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 clausoline-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; clausoline; coumarin; 5-geranyloxy-7-hydroxycoumarin; Rutaceae

*Clausena excavata* Burm. f. (Rutaceae), widely distributed in southern Asia, are shrubs, and their branchlets are pubescent. 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. The plants of *Clausena* species are known to be rich sources of carbazole alkaloids and coumarins. We have studied the constituents of *Murraya koenigii* (L.) Spreng and *Murraya euchrestifolia* Hayata, 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 Clausoline-A (1) and Clausoline-B (2)**

Clausoline-A (1) was obtained as a pale yellow powder. The molecular formula was determined as C_{23}H_{33}NO_{3} by high-resolution (HR)-MS. The UV spectrum showed typical absorption of a carbazole nucleus. The IR spectrum showed bands at ν_{max} 3450 and 3440 (br) cm^{-1} due to an imino group and a hydroxyl group, respectively. The ^1H-NMR spectrum showed two 1H-singlets attributable to a formyl group [δ 9.80 (s)] and a typically deshielded H-4 [δ 7.98 (s)] on the carbazole nucleus, as well as an imino [δ 8.31 (1H, brs)] and a strongly hydrogen-bonded hydroxyl group [δ 11.64 (1H, s)]. Observations of a singlet (6H) at δ 1.53 assignable to geminal dimethyls attached to an oxygenated carbon and AB-type doublets at δ 6.48 and 5.63 (each 1H, J = 9.9 Hz), together with those of ortho-coupled protons [δ 7.44 and 6.90 (each 1H, J = 7.7 Hz)] including a lower-field H-5 proton at δ 7.44, 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 (δ 6.90) and H-4 (δ 6.48) signals. Further, in the NOE experiment, irradiation of H-4 at δ 7.98 showed enhancements both at the CHO (δ 9.89) and the H-5 (δ 7.44) signals, indicating

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![Chart 1. Isolation of Carbazole Alkaloids and Coumarin from *Clausena excavata*](chart1.png)

a) New carbazole alkaloids. b) New coumarin.

* To whom correspondence should be addressed. © 1996 Pharmaceutical Society of Japan
the location of the formyl group to be at C-3, and thus suggesting the location of a hydroxyl group (δ 11.64) at C-2. The presence of a prenyl group was revealed by the appearance of 1H-NMR signals at δ 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^+ - CH = C(CH3)2] in the electron impact (EI)-MS. These spectral data led us to propose the structure I for clauszoline-A.

Clauszoline-B (2) was isolated as a yellow oil, and its molecular formula was found to be C18H13NO3 by HR-MS. The UV spectrum showed a close resemblance to that of I, suggesting the pyranocarbazole structure for this compound, as in the case of I. The 1H-NMR spectrum also showed a similar signal pattern to that of I, except for the appearance of a higher-field sharp 1H singlet at δ 6.83 in the aromatic proton region, instead of signals due to the prenyl side chain [−CH2CH = C(CH3)2]. In the NOE experiments, irradiation of a lower-field 1H singlet at δ 8.07, a typically deshielded H-4 on the carbazole nucleus, caused 4 and 16% enhancements of one of the doublets at δ 7.43 (H-5) and a singlet at δ 9.89 (3-CHO), respectively. Irradiation of the doublet at δ 6.45 (H-4') showed 5% enhancement of the doublet at δ 6.90 (H-6), in the case of I. Based on these results, we assigned the structure 2, corresponding to the deprenylated derivative of I, 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 C14H13NO4 was confirmed by HR-MS. The UV spectrum suggested the presence of a carbazole nucleus. The IR spectrum showed an absorption band due to an NH group at νmax 3467 cm⁻¹. In the 1H-NMR spectrum, ABC-type signals at δ 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
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.\(^{3,5}\) 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 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 cm$^{-1}$, two significant mass fragments at $m/z$ 254 [M$^+$ - OCH$_3$] and 223 [M$^+$ - COOCH$_3$ - H] in the EI-MS, and $^{13}$C-NMR signals at $\delta$ 167.41 and 51.62 suggested the presence of a carboxyethoxyl group on the carbazole nucleus. Based on these results, we assigned the structure 3 to clausazolene-C.

**Structures of Clausazolene-D (4), -E (5), and -F (6)**

Clausazolene-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.\(^{14}\) Further, in the $^1$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.

