

PYRANO- AND FUROCARBAZOLE ALKALOIDS FROM THE ROOT BARK OF *CLAUSENA EXCAVATA*

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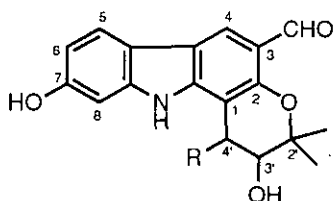
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Abstract -- Two pyranocarbazole alkaloids, clausine-W (1) and clausine-T (2), as well as two furocarbazole alkaloids, furoclausine-A (3) and furoclausine-B (4), were isolated and identified from the acetone extract of the root bark of *Clausena excavata*. Their structures have been elucidated by spectroscopic analyses.

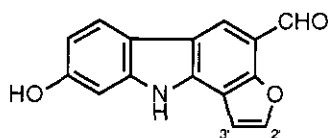
INTRODUCTION

We have reported the isolation of a strong antibacterial compound along with some coumarins and carbazole alkaloids from the root bark of *Clausena excavata* Brum. f.^{1,2} After extensive investigation about the separation of the acetone extract of the same part, two pyranocarbazole alkaloids, clausine-W (1) and clausine-T (2), as well as two furocarbazole alkaloids, furoclausine-A (3) and furoclausine-B (4), were obtained. Here we report the structural elucidation of the four new carbazole alkaloids by spectroscopic analyses.

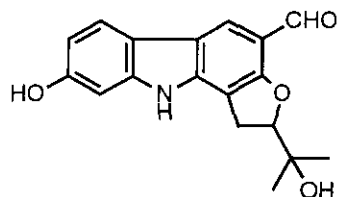


1: R = *trans*-OH

2: R = H



3



4

RESULTS AND DISCUSSION

Clausine-W (**1**) was isolated as optically active yellow granules. The FABMS exhibited a pseudo molecular ion at m/z 328 for $[M + 1]^+$ which suggested the molecular formula as $C_{18}H_{17}NO_5$. The IR carbonyl absorption at 1630 cm^{-1} , the ^1H NMR aldehydic signal at δ 10.41 together with the UV bands at 202, 224, 237, 243, 250, 287, 301, 349 nm indicated that compound (**1**) would be a 2,7-dioxygenated 3-formylcarbazole alkaloid (Table 1).³ In the aromatic region of the ^1H NMR spectrum, a set of ABC type signals at δ 6.76 (*dd*, $J = 8.4, 2.2\text{ Hz}$), 7.03 (*d*, $J = 2.2\text{ Hz}$) and 7.88 (*d*, $J = 8.4\text{ Hz}$) typically arise from H-6, H-8 and H-5, respectively. An additional singlet at δ 8.26 was assigned for H-4. Two D_2O exchangeable signals at δ 10.13 and 8.41 could be attributed to a carbazole NH and a phenolic OH at C-7, respectively. In the heteroaliphatic region, there were four mutually coupled protons at δ 3.81 (*dd*, $J = 7.5, 5.2\text{ Hz}$), 4.58 (*d*, $J = 7.0\text{ Hz}$), 4.86 (*d*, $J = 5.2\text{ Hz}$) and 4.92 (*dd*, $J = 7.5, 7.0\text{ Hz}$). The middle two signals at δ 4.58 and 4.86 showed D_2O exchangeable property and the other two protons at δ 3.81 and 4.92 exhibited an axial-axial coupling constant of 7.5 Hz with each other which indicated the presence of a *trans*-diol, $-\text{CH}(\text{OH})\text{CH}(\text{OH})-$, moiety.⁴ The existence of two methyl singlets at δ 1.31 and 1.55 can be attached to a tertiary carbon bearing an oxygen functionality. Based on the above analyses, a *trans*-2',2'-dimethyl-3',4'-dihydroxydihydropyrano ring was suggested to be fused to this carbazole nucleus using the bond between C-1 and C-2. The complete assignments of the ^1H and ^{13}C NMR spectra were further confirmed by HMQC, HMBC and NOESY experiments (Figures 1 and 2). Consequently, the structure of the pyranocarbazole **1** was deduced for clausine-W.

