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Carbazole-Pyranocoumarin Dimer and Binary Carbazole Alkaloid from *Clausena excavata*

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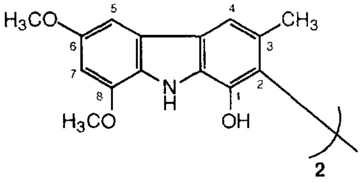
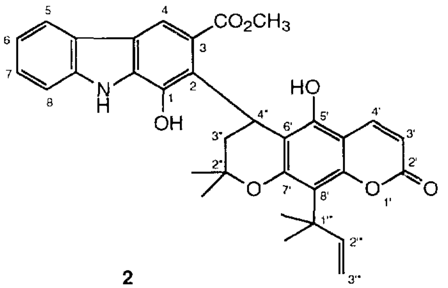
Abstract. Examination of the extract from the stem bark and root bark of *Clausena excavata*, a binary carbazole alkaloid, clausenamine-A (**1**), and a carbazole-pyranocoumarin dimer, carbazomarin-A (**2**), were isolated, respectively. Their structures were elucidated by spectroscopic analyses. Copyright © 1996 Elsevier Science Ltd

We have reported the isolation and the pharmacological evaluation of many carbazole alkaloids from the leaves, stem barks and root barks of *Clausena excavata*.[1-4] In the continuing search for bioactive components from natural source, a binary carbazole alkaloid, clausenamine-A (**1**), and a carbazole-pyranocoumarin dimer, carbazomarin-A (**2**), were isolated from the stem and root bark of *C. excavata*, respectively. Here we deal with the structural elucidation of **1** and **2**.

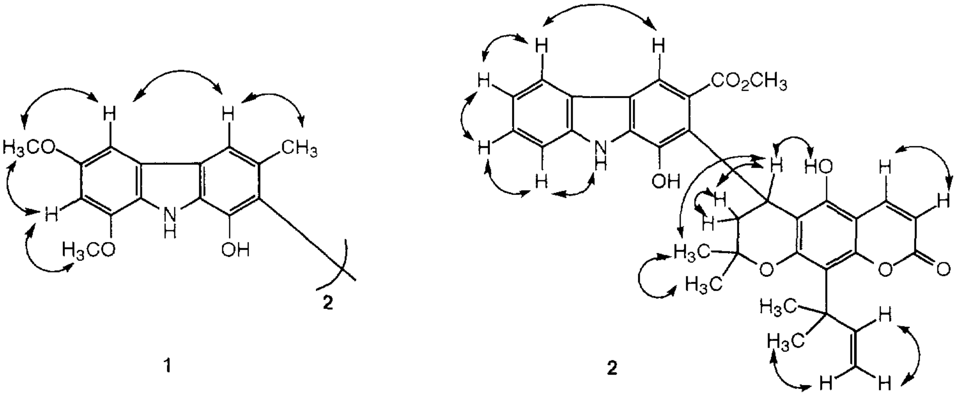


1



2

Clausenamine-A (**1**),[5] colorless granules, was isolated from the methanol extract of the stem barks of *C. excavata* in (0.00007%) yield. The molecular formula $C_{30}H_{28}N_2O_6$ was established by high resolution mass spectrometry ([M$^+$]: calcd 512.1948, found 512.1947). The UV maxima at 218, 235, 257, 307 and 343 nm together with the ^{1}H NMR singlets at δ 2.44 and 8.95 typically for aromatic methyl and carbazole NH, respectively, indicated the presence of a 3-methylcarbazole nucleus in the structure formula of **1**. Even number of molecular weight and the diagnostic ion peak at m/z 256, half of the molecular weight, in the mass spectrum as well as only one set of carbazole signals in the ^{1}H NMR spectrum suggested that its structure should be a highly symmetrical carbazole alkaloid dimer. Examining the ^{1}H NMR spectrum of **1**, a lowfield singlet at δ 7.81 was assigned for H-4, meta-coupled protons at δ 6.45 and 7.13 (*d*, *J* = 2.0 Hz) were attributed to H-7 and H-5, respectively, which were supported by the existence of NOE of the signal at δ 7.81 (H-4) with the signal at δ 7.13 (H-5) (Figure 1). Two methoxyls at δ 3.77 and 3.82 located at C-8 and C-6, respectively, on the basis of the NOEs for the methoxyl at δ 3.77 to H-7 along with the other methoxyl at δ 3.82 and H-5, H-7 (Figure 1). In addition, the signal at δ 7.33 which showed D_2O exchange suggested for a hydroxyl substituted on C-1 or C-2. In order to determine the regiochemistry of this substituent, the HMBC experiment was acquired (Figure 2). The ^{1}H-^{13}C long range correlation between the NH (δ 8.95) and a carbon signal at δ 152.2 whose lowerfield chemical shift indicated the hydroxyl group must sited at C-1. Thus the C-2 carbon-carbon linkage between two carbazole moieties completed the structure of **1** for clausenamine-A.



