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# CARBAZOLE ALKALOIDS FROM STEM BARK OF *CLAUSENA EXCAVATA*

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**Key Word Index**—*Clausena excavata*; Rutaceae; stem bark; carbazole alkaloids; clausines A, C, G and J.

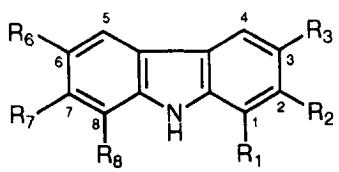
**Abstract**—Four new carbazole alkaloids, clausines A, C, G and J were identified as 3-formyl-2-hydroxy-8-methoxycarbazole 3-carbomethoxy-7-methoxycarbazole, 3-carbomethoxy-1-hydroxy-6-methoxycarbazole and 3-formyl-1, 7-dihydroxy-6-methoxycarbazole, respectively, from a methanol extract of the stem bark of *Clausena excavata*. Structures were established by spectroscopic analyses. Copyright © 1996 Elsevier Science Ltd

## INTRODUCTION

In a previous paper, we reported the isolation and antiplatelet aggregating action of carbazole alkaloids from the stem bark of *Clausena excavata* [1]. An extensive reinvestigation of the isolated unknown compounds **a**(**1**), **b**(**2**), **d**(**3**) and **e**(**4**) led to the identification of their structures as clausines A, C, G and J, respectively. We describe the structural elucidation of these new carbazole alkaloids.

## RESULTS AND DISCUSSION

Clausine A (**1**) was determined to have the molecular formula $C_{14}H_{11}NO_3$ by high-resolution mass spectrometry. The UV spectrum, with absorption bands at 202, 218, 241, 268 (sh), 275, 288 and 352 nm, and the IR spectrum, with bands at 3400 and 3225 $cm^{-1}$, indicated that the structure of compound **1** was very close to the carbazole alkaloid, mukonal (**5**) [2]. In the $^1H$ NMR spectrum, a characteristic aldehydic signal at $\delta$ 9.99 appeared on C-3, as well as a phenolic hydroxyl at $\delta$ 11.42 located on C-2 shifted to lowerfield, indicating intramolecular hydrogen bonding. The presence of a 3-CHO and a 2-OH caused the chemical shift of the neighbouring proton H-1 ($\delta$ 6.94) to move upfield and H-4 ($\delta$ 8.44) to shift downfield in the aromatic region. Except for these two singlets for ring-C protons, there were three mutually coupled proton signals at $\delta$ 6.99 (1H, *d*, $J$ = 8.0 Hz, H-7), 7.17 (1H, *t*, $J$ = 8.0 Hz, H-6) and 7.68 (1H, *d*, $J$ = 8.0 Hz, H-5) assignable for H-7, H-6 and H-5 in ring-A, respectively. The remaining substituent on C-8 was found to be a methoxyl group because of the presence of a three-proton singlet at $\delta$ 3.99. On the basis of the above evidence, the structure 3-formyl-2-hydroxy-8-methoxycarbazole (**1**) was deduced for clausine A.




| | $R_1$ | $R_2$ | $R_3$ | $R_6$ | $R_7$ | $R_8$ |
|---|---|---|---|---|---|---|
| **1** | H | OH | CHO | H | H | OMe |
| **2** | H | H | $CO_2Me$ | H | OMe | H |
| **3** | OH | H | $CO_2Me$ | OMe | H | H |
| **4** | OH | H | CHO | OMe | OH | H |
| **5** | H | OH | CHO | H | H | H |
| **6** | OH | H | CHO | OMe | H | H |

Clausine C (**2**) had the molecular formula $C_{15}H_{13}NO_3$. In addition to two close methoxyls at $\delta$ 3.87 and 3.89, two sets of ABX-pattern signals at $\delta$ 6.87 (*dd*, $J$ = 8.6, 2.2 Hz), 7.08 (*d*, $J$ = 2.2 Hz) and 8.09 (*d*, $J$ = 8.6 Hz); 7.49 (*d*, $J$ = 8.1 Hz), 7.98 (*dd*, $J$ = 8.1, 1.5 Hz) and 8.68 (*d*, $J$ = 1.5 Hz) appeared in the aromatic region of its $^1H$ NMR spectrum. The lower field shift signals in the latter set and the IR absorption at 1700 $cm^{-1}$, as well as the mass spectral fragment ion at $m/z$ 196 ($[M - CO_2Me]^+$, indicated the presence of a carbomethoxyl substituent on C-3. The other methoxyl on C-7 was confirmed by the upfield shift signal of H-8 ($\delta$ 7.08) and the downfield shift signal of H-5 ($\delta$ 8.09) in the former set. Consequently, the structure of 3-carbomethoxy-7-methoxycarbazole (**2**) was deduced for clausine C.