Clausazolene-D (4) was isolated as a yellow oil, [x]$_D$ 0°, and the molecular formula was determined as C$_{19}$H$_{19}$NO$_3$ by HR-MS. The structure of the substituent at C-1 was suggested by the following $^1$H-NMR and EI-MS results. In the $^1$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, [M$^+$ - CO(OH)(CH$_3$)$_2$] and [M$^+$ - CH(OH)OC(OH)(CH$_3$)$_2$], respectively. This let us conclude that clausazolene-D has the structure 4.

Clausazolene-E (5) was isolated as a yellow powder, [x]$_D$ 0°, having the molecular formula C$_{19}$H$_{19}$NO$_3$ by HR-MS. In the $^1$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 D$_2$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-hydroxy-dihydropyran ring system. Based on these spectral data, coupled with the results of the NOE (Experimental) and $^1$H-detected heteronuclear multiple bond connectivity (HMB) experiments, shown by arrows in Fig. 1, the structure of clausazolene-E was concluded to be 5.

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

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**Table 1. $^1$H-NMR Data (in CDCl$_3$) of New Carbazole Alkaloids**

<table>
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<th>3</th>
<th>4</th>
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<td>H-1</td>
<td>—</td>
<td>6.83 (s)</td>
<td>7.10 (s)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>-R</td>
<td>11.64 (s)</td>
<td>11.42 (s)</td>
<td>3.89 (3H, s)</td>
<td>11.77 (s)</td>
<td>—</td>
<td>11.62 (s)</td>
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<td>2-2OH</td>
<td>2-OMe</td>
<td>2-OMe</td>
<td>2-OMe</td>
<td>2-OMe</td>
<td>—</td>
<td>2-OH</td>
</tr>
<tr>
<td>3-CHO</td>
<td>9.89 (s)</td>
<td>9.89 (s)</td>
<td>—</td>
<td>9.97 (s)</td>
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<td>10.47 (s)</td>
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<td>8.07 (s)</td>
<td>8.40 (s)</td>
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<td>8.34 (s)</td>
<td>8.02 (s)</td>
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<td>7.43 (d, 7.7)</td>
<td>7.94 (d, 7.7)</td>
<td>7.57 (d, 7.7)</td>
<td>7.58 (d, 7.7)</td>
<td>7.57 (d, 7.7)</td>
<td>7.61 (d, 7.7)</td>
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<tr>
<td>H-6</td>
<td>6.90 (d, 7.7)</td>
<td>6.90 (d, 7.7)</td>
<td>6.82 (dd, 7.7, 2.4)</td>
<td>7.05 (t, 7.7)</td>
<td>7.03 (t, 7.7)</td>
<td>7.10 (t, 7.7)</td>
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<td>—</td>
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<td>6.85 (d, 7.7)</td>
<td>6.83 (d, 7.7)</td>
<td>6.83 (d, 7.7)</td>
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<tr>
<td>H-8</td>
<td>—</td>
<td>—</td>
<td>7.02 (d, 2.4)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<td>NH</td>
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<td>8.34 (br s)</td>
<td>10.36 (br s)</td>
<td>10.43 (br s)</td>
<td>10.43 (br s)</td>
<td>8.45 (br s)</td>
<td>10.58 (br s)</td>
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<td>Others</td>
<td>5.36 (s, 7.3, 2-H')</td>
<td>6.45 (d, 9.9, H-4')</td>
<td>3.86</td>
<td>3.73 (brd, 9.5, H-2')</td>
<td>4.01 (m, 3-H')</td>
<td>5.53 (t, 7.0, H-2')</td>
<td>8.90 (br, 8-0H)</td>
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Values in (δ) 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$_6$.
HR-MS. The $^1$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, $J=7.00$ Hz), 5.07 (1H, m), and 5.35 (1H, $t$, $J=7.00$ Hz)] assignable to the side chain $[-CH_2CH=C(CH_3)-CH_2CH_2CH=C(CH_3)_3]$ instead of the signals due to the side chain $[-CH_2CH(OMe)-C(OH)(CH_3)_2]$ in the spectrum of 4. The appearance of mass fragment ions at $m/z$ 294 and 240 arising from loss of $[\cdot CH_2H_2]$ and $[\cdot CH_2H_2]$ 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 $^1$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 clausozoline-P has the structure 6.

