STRUCTURES OF A NEW COUMARIN AND A NEW ACRIDONE DIMER FROM A *CITRUS* PLANT[1]

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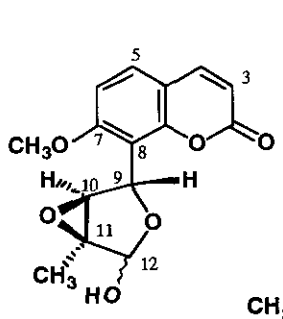
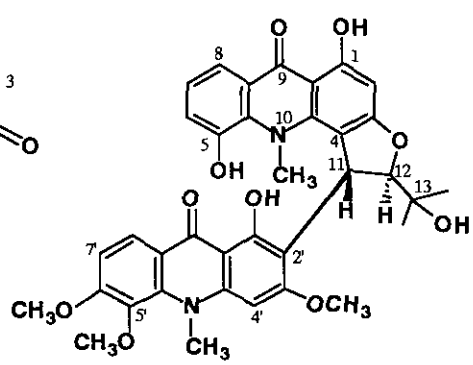
Abstracts ——A new coumarin, marshrin (**1**) and a new dimeric acridone alkaloid, citbismine-F (**2**) were isolated from the roots of Marsh grapefruit (*Citrus paradisi* Macf.). Structures of these new compounds were elucidated by spectral analyses especially by HMQC, HMBC and NOE experiments.

In the previous papers, we reported the isolation and structure elucidation of many new monomeric acridone alkaloids,[2] dimeric acridone alkaloids,[3] bicoumarins[4] and acridone-coumarin dimers[1] from the roots of Marsh grapefruit (*Citrus paradisi* Macf.). In continuing studies on the constituents of this plant, we isolated a new coumarin, marshrin (**1**) and a new dimeric acridone alkaloid, citbismine-F (**2**).

Marshrin (**1**) was obtained as colorless cubes, mp 200-203 ℃, $[\alpha]_D^{24}$ +2.6°. The molecular formula $C_{15}H_{14}O_6$ was obtained by HR-MS which showed molecular ion peak at m/z 290.0794. The IR (1720, 1605 cm^{-1}) and UV [218 (sh), 251, 257, 322 nm] absorptions indicated the presence of 7-oxygenated 8-substituted coumarin skeleton.[5] The ^{1}H-NMR spectrum showed characteristic signals due to H-4, H-3,



(**1**)



(**2**)

H-5 and H-6 [δ 7.97 and 6.27 (each 1H, d, J= 9.4 Hz), 7.68 and 7.10 (each 1H, d, J= 8.6 Hz)] of coumarin skeleton. On the NOE experiment, irradiation of the methoxy signal at δ 3.87 showed 17% increment of the signal at δ 7.10 (H-6) indicating the location of methoxy group at C-7. The signals at δ_H 6.72 (1H, d, J=5.9 Hz, disappeared with D_2O), 5.44 (1H, s), 5.09 (1H, d, J=5.9 Hz), 3.90 (1H, s), 1.48 (3H, s) and the signals at δ_C 96.26 (d), 69.75 (d), 63.49 (s), 61.21 (d), 12.44 (q) were considered due to C5 unit at C-8. The relationship of these signals was clarified by HMQC and HMBC experiments. The signal at δ 5.44 (H-9) showed ^2J and ^3J-connectivity with C-7, C-8, C-8a indicating that this proton locates at benzylic position. The connectivities were also observed between H-12 (δ 5.09) / C-9 and C-10; 11-Me (δ 1.48) / C-12 and C-10; 12-OH (δ 6.72) / C-11 and C-12 (Figure 1). These results suggested the presence of 3,4-epoxy-2-hydroxy-3-methyltetrahydrofuran moiety at C-8. Although NOEs were observed between 11-Me (δ 1.48) / H-10 (δ 3.90) and H-12 (δ 5.09); H-10 / H-9 (δ 5.44), the assignment of the stereochemistry of the five-membered epoxy lactone ring system was impossible. The relative stereochemistry of H-9 and H-10 was assigned as *trans* (dihedral angle 90°) from the absence of observable coupling similar to the case of micromelin[6] and acetyldihydromicromelin-A.[7] From the above results, we assigned the structure (**1**) to marshrin except for the stereochemistry at C-12.