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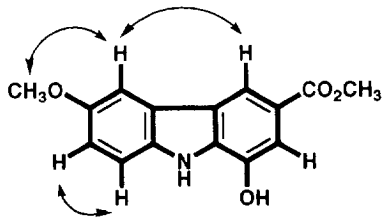


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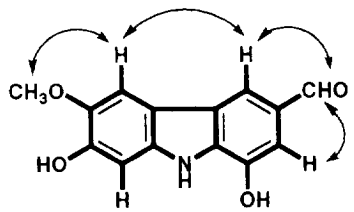


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Fig. 1. NOESY spectra of compounds **3** and **4**.

Clausine G (**3**) was found to have the molecular formula $C_{15}H_{13}NO_4$. In the aromatic region of the $^1H$ NMR spectrum, the chemical shifts and splitting patterns were very similar to those of clausine I (**6**) [1]. A pair of *meta*-coupled protons at $\delta$ 7.55 (*d*, $J$ = 1.4 Hz) and 8.36 (*d*, $J$ = 1.4 Hz) for H-2 and H-4, respectively, and ABX protons at $\delta$ 7.07 (*dd*, $J$ = 8.5, 2.4 Hz), 7.51 (*d*, $J$ = 8.5 Hz), 7.75 (*d*, $J$ = 2.4 Hz) for H-7, H-8 and H-5, respectively, were present. In addition, a methoxyl at $\delta$ 3.92 on C-6 was supported by the presence of a NOE cross-peak between this signal and the signal at $\delta$ 7.75 (H-5) (Fig. 1). The presence of a carbomethoxyl group on C-3 was proved by the existence of a methoxyl signal at $\delta$ 3.89 in the $^1H$ NMR spectrum, a carbonyl signal at $\delta$ 168.0 in the $^{13}C$ NMR spectrum and a band at 1670 cm$^{-1}$ in the IR spectrum. Accordingly, the extra hydroxyl at $\delta$ 9.29 should be located on C-1. The full assignments of $^1H$ and $^{13}C$ NMR signals were further confirmed by the $^1H$–$^{13}C$ long-range correlations in the HMBC spectrum (Fig. 2). Therefore, the above spectral data indicated the structure of clausine-G (**3**) as 3-carbomethoxy-1-hydroxy-6-methoxycarbazole.

Clausine J (**4**) had the molecular formula $C_{14}H_{11}NO_4$. From the $^1H$ NHR spectrum, the 3-CHO ($\delta$ 9.94) and 1-OH ($\delta$ 9.31) signals, together with those of two meta-coupled protons, H-2 ($\delta$ 7.30) and H-4 ($\delta$ 8.10), enabled the partial structure 1-hydroxy-3-formyl on ring-C to be deduced. This was supported by the aldehyde signal showing NOEs with the two protons, H-2 and H-4 (Fig. 1). Hence, the two lone singlets at $\delta$ 7.09 and $\delta$ 7.74 were attributed to H-8 and H-5, respectively. The remaining two substituents, a hydroxyl at $\delta$ 7.80 and a methoxyl at $\delta$ 3.98, were found in the $^1H$ NMR spectrum. The existence of NOEs between the methoxyl and H-5 suggested that this methoxyl was attached to C-6, whereas the hydroxyl was located on C-1 (Fig. 1). On the basis of the foregoing spectral analyses, the structure of clausine J (**4**) was established as 3-formyl-1,7-dihydroxy-6-methoxycarbazole.

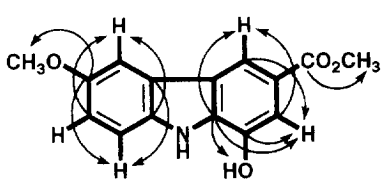


Fig. 2. H–$^{13}C$ long-range correlations in HMBC spectrum of compound **3**.

## EXPERIMENTAL

*General.* Mps: uncorr. UV: MeOH. IR: KBr. $^1H$ and $^{13}C$ NMR: $Me_2CO$-$d_6$ with TMS as int. ref., except where noted. MS: direct inlet.

*Plant material, extraction and isolation.* The isolation procedure was as described in the ref. [1] Four unidentified compounds **a, b, d** and **e** from the MeOH extract of the stem bark of C. *excavata* were determined as **1, 2, 3** and **4**.