Structure of Clausozoline-G (7) Clausozoline-G (7) was obtained as a yellow powder. The HR-MS analysis indicated the molecular formula to be $C_{18}H_{15}N_2O_3$, a difference of $H_2O$ compared with 5. The UV spectrum and IR bands (see Experimental) suggested the presence of a carbozole skeleton. 3,5 The $^1$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, $J=9.99$ Hz), 7.18 (1H, d, $J=9.99$ Hz), 1.56 (6H, s)], instead of signals due to the 2,2-dimethyl-3-hydroxy-dihydropyran ring. On the basis of these spectral data, we propose the structure 7 for clausozoline-G.

Structure of 5-Geranyloxy-7-hydroxyxocarmin (8) This compound (8) was isolated as a colorless powder. The molecular formula was determined as $C_{19}H_{25}O_4$ by HR-MS. The UV spectrum ($\lambda_{max} nm$: 211, 226 (sh), 250, 257, 331) was similar to that of 5,7-dihydroxycarmin (10, 15) and IR bands appeared at $\nu_{max}$ 3247 and 1718 cm$^{-1}$ (a hydroxyl group and an $\alpha$, $\beta$ unsaturated lactone). The $^1$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, 10 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 $^1$H-NMR spectrum, coupled with two characteristic ions at $m/z$ 245 and 191 arising from loss of $[\cdot CH_2H_2]$ and $[\cdot CH_2H_2]$ 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 $[-CH_2CH=C(CH_3)-CH_2CH_2CH=C(CH_3)_3]$ 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$_3$) 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-hydroxyxocarmin 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, 16 clausine-L, 17 heptazoline, 18 2-hydroxy-3-formyl-7-methoxyxocarmin, 19 3-formyl-2,7-dimethoxyxocarmin, 19 heptaphylline, 20 and 7-methoxyheptaphylline 18 by comparisons of the $^1$H-NMR and IR data with those reported in the literature. 14, 16, 20

Experimental

Melting points were measured on a microtempering point hot-stage apparatus (Yanagimoto). 1H-NMR spectra were recorded on an A-400 (JEOL) spectrometer in 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 UV-6100C double-beam spectrophotometer (JASCO) in methanol; IR spectra on an IR-230 (JASCO) in CHCl$_3$, and optical rotations on a DIP-370 (JASCO) in CHCl$_3$, at 25°C. Preparative TLC was done on Kieselgel 60 F$_{254}$ (Merck).

Extraction and Isolation

The dried stem bark (300g) of Clausena excavaata 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-isopro-O (1:1); isopro-O-EtOAc (4:1); isopro-O-EtOAc (7:3); isopro-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, CH$_2Cl_2$, isopro-O, benzene, CHCl$_3$, and acetone as developing solvents to give seven new carboxyls and a new coumarin, as well as seven known carboxyls, as stated below. From the n-hexane-isopro-O (1:1) eluate: clausozoline-A (1) (1.5 mg), clausozoline-B (2) (0.6 mg), and heptaphylline (1.0 mg). From the isopro-O-EtOAc (4:1) eluate: clausozoline-D (4) (1.2 mg), clausozoline-E (5) (23 mg), clausozoline-F (6) (3.6 mg), clausozoline-G (7) (3.0 mg), 2,3-dihydroxy-3-formyl-1-(3'-methyl-2'-butenyl)carbazole (1.2 mg), heptaphylline (89 mg), 2,3-dihydroxy-3-formyl-7-methoxyxocarmin (2.2 mg), 7-methoxyheptaphylline (1.5 mg), and 5-geranyloxy-7-hydroxyxocarmin (8) (1.5 mg). From the isopro-O-EtOAc (7:3) eluate: clausozoline-C (3) (1.0 mg) and 3-formyl-2,7-dimethoxyxocarmin (1.0 mg). Known components were fully characterized by comparisons of the $^1$H-NMR and IR data with those reported in the literature. 14, 16, 20

Clausozoline-A (1) Pale yellow powder. UV $\lambda_{max}$ nm: 204, 240, 265, 306, 375. IR $\nu_{max}$ cm$^{-1}$: 3450, 3440 (br), 1630. EI-MS m/z (%): 361 (M$^+$, 50), 346 (100), 318 (12), 3069, 2932, 292 (23), 246 (9), 224 (14), 2291, 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' ($\delta 6.68$) gave 6% NOE at H-6 ($\delta 6.90$). HR-MS Calcd for C$_{23}$H$_{25}$O$_5$: 361.1676. Found: 361.1651.

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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. 

References and Notes