CLAUSINE-D AND -F, TWO NEW 4-PRENYLCARBAZOLE ALKALOIDS FROM *CLAUSENA EXCAVATA*

Tian-Shung WU*,[a] and Shiow-Chyn HUANG[b]

Department of Chemistry, National Cheng Kung University,[a] Tainan, Taiwan, R.O.C. and Department of Applied Chemistry, Providence University,[b] Taichung Hsien, Taiwan, R.O.C.

Two new 4-prenylcarbazole alkaloids, clausine-D (**1**) and -F (**3**) were isolated from the stem bark of *Clausena excavata*. Their structures were elucidated by spectral methods. Both of clausine-D (**1**) and -F (**3**) showed significant antiplatelet aggregation activity.

KEYWORDS clausine-D; cluasine-F; carbazole alkaloid; *Clausena excavata* ; Rutaceae; HMBC; antiplatelet activity

Clausena excavata (Rutaceae) is a wild shrub which has been claimed to be a useful folk medicine in the treatment of snakebite, for abdominal pain, and as a detoxification agent.[1] We have reported the isolation of coumarins and carbazole alkaloid from the root bark of this plant.[2] In the course of a continuing search for bioactive constituents from natural sources, we were interested in the stem bark constituents of *Clausena excavata* due to its antiplatelet activity. We now describe the structural elucidation of two new 4-prenylcarbazole alkaloids: clausine-D (**1**) and clausine-F (**3**), which were isolated from the stem bark of *Clausena excavata* collected in Taiwan.

Clausine-D (**1**) was isolated as brown powder, mp > 300°C.The molecular formula of this alkaloid was established as $C_{18}H_{17}NO_2$ by high resolution mass spectrometry (HR-MS) [M^+, 279.1260]. The UV spectrum of **1** at λ_{max}(log ε) : 225(4.27), 242(4.33), 253(4.27, sh), 276(4.40), 289(4.28), and 351(4.00) nm was similar to that of murrayanine (**2**)character- istic of an 1-oxygenated-3-formylcarbazoles.[3,4] The IR absorption bands at 3380, 3350 and 1655 cm^{-1} coupled with the signals at δ10.79 and 9.25(each 1H, brs., exchangeable with D_2O) and 10.34 (1H, s, CHO) in the ^{1}HNMR spectrum revealed the presence of NH and /or OH and CHO groups in the molecule. The lack of substituent on ring A was suggested by the four mutually coupling aromatic protons at δ 7.20 (1H, ddd, J=7.0, 1.4, 7.7 Hz), 7.40 (1H, ddd, J=7.7, 7.0, 1.4 Hz), 7.63(1H, dd, J=7.7, 1.4 Hz) and 8.11 (1H, brd, J=7.7 Hz), the lowest field signal at δ 8.11 is a character of 5-H of carbazoles which was well known. The presence of a 3,3-dimethylallyl group in **1** was suggested by the ^{1}HNMR signals: two singlet methyl signals at δ1.65 and 1.88; a benzylic methylene doublet at δ 4.33 (J=7 Hz) ; a multiplet for vinylic proton at δ 5.26 and the mass fragmentation ion at m/z 223 [M- $CH=C(Me)_2$-H]$^+$, coupled with the appearance of the carbon signals at δ 27.2(t), 123.9(d), 132.7(s), 18.3(q) and 25.6(q) in the ^{13}CNMR spectrum. The downfield shift benzylic protons of a 3,3-dimethylallyl group at δ4.33 together with the absence of a strongly deshielded H-4 resonance in its ^{1}HNMR spectrum indicated the location of the 3,3-dimethylallyl group at C-4. Therefore, an isolated aromatic proton at δ7.37 was attributed to H-2. These spectral data suggested the structure of clausine-D as **1**. For clarity, the proposed gross structure was unambiguously confirmed by the NOESY and HMBC spectra of **1**. The relative substituents at C-1, C-3 and C-4 were deduced from a NOESY spectrum (Fig. 1), which showed H_2-1' to be within nOe distance from H-5 and 3-CHO, 3-CHO from H-2 and H_2-1'. In the ^{1}H detected heteronuclear multiple bond connectivity(HMBC) spectrum of **1** (Fig. 2) which displayed the presence of the following significant correlations (2J or 3J H-C) each of C-9a (δ_C135.09) and H-2 (δ_H 7.37), C-4(δ_C123.75) and H-2 (δ_H7.37), 3-CHO(δ_C190.54) and H-2 (δ_H 7.37) , C-2(δ_C110.1) and 3-CHO (δ_H10.34), C-1 (δ_C142.19) and H-2(δ_H7.37), C-4 (δ_C123.75) and H_2-1'(δ_H4.33), respectively. On the basis of the above results, we could assign the structure **1** for clausine-D.