Clausine-T (**2**), an optically active compound, was determined to have the molecular formula as $C_{18}H_{17}NO_4$ by HRMS spectrometry. The spectral data including UV, IR, and ^1H NMR were almost the same as those for **1**, suggesting a 3-formyl-7-hydroxypyranocarbazole alkaloid (Table 1). The only difference was an aliphatic proton in **2** replaced the hydroxyl signal in **1**. The location of upshifted two protons at δ 2.88 (*dd*, $J = 16.0, 8.0\text{ Hz}$) and 3.21 (*dd*, $J = 16.0, 6.0\text{ Hz}$) was attributed to H-4' which was proved by the presence of NOEs of this methylene with carbazole NH (δ 10.35) (Figure 1). Hence, the pyranocarbazole structure of **2** was assigned for clausine-T.

Furoclausine-A (**3**) possessed the molecular formula as $C_{15}H_9NO_3$ by HRMS spectrometry. The basic carbazole moiety was the same as that of **1** and **2** in 1-alkyl-2-alkoxy-3-formyl-7-hydroxycarbazole by the comparison of the spectral data (Table 1). The apparent difference was the disappearance of all aliphatic signals and the appearance of two extra aromatic doublets at δ 7.27 and 7.99 (*d*, $J = 2.3\text{ Hz}$). The characteristic small vicinal coupling constant suggested a furan ring fused to the carbazole. The NOEs of the signal at δ 8.48 (7-OH) with those at δ 6.87 (H-6) and 7.04 (H-8), the signal at δ 8.46 (H-4) with those at δ 8.04 (H-5) and 10.42 (3-CHO), as well as the 3J long range ^{13}C - ^1H correlations of the carbon at δ 119.4 (C-1) with two aromatic protons at δ 7.27 (H-3') and 7.99 (H-2') further supported the regiochemistry of these substituents. The full assignments of the ^1H and ^{13}C NMR signals were confirmed by HMQC, HMBC and NOESY spectra (Figures 1 and 2). Therefore, these spectral data afforded the

structure of furoclausine-A as **3**.

Furoclausine-B (**4**), another optically active yellow oil, have the molecular formula $C_{18}H_{17}NO_4$ by FABMS spectrometry. From the 1H NMR spectrum, the carbazole skeleton is the same as that of **1-3** in 3-formyl at δ 10.26, 7-hydroxyl at δ 8.45, H-4 at δ 8.13 and an ABC protons at δ 6.76 (*dd*, $J = 8.4, 2.1$ Hz, H-6), 6.91 (*d*, $J = 2.1$ Hz, H-8) and 7.88 (*d*, $J = 8.4$ Hz, H-5). In addition, two diastereotopic protons for a methylene at δ 3.40 (*d*, $J = 8.3$ Hz) and 3.41 (*d*, $J = 8.0$ Hz) coupled with an oxygenated signal at δ 4.92 (1H, *dd*, $J = 8.3, 8.0$ Hz), two dimethyl singlets at δ 1.21 and a hydroxyl at δ 3.60 were organized into a (2-hydroxyisopropyl) dihydrofuran ring. Consequently, furoclausine-B possessed the structure as **4**.

Table 1. 1H NMR spectral data for compounds (**1-4**) (acetone- d_6 , δ , multiplicity, J , Hz)