*Clausine A* (**1**). Yellowish needles ($Me_2CO$), mp 184–186°. HR-MS: calcd for $C_{14}H_{11}NO_3$, *m/z* 241.0739 $[M]^+$, found 241.0738. UV $\lambda_{max}$ nm (log $\varepsilon$): 202 (4.27), 218 (4.33), 241 (4.52), 268 (4.42, sh), 275 (4.53), 288 (4.38), 352 (4.04). IR $\nu_{max}$ cm$^{-1}$: 3400, 3225, 1675, 1650, 1625, 1590. EI-MS *m/z* (rel. int.): 241 ($[M]^+$, 100), 226, (32), 198 (52), 141 (11), $^1H$ NMR: $\delta$ 3.99 (3H, *s*, 8-OMe), 6.94 (1H, *s*, H-1), 6.99 (1H, *d*, $J$=8.0 Hz, H=7), 7.17 (1H, *t*, $J$=8.0 Hz, H-6), 7.68 (1H, *d*, $J$=8.0 Hz, H-5), 8.44 (1H, *s*, H-4), 9.99 (1H, *s*, CHO), 10.85 (1H, *br s*, NH), 11.42 (1H, *s*, 2-OH).

*Clausine C* (**2**). Yellowish needles ($Me_2CO$), mp 195–197°. HR-MS: calcd for $C_{15}H_{13}NO_3$, *m/z* 255.0895 $[M]^+$, found 255.0891. UV $\lambda_{max}$ nm: 220, 239 (sh), 249, 282, 308 (sh), 320 (sh). IR $\nu_{max}$ cm$^{-1}$: 3280, 1700, 1605. EI-MS *m/z* (rel. int): 255 ($[M]^+$, 100), 240 (21), 224 (39), 212 (24), 196 (17), 181 (12). $^1H$ NMR: $\delta$ 3.87 and 3.89 (each 3H, *s*, 3-$CO_2$Me and 7-OMe), 6.87 (1H, *dd*, $J$=8.6, 2.2 Hz, H-6), 7.08 (1H, *d*, $J$=2.2 Hz, H-8), 7.49 (1H, *d*, $J$=8.1 Hz, H-1), 7.98 (1H, *dd*, $J$=8.1, 1.5 Hz, H-2), 8.09 (1H, *d*, $J$=8.6 Hz, H-5), 8.68 (1H, *d*, $J$=1.5 Hz, H-4) 10.65 (1H, *s*, NH).

*Clausine G* (**3**). Granules ($Me_2CO$), mp>280°. HR-MS: calcd for $C_{15}H_{13}NO_4$, m/z 271.0847 $[M]^+$, found 271.0845. UV $\lambda_{max}$ nm: 224, 272, 282, 316 (sh), 353 (sh). IR $\nu_{max}$ cm$^{-1}$: 3350, 1690, 1670. EI-MS *m/z* (rel. int.): 271 ($[M]^+$, 100) 256 (71), 240 (15). $^1H$ NMR: $\delta$ 3.89 (3H, *s*, 3-OMe), 3.92 (3H, *s*, 6-OMe), 7.07 (1H, *dd*, $J$=8.5, 2.4, Hz, H-7), 7.51 (1H, *d*, $J$=8.5 Hz, H-8), 7.55 (1H, *d*, $J$=1.4 Hz, H-2), 7.75 (1H, *d*, $J$=2.4 Hz, H-5), 8.36 (1H, *d*, $J$=1.4 Hz, H-4), 9.29 (1H, *br s*, 1-OH), 10.50 (1H, *s*, NH). $^{13}C$ NMR: $\delta$ 51.9 (*q*, 3-OMe), 56.1 (*q*, 6-OMe), 103.7 (*d*, C-5), 111.2 (*d*, C-2), 113.2 (*d*, C-8), 115.7 (*d*, C-4), 116.6 (*d*, C-7), 122.0 (*s*, C-4a), 125.0 (*s*, C-3 and C-5a), 134.4 (*s*, C-1a), 136.1

(*s*, C-8a), 143.5 (*s*, C-1) 155.3 (*s*, C-6), 168.0 (*s*, C=O).

*Clausine J* (**4**). Powder ($Me_2CO$), mp>290°. HR-MS: calcd for $C_{14}H_{11}NO_4$, *m/z* 257.0688 $[M]^+$, found 257.0689. UV $\lambda_{max}$ nm: 235, 255 (sh), 289, 305, 347 (sh). IR $\nu_{max}$ $cm^{-1}$: 3430, 1650. EI-MS *m/z* (rel. int.): 257 ($[M]^+$, 80), 242 (100), 214 (36), 57 (17). $^1H$ NMR: $\delta$ 3.98 (3H, *s*, 6-OMe), 7.09 (1H, *s*, H-8), 7.30 (1H, *d*, *J*=1.0 Hz, H-2), 7.74 (1H, *s*, H-5), 7.80 (1H, *br s*, 7-OH), 8.10 (1H, *br s*, H-4), 9.31 (1H, *br s*, 1-OH), 9.94 (1H, *s*, 3-CHO), 10.46 (1H, *br s*, NH).

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