Clausine-F (**3**) was obtained as a colorless powder, mp 200-202°C. The molecular formula of **3** has been determined as $C_{19}H_{19}NO_3$ from the HRMS(M^+, 309.1365) analysis. The UV spectrum of **3** suggested that clausine-F possessed the same 1-oxygenated-3-carboxy-carbazole

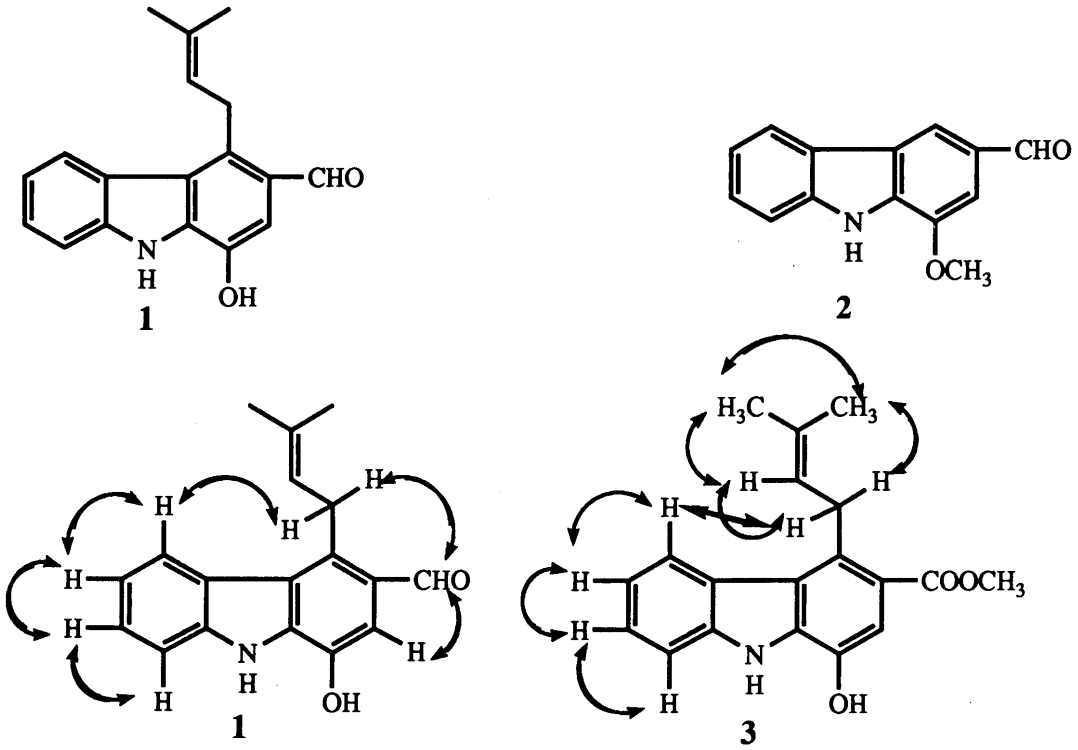


Fig. 1. The NOESY Spectra of Clausine-D (**1**) and -F (**3**)

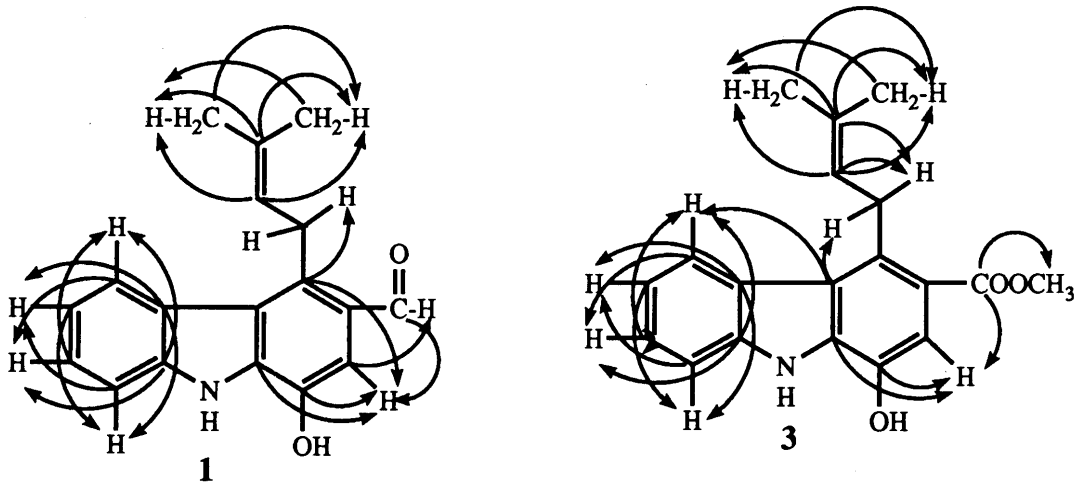
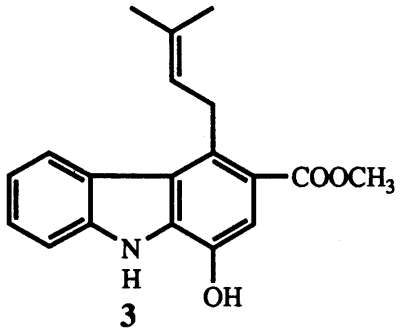