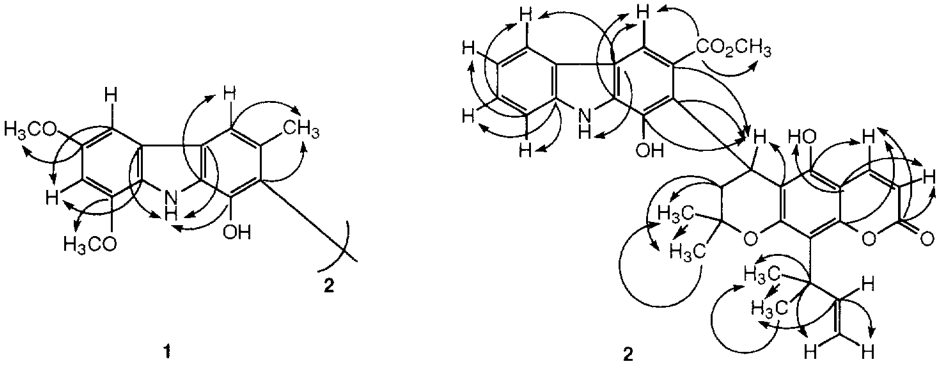
1 **2**

Figure 1. The NOESY spectral data of **1** and **2**

Carbazomarin-A (**2**),[5] an optically active yellow powder, was isolated from the acetone extract of the root barks of *C. excavata* with the molecular formula as $C_{33}H_{31}NO_7$ in (0.00005%) yield. The UV spectrum (absorptions at 215, 240, 253, 270, 326, 338, 356 nm) and the IR spectrum (bands at 3400, 1670 cm^{-1}) revealed that compound **2** consisted of a 3-carbonyl carbazole alkaloid unit. In the aromatic region of the ^{1}H NMR spectrum, there were four mutually coupled protons at δ 7.26 (1H, *m*), 7.43 (2H, *m*) and 8.03 (1H, *d*, *J* = 8.0 Hz) assignable for a nonsubstituted ring A; a characteristic peak at δ 8.21 attributable to H-4 in this carbazole moiety. The singlet pattern of the latter peak suggested that only C-4 was not substituted in ring C. The ^{1}H-^{13}C long range correlations between the carbonyl carbon (δ 173.3) and H-4 (δ 8.21) or the methoxyl protons (δ 4.11) suggested a carbomethoxy substituent on C-3 (Figure 2). Rest of the signals in the olefinic and aromatic region were found very close to those of nordentatin as a 5'-hydroxy-8'-dimethylallyldimethyl-pyranocoumarin nucleus.[6] In contrast with nordentatin, one double bond in pyran ring was reduced to single bond which presented a -CH_2CH- type protons at δ 2.11 and 2.25 (*dd*, *J* = 13.8, 9.7 Hz and 13.8, 10.4 Hz, H-3"), 5.17 (*dd*, *J* = 10.4, 9.7 Hz, H-4"). Two benzene rings adjacent to H-4" gave rise to a downfield signal of

H-4". The observed NOE between H-4" (δ 5.17) and 5'-OH (δ 9.38) as well as the long range ^{1}H-^{13}C correlations for H-4" with C-1 (δ 140.6), C-2 (δ 121.0) and C-3 (δ 125.5) supported a carbon-carbon linkage between C-2 and C-4" in the carbazole and dihydropyranocoumarin units (Figure 1 and 2). The cyclohexene-like dihydropyran ring caused two large coupling constants in pseudo-axial and pseudo-equatorial H-3"s with H-4" (9.7 and 10.4 Hz).[7] However, only one of the upfield signals in H-3" and 2"-Me showed NOEs with H-4", which suggested that the orientation of H-4" is located toward axial direction. It also suggests that a hydroxyl at δ 5.76 on C-1 caused the lowfield shift of this carbon. The chemical shift of C-1 in **2** is not as lowfield as that in **1** (δ 140.6 in **2** vs. 152.2 in **1**) due to the anisotropic effect of a electron-withdrawing group CO_2CH_3 at C-3. Finally this kind of dimer was further proved by the strong MS peak at m/z 241 for dihydropyranocoumarin nucleus minus a hydrogen and the fragment peak at m/z 312 for carbazole nucleus plus a hydrogen. The full ^{13}C NMR chemical shift assignments were made with NOESY and HMBC spectral analyses. Based on the above results, the structure of carbazomarin-A was deduced as **2**.