	1	2	3	4
3-CHO	10.41 <i>s</i>	10.44 <i>s</i>	10.42 <i>s</i>	10.26 <i>s</i>
H-4	8.26 <i>s</i>	8.19 <i>s</i>	8.46 <i>s</i>	8.13 <i>s</i>
H-5	7.88 <i>d</i> (8.4)	7.86 <i>d</i> (8.0)	8.04 <i>d</i> (8.0)	7.88 <i>d</i> (8.4)
H-6	6.76 <i>dd</i> (8.4, 2.2)	6.76 <i>dd</i> (8.0, 2.2)	6.87 <i>dd</i> (8.0, 2.2)	6.76 <i>dd</i> (8.4, 2.1)
7-OH	8.41 <i>s</i>	8.46 <i>s</i>	8.48 <i>s</i>	8.45 <i>s</i>
H-8	7.03 <i>d</i> (2.2)	6.92 <i>d</i> (2.2)	7.04 <i>d</i> (2.2)	6.91 <i>d</i> (2.1)
2',2'-dimethyl or H-2'	1.31 and 1.55 <i>s</i>	1.41 and 1.50 <i>s</i>	7.99 <i>d</i> (2.3)	4.92 <i>dd</i> (8.3, 8.0)
2'-methylhydroxyethyl	—	—	—	1.21 <i>s</i> (2xMe) and 3.60 <i>s</i> (OH)
H-3' and/or 3'-OH	3.81 <i>dd</i> (7.5, 5.2) 4.86 <i>d</i> (5.2, OH)	3.99 <i>dd</i> (8.0, 6.0) 4.51 <i>br s</i> (OH)	7.27 <i>d</i> (2.3)	3.40 <i>d</i> (8.3), 3.41 <i>d</i> (8.0)
H-4' and/or 4'-OH	4.92 <i>dd</i> (7.5, 7.0) 4.58 <i>d</i> (7.0, OH)	2.88 <i>dd</i> (16.0, 8.0) 3.21 <i>dd</i> (16.0, 6.0)	—	—
NH	10.13 <i>br s</i>	10.35 <i>br s</i>	11.10 <i>br s</i>	10.36 <i>br s</i>

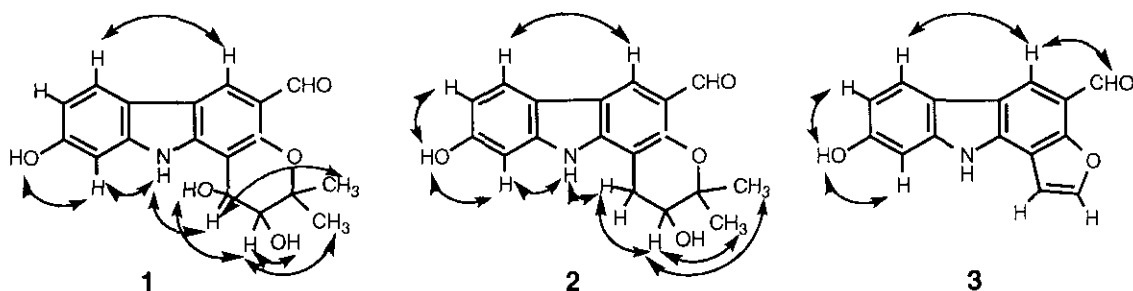


Figure 1. The NOESY spectra of compounds (**1-3**)

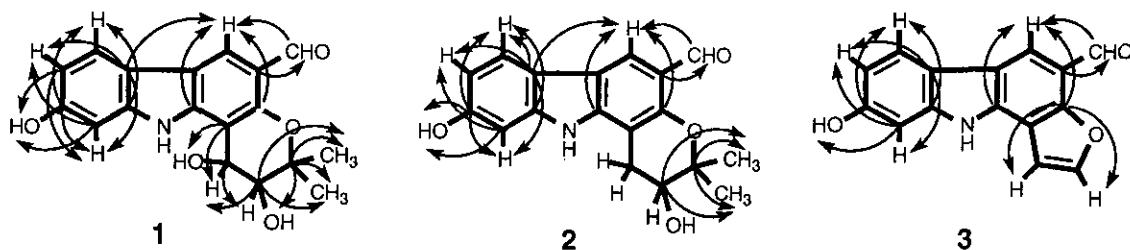


Figure 2. The HMBC spectra of compounds (1-3)

EXPERIMENTAL

Melting point was measured on a Yanagimoto MP-S₃ micromelting point apparatus. The ¹H and ¹³C NMR spectra were recorded on Bruker AC-200, AMX-400 and Varian-400 Unity Plus spectrometers in acetone-*d*₆. Chemical shifts are shown in δ values with TMS as internal reference. MS were obtained on a VG 70-250 S spectrometer by direct inlet system. UV spectra were recorded on a Hitachi UV-3210 spectrophotometer in MeOH. IR spectra were recorded on a Jasco IR Report-100 spectrophotometer in KBr disc. Specific rotations were recorded on a Jasco DIP-370 polarimeter.

Plant Material

Clausena excavata Brum. f. was collected from San Dei Men, Pingtung Hsien, Taiwan in June 1989 and verified by Prof. C. S. Kuoh. The specimen of this plant is deposited in the herbarium of National Cheng Kung University, Tainan, Taiwan.

