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STRUCTURE AND SYNTHESIS OF CLAUSENAQUINONE-A. A NOVEL CARBAZOLEQUINONE ALKALOID AND BIOACTIVE PRINCIPLE FROM *CLAUSENA EXCAVATA*

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Abstract. A new carbazolequinone alkaloid, clausenaquinone-A, has been isolated from the stem bark of *Clausena excavata*. The structure has been established from spectral data and total synthesis. Clausenaquinone-A shows potent inhibitory activity of the rabbit platelet aggregation as well as cytotoxicity in HCT-8, RPMI-7951, and TE671 tumor cells.

Clausena excavata (Rutaceae), a plant widely distributed in southern Taiwan, is currently used as a folk medicine for the treatment of snakebite and abdominal pain and as a detoxification agent.[1] In previous papers, we have reported the isolation of carbazole alkaloids, coumarins, flavonoids, chlorophylls, steroids and triterpenoids from the leaves, roots and stem bark of this plant.[2-5] In the course of our continuing search for bioactive constituents from natural sources, we were interested in the stem bark constituents of *Clausena excavata* due to its antiplatelet activity and cytotoxicity. A further examination led to the isolation of thirty-two compounds from the stem bark of this plant.[6] We report herein on the structural elucidation of a new carbazole-5,8-quinone alkaloid, clausenaquinone-A (1), and the pharmacological evaluation of **1** and its isomer **4**.

Clausenaquinone-A was isolated as a brown powder with the molecular formula, $C_{14}H_{11}NO_4$, as determined from high resolution mass spectrometry (m/e M^+, 257.0688). Comparison of its UV absorptions [λ_{max} (CH_3OH) 200.8, 227.6, 265.2, 294.8, 320.0 and 419.2 nm] and IR bands [υ_{max} (KBr) 3370 (OH), 3260 (NH), 1660, 1625 and 1600 cm^{-1}] with those in the literature suggested that **1** contained a carbazole-5,8-quinone nucleus.[7,8] A tri-substituted carbazolequinone skeleton was further established from three low field signals in

the ^1H NMR spectrum. In order to confirm the position of the substituents, an HMBC (proton detected heteronuclear multiple-bond correlation) experiment, coupled with biogenetic consideration, was conducted. The result is shown in Fig. 1. In the ^1H NMR spectrum, two singlet protons in the aromatic region at δ7.04 and 8.17 and a singlet vinyl proton at δ5.78 were assigned to H-1, H-4 and H-7, respectively. The presence of a hydroxyl substituent at C-2 was shown by a D_2O-exchangeable signal at δ7.82. Two three-proton singlets at δ3.88 and 2.33 were assigned as OCH_3 and CH_3 groups attached at C-6 and C-3, respectively. On the basis of the above data, the structure of clausenaquinone-A was assigned as **1**. To confirm this proposal, a total synthesis of **1** was established as follows.

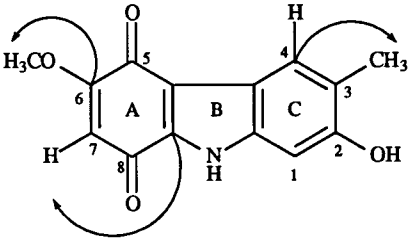
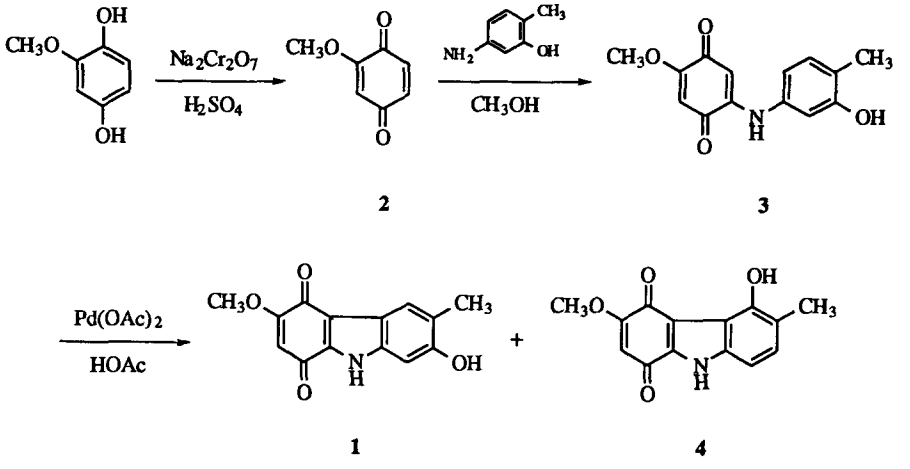