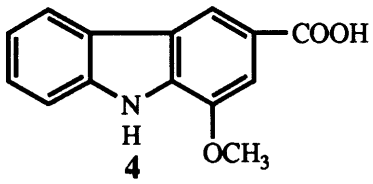
Fig. 2. C-H Correlation in HMBC Spectra of Clausine-D (**1**) and -F (**3**) (J=5 & 8 Hz)

nucleus as mukoeic acid (**4**)[4,6,7]. The ^{1}HNMR features of **3** showed similar signal patterns to that of **1**, except for a carbomethoxy signal at δ3.83 in **3** instead of an aldehyde signal at δ10.34 in **1**. The presence of a carbomethoxy group was also supported by the following data: a carbonyl carbon and methoxy carbon signals at δ168.85 and 51.53 in ^{13}CNMR

spectrum; an aromatic ester band at 1670 cm^{-1} in IR spectrum; a fragment ion at m/z 250 by loss of a carbomethoxy moiety in mass spectrum; the H-C three-bond correlation between a methoxy proton at δ3.83 and a carbonyl carbon at δ168.85 in HMBC spectrum. The other ^{1}HNMR signals of **3** appeared at δ1.66 (3H,d, J=1.4 Hz), 1.89(3H,s), 4.32(2H,d,J=5.9 Hz) and 5.23(1H,m) for a prenyl group, δ 7.20(1H,dt,J=1.2 and 7.4 Hz), 7.42(1H,dt,J=1.4 and 7.4 Hz), 7.64(1H,dd,J=1.2 and 7.4 Hz) and 8.12(1H,brd, J=7.4 Hz) for H-6,7,8 and 5 in ring A, respectively, a singlet signal at δ7.46 for H-2, and the hydroxyl and imino groups at δ8.99 and 10.62, respectively. The relative substituted position of the substituents in the molecule were confirmed by the NOESY (Fig. 1) and HMBC (Fig. 2) experiments. On the basis of the spectral results mentioned above, we assigned structure **3** to clausine-F.[8]



3



4

It is worthy of note that this is the second report of the isolation of such 4-prenylcarbazole alkaloids from a natural source. The aggregation of rabbit platelets induced by arachidonic acid showed 53% and 37% inhibition by clausine-D(**1**) and-F (**3**) at 1 μg/ml , respectively. In the case of collagen instead of arachidonic acid as platelet aggregation agent, compound **1** and **3** at 10 μg/ml displayed 66% and 48% inhibition, respectively. This is first demonstration of the antiplatelet activity of carbazole alkaloids.

ACKNOWLEDGEMENT Financial support of this project was by a grant (NSC 80-0420-B-006-07) of the National Science Council of R. O. C. to T. S. Wu, which is gratefully acknowledged. We further thank Miss J. Z. Wu and Miss L. N. Lai, National Cheng Kung University, for measuring the NMR and mass spectra, respectively.

REFERENCES AND NOTES

1) S. Sasaki, "*Khoyo Taiwan Minkan Yakyo Shokubutsu Shi*", Khobunkan, Taipei, *1924*, p36.
2) T. S. Wu and H. Furukawa, *J. Nat. Prod.*, **45** , 718 (1982).
3) D. P. Chakraborty, B. K. Barman and P. K. Bose, *Tetrahedron* , **21**, 681 (1965).
4) D. P. Chakraborty, *Fortschr. Chem. Org. Naturst.*, **34** , 299 (1977).
5) **1** : EIMS m/z (%): 279(M^+,100), 264(31), 224(20), 223(49), 210(3), 195(89), 69(11).
6) B. K. Chowdhury and D. P. Chakraborty, *Chem. Ind., 1969*, 549.
7) B.K. Chowdhury and D. P. Chakraborty, *Phytochemistry* , **10** , 1967 (1971).
8) **3** : λ_{max} nm(log ε):225.4(4.31,sh), 244.2(4.45,sh), 251 (4.46,sh), 269.4(4.59), 312.8 (3.88),322.8(3.86),339.6(3.77).ν_{max} cm^{-1}:3410,3350,1670,1630,1585.EIMSm/z(%) :309(M^+,100),277(66), 266(88), 262(57), 255(15), 254(27), 250(28), 234(34).

(Received December 24, 1991)