Extraction and Isolation

The acetone extract (98 g) of the root bark of *C. excavata* (0.8 kg) was subjected to silica gel column chromatography and eluted with CHCl₃-MeOH (25:1) and *i*-Pr₂O-MeOH-C₆H₁₄ (9:1:1) or CHCl₃-MeOH (30:1) to give **1** (10.8 mg), **2** (0.5 mg), **3** (5.8 mg) and **4** (0.3 mg). Several other carbazole alkaloids were also found which will be elucidated elsewhere.

Clausine-W (**1**)

Yellow granules, mp 178–179 °C (Me₂CO); $[\alpha]_D$: -3.41° (c 1.002, MeOH); HRMS 327.1108 (calcd for C₁₈H₁₇NO₅ 327.1107); UV λ_{\max} (log ϵ) 202 (4.35), 224 (sh, 4.34), 237 (sh, 4.45), 243 (4.48), 250 (sh, 4.47), 287 (sh, 4.48), 301 (4.65), 349 (4.04) nm; IR ν_{\max} 3350, 1630, 1590 cm⁻¹; MS *m/z* 327 (M⁺, 2%), 295 (100), 280 (17), 252 (21), 240 (84), 239 (26), 238 (913), 211 (17), 183 (21); FABMS *m/z* 328 (M⁺ + 1, 100%); ¹³C NMR δ 19.4 and 26.5 (2 x 2'-Me), 69.4 (C-4'), 76.8 (C-3'), 80.7 (C-2'), 98.5 (C-8), 107.4 (C-1), 110.2 (C-6), 117.1 (C-4b), 118.8 (C-4a), 118.9 (C-3 and C-4), 121.3 (C-5), 143.3 (C-8a), 144.7 (C-9a), 154.0 (C-2), 157.5 (C-7), 188.8 (3-CHO).

Clausine-T (2)

Yellowish granules, mp 155—158°C (Me₂CO); [α]_D: - 82.1° (c 0.0341, MeOH); HRMS 311.1159 (calcd for C₁₈H₁₇NO₄ 311.1158); UV λ_{\max} 202, 242, 249, 274, 285 (sh), 302, 345 nm; IR ν_{\max} 3400, 1610, 1590 cm⁻¹; MS m/z 311 (M⁺, 59%), 291 (34), 263 (16), 240 (100), 211 (27), 183 (51); ¹³C NMR δ 20.6 and 25.8 (2 x 2'-Me), 28.1 (C-4'), 69.1 (C-3'), 78.9 (C-2'), 98.1 (C-8), 103.8 (C-1), 110.1 (C-6), 116.5 (C-4a), 117.5 (C-4), 117.9 (C-4b), 119.6 (C-3), 121.4 (C-5), 143.4 (C-8a), 145.8 (C-9a), 153.6 (C-2), 157.4 (C-7), 188.9 (3-CHO).

Furoclausine-A (3)

Yellow oil; HRMS 251.0580 (calcd for C₁₅H₉NO₃ 251.0558); UV λ_{\max} 221, 239, 288 (sh), 301, 345 nm; IR ν_{\max} 3300, 2930, 1650, 1645, 1630 cm⁻¹; MS m/z 251 (M⁺, 100%), 250 (55), 222 (24), 194 (19), 139 (10); ¹³C NMR δ 98.3 (C-8), 104.7 (C-3'), 110.8 (C-6), 116.2 (C-3), 117.6 (C-4b), 119.4 (C-1 and C-4a), 119.8 (C-4), 121.6 (C-5), 137.8 (C-9a), 142.6 (C-8a), 146.0 (C-2'), 154.1 (C-2), 157.5 (C-7), 187.7 (3-CHO).

Furoclausine-B (4)

Yellow oil; [α]_D: - 32.73° (c 0.022, MeOH); UV λ_{\max} 202, 218 (sh), 231 (sh), 252, 275 (sh), 286 (sh), 301, 339 nm; IR ν_{\max} 3400, 2930, 1650, 1630, 1600 cm⁻¹; FABMS m/z 312 (M⁺ + 1, 10%).

ACKNOWLEDGMENT

We thank National Science Council, R. O. C. (NSC 81-0420-B-006-10) for support of this research.

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Received, 3rd March, 1997