8)** Colorless powder. UV  $\lambda_{\max}$  nm: 211, 226 (sh), 250, 257, 331. IR  $\nu_{\max}$   $\text{cm}^{-1}$ : 3247, 1718, 1612. EI-MS  $m/z$  (%): 314 ( $M^+$ , 3), 254 (2), 245 (2), 240 (2), 231 (3), 229 (3), 203 (3), 191 (5), 178 (100), 161 (2).  $^1\text{H}$ -NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 8.06 (1H, d,  $J=9.5$  Hz, H-4), 6.62 (1H, d,  $J=2.2$  Hz, H-6), 6.32 (1H, d,  $J=2.2$  Hz, H-8), 6.15 (1H, d,  $J=9.5$  Hz, H-3), 5.48 (1H, t,  $J=6.6$  Hz), 5.09 (1H, m), 4.61 (2H, d,  $J=6.6$  Hz), 2.11 (4H, m), 1.75, 1.68, 1.61 (each 3H, s).  $^{13}\text{C}$ -NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta_c$ : 162.64 (s), 160.92 (s), 156.80 (s), 156.46 (s), 142.24 (s), 139.89 (d), 131.97 (s), 123.57 (d), 118.37 (d), 109.94 (d), 104.02 (s), 96.25 (d), 95.69 (d), 65.76 (t), 39.48 (t), 26.22 (t), 25.66 (q), 17.70 (q), 16.73 (q). NOE: irradiation of H-1' ( $\delta$  4.61) gave 6, 10 and 14% NOE at 3'-Me ( $\delta$  1.75), H-2' ( $\delta$  5.48) and H-6 (d 6.32), respectively. HR-MS Calcd for  $C_{19}H_{22}O_4$ : 314.1516. Found: 314.1513.

**O-Methylation of 8 with Diazomethane** A large excess of ethereal diazomethane was added to a methanolic solution (20 ml) of **8** (4 mg), and the mixture was left overnight at room temperature. The solvent was evaporated off, and the residue was purified by preparative TLC to give **9** almost quantitatively: Colorless oil. UV  $\lambda_{\max}$  nm: 207, 247, 255, 325. IR  $\nu_{\max}$   $\text{cm}^{-1}$ : 1724, 1610. EI-MS  $m/z$  (%): 328 ( $M^+$ , 4), 256 (5), 192 (100), 164 (18).  $^1\text{H}$ -NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 7.99 (1H, d,  $J=9.5$  Hz, H-4), 6.39 (1H, br s, H-6), 6.26 (1H, br s, H-8), 6.13 (1H, d,  $J=9.5$  Hz, H-3), 5.45 (1H, t,  $J=6.6$  Hz), 5.07 (1H, m), 4.58 (2H, d,  $J=6.6$  Hz), 3.83 (3H, s, 7-OMe), 2.08 (4H, m), 1.72, 1.66, 1.59 (each 3H, s). NOE: irradiation of 7-OMe ( $\delta$  3.83) gave 2% NOE at H-8 ( $\delta$  6.26) and 16% NOE at H-6 ( $\delta$  6.39).

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**Added in Proof** (October 4, 1996) After this paper was submitted, clauszoline-C (**3**) was also isolated recently by Wu T.-S. *et al.* [Wu T.-S., Huang S.-C., Wu P.-L., Teng C.-M., *Phytochemistry*, **43**, 133–140 (1996).]

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