Figure 1. ^{13}C-^1H long range correlations from the HMBC experiment of clausenaquinone (**1**)

Methoxyquinone (**2**) was readily obtained from the oxidation of methoxyhydroquinone[9] with chromic acid. Then, **2** was condensed with 5-amino-o-cresol[9] in methanol at room temperature for 5 h to give 2-(4-methyl-3-hydroxyanilino)-5-methoxy-1,4-benzoquinone (**3**)[10] as violet crystals. Treatment of **3** with acetic acid in the presence of palladium acetate under reflux for 1.5 h led to the formation of two regioisomeric cyclized products, **1** and **4**,[11] in an almost 1:1 ratio. The two products were separated by column chromatography on silica gel eluting with CH_2Cl_2:CH_3OH (25:1). TLC, mixed mp and spectral (UV, IR, ^1H NMR and EIMS) comparison of this synthetic compound **1** with naturally occurring clausenaquinone-A showed that they are identical.



At 10 μg/mL, clausenaquinone-A (**1**) showed 100 ± 0.8% inhibition of the rabbit platelet aggregation induced by arachidonic acid (100 μM). Compound **1** also showed significant cytotoxicity in HCT-8, RPMI-7951, and TE671 cell lines at 0.92, 0.22 and 3.82 μg/ml, respectively. Its synthetic isomer, compound **4**, exhibited potent cytotoxicity in HCT-8, P-388, RPMI-7951 and TE671 cell lines at IC_{50} values of 1.82, 2.24, 0.33 and 0.17 μg/mL, respectively.

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References and Notes

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6. These results will be published elsewhere.
7. mp: >290°C. HRMS m/e $C_{14}H_{11}NO_4$ obs. 257.0688, calcd. 257.0688. EIMS m/z (relative intensity) 257 (100, M^+), 228 (26), 214 (14), 200 (25), 190 (14), 144 (17), 119 (43), 57 (47).
8. Wu, T.S.; Ohta, T.; Furukawa, H. Heterocycles **1983**, *20*, 1267.
9. Methoxyhydroquinone and 5-amino-o-cresol are commercially available.

10. Compound **3**: recrystallized from acetone, mp 233-235°C. HRMS m/e $C_{14}H_{13}NO_4$ obs. 259.0844, calcd. 259.0845. UV λ_{max} (log ε) in CH_3OH 228.4 (4.65), 265.2 (4.64), 290.8 (4.27, sh), 320.0 (3.91), 419.2 (3.89) nm. IR υ_{max} (KBr) 3320, 3230, 1660, 1600 cm^{-1}. ^1H NMR (acetone-d_6, 200 MHz) δ 2.19 (3H, s, CH_3), 3.86 (3H, s, OCH_3), 5.89 (1H, s, H-3), 5.95 (1H, s, H-6), 6.78 (1H, dd, J=8.0, 2.1 Hz, H-6'), 6.88 (1H, d, J=2.1 Hz, H-2'), 7.44 (1H, d, J=8.0 Hz, H-5'), 8.13 (1H, brs, NH), 8.58 (1H, s, OH). EIMS m/z (relative intensity) 259 (100, M^+), 244 (16), 230 (11), 202 (12), 200 (12), 174 (62).
11. Compound **4**: mp 270-273°C. HRMS m/e $C_{14}H_{11}NO_4$ obs. 257.0685, calcd. 257.0688. UV λ_{max} (log ε) in CH_3OH 228.4 (4.91), 255.2 (4.49 sh), 262.4 (4.55), 288.4 (4.62), 327.6 (3.86, sh), 433.6 (3.87) nm. IR υ_{max} (KBr) 3300, 1655, 1630, 1595 cm^{-1}. ^1H NMR (acetone-d_6, 200 MHz) δ 2.26 (3H, s, CH_3), 3.95 (3H, s, OCH_3), 5.92 (1H, s, H-7), 6.99 (1H, d, J=8.0 Hz, H-1), 7.17 (1H, d, J=8.0 Hz, H-2), 9.74 (1H, s, OH), 11.74 (1H, brs, NH). EIMS m/z (relative intensity) 257 (100, M^+), 242 (18), 228 (9), 214 (12), 200 (26).

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