The carbon-carbon linkage between carbazole alkaloid and coumarin was found for the first time in nature, though we have isolated several bis- or dimeric carbazole alkaloids from *Murraya euchrestifolia*.[8]



1   **2**

Figure 2. The HMBC spectral data of 1 and **2**

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5. **Clausenamine-A** (**1**): Colorless granules, mp 278—280 °C. HRMS for $C_{30}H_{28}N_2O_6$: calcd 512.1948, found 512.1947. UV $\lambda_{\max}^{MeOH}$ nm: 218 (sh), 235, 257 (sh), 307, 343 (sh). IR $\upsilon_{\max}^{KBr}$ cm^{-1}: 3380, 1590, 1505. EIMS *m/z* (rel. int.) 512 (M^+, 75), 497 (7), 482 (9), 256 (15), 115 (25), 91 (30), 69 (48), 53 (100). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.39 (6H, s, 2 x Me), 3.77 (6H, *s*, 2 x 8-OMe), 3.82 (6H, *s*, 2 x 6-OMe), 6.45 (2H, *d*, J = 2.2 Hz, 2 x H-7), 7.13 (2H, *d* J = 2.2 Hz, 2 x H-5), 7.33 (2H, *br. s*, 2 x 1-OH), 7.81 (2H, *s*, 2 x H-4), 8.95 (2H, *s*, 2 x NH). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 17.8 (q, 2 x Me), 55.4 (q, 2 x 8-OMe), 55.8 (q, 2 x 6-OMe), 94.0 (d, 2 x C5), 95.7 (d, 2 x C7),104.7 (s, 2 x C3), 116.4 (s, 2 x C4a), 117.5 (s, 2 x C2), 120.3 (d, 2 x C4), 124.3 (s, C8a), 124.4 (s, C5a), 139.5 (s, C1a), 145.8 (s, C8), 152.2 (s, C1), 154.1 (s C6).

   Carbazomarin-A (**2**): Yellow powder, mp 238—239 °C. [α] - 27.03° (c, 0.0259, CHCl$_3$). HRMS for $C_{33}H_{31}NO_7$: calcd 553.2101, found 553.2101. UV $\lambda_{\max}^{MeOH}$ (log ε) nm: 215 (4.47), 240 (4.40), 253 (4.38, sh), 267 (4.58), 326 (4.12, sh), 338 (4.14), 356 (3.93, sh). IR $\upsilon_{\max}^{KBr}$ cm^{-1}: 3400, 1670, 1610, 1595. EIMS *m/z* (rel. int.) 553 (M^+, 3), 312 (36), 298 (20), 297 (100), 242 (15), 241 (98), 210 (55), 182 (17), 121 (29). ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.29 and 1.50 (each 3H, *s*, 2 x 2"-Me), 1.65 and 1.66 (each 3H, *s*, 2 x 1"'-Me), 2.11 and 2.25 (each 1H, *dd*, J = 13.8, 9.7 and 13.8, 10.4 Hz, 2 x H-3"), 4.11 (3H, *s*, CO$_2$Me), 4.82 and 4.86 (each 1H, *dd*, J = 10.6, 2.7 and 17.3, 2.7 Hz, H-3"'), 5.17 (1H, *dd*, J = 10.4, 9.7 Hz, H-4"), 5.76 (1H, *br. s*, 1-OH), 6.07 (1H, *d*, J = 9.6 Hz, H-3'), 6.25 (1H, *dd*, J = 17.3, 10.6 Hz, H-2"), 7.26 (1H, *m*, H-6), 7.43 (2H, *m*, H-7, and H-8), 7.98 (1H, *d*, J = 9.6 Hz, H-4'), 8.03 (1H, *d*, J = 8.0 Hz, H-5), 8.21 (1H, *s*, H-4), 8.63 (1H, *br. s*, NH), 9.38 (1H, *s*, 5'-OH). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 22.7 and 29.3 (q, 2 x 2"-Me), 29.0 (d, C4"), 29.6 and 30.1 (q, 2 x 1"'-Me), 40.2 (t, C3"), 41.2 (s, C1"'), 53.4 (q, OMe), 77.0 (s, C2"), 104.7 (s, C4'a), 106.4 (s, C6'), 108.0 (t, C3"'), 110.0 (d, C3'), 111.3 (d, C8), 115.6 (s, C8'), 116.5 (d, C4), 120.4 (d, C6), 120.5 (d, C5), 121.0 (s, C2), 122.8 (s, C4a), 123.3 (s, C5a), 125.5 (s, C3), 126.8 (d, C7), 133.5 (s, C1a), 139.8 (s, C8a), 140.2 (d, C4'), 140.6 (s, C1), 150.3 (d, C2"'), 151.9 (s, 5'), 153.4 (s, C8'a), 156.5 (s, C7'), 161.8 (s, 2'-CO), 173.3 (s, 3-CO).

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