Citbismine-F (**2**) was obtained as optically inactive yellow cubes, mp 330-341° (decomp). The molecular formula $C_{36}H_{34}N_2O_{10}$ was established by the molecular ion peak at m/z 654.2220 obtained by HR-MS. The IR (1630, 1590 cm^{-1}) and UV [223, 265, 276 (sh), 333, 396 nm] absorptions suggested the presence of acridone skeleton.[8] The ^{1}H-NMR spectrum showed signals assignable to an ABC [δ 7.56 (1H, dd, J=

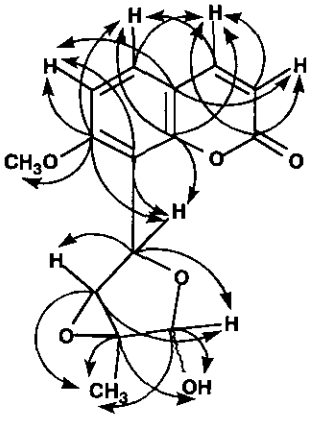


Figure 1. C-H Long-Range Correlations in the HMBC spectrum of marshrin (**1**)

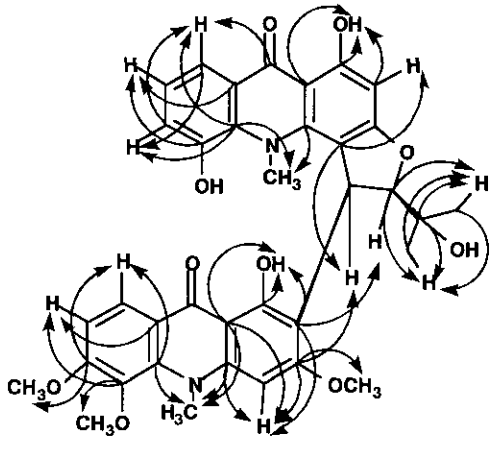


Figure 2. C-H Long-Range Correlations in the HMBC spectrum of citbismine-F (**2**)

1.1, 7.7 Hz), 7.03 (1H, t, J= 7.7 Hz), 6.94 (1H,br d, J= 7.7 Hz)], an AB [δ 8.12 (1H, d, J= 9.2Hz),7.25 (1H, d, J= 9.2 Hz)] and two isolated [δ 6.18, 6.38 (each 1H, s)] aromatic protons. The lower signals at δ 7.56 and 8.12 were considered to be deshielded by 9-carbonyl group and were assigned as H-8 and H-8' of acridone moieties. Two hydrogen-bonded signals at δ 14.86 and 15.62 along with molecular ion suggested that **2** is a dimeric acridone alkaloid. The signals at δ_H 5.57 (1H, d, J= 5.1 Hz), 4.35 (1H, d, J= 5.1 Hz), 1.27, 1.22 (each 3H, s) and δ_C 37.6 (d), 96.7 (d), 70.8 (s), 25.9 (q), 24.7 (q) indicated the presence of 2-(2-hydroxy-2-methyl)ethyldihydrofuran moiety. The presence of three methoxy and two *N*-methyl groups was apparent from the signals at δ_H 3.97, 3.91, 3.79, 3.70 and 3.52, and δ_C 61.0, 56.4, 55.8, 44.4 and 40.0. In the NOE experiment, irradiation of the *N*-methyl signal at δ 3.79 induced a 6% increment of the methine proton signal at δ 5.57 suggesting the angular orientation of dihydrofuran ring. When the methoxy signal at δ 3.52 and the *N*-methyl signal at δ 3.91 were irradiated, 7 and 12% enhancement of the signal at δ 6.38 (H-4') was observed, respectively. Thus the linkage between C-11 and C-2' of two acridone moieties was suggested. Further structure elucidation was performed with the aid of HMQC and HMBC experiments. As shown in Figure 2, the ^2J and ^3J- connectivities of H-11 (δ_H 4.35) to C-4 (δ_C 106.6) and C-3' (δ_C 164.8) enabled us to establish the linkage of two acridone skeletons and other correlations supported the structure of both acridone moieties. Because no increments were observed between H-11 and H-12 in the NOE experiment, the relative stereochemistry of these methine protons was assigned as *trans*. From the above results, we concluded the structure of citbismine-F as **2**.

EXPERIMENTAL

Isolation: The CH_2Cl_2 eluate[3a] was treated repeatedly by silica gel chromatography and preparative TLC [solvent systems: acetone - benzene (2:8), acetone - $CHCl_3$ (1:9), MeOH - $CHCl_3$ (1:19)] to give marshrin (**1**) (62.1 mg) and citbismine-F (**2**) (4.7 mg).

Marshrin (**1**): Colorless cubes, mp 200-203 °C, $[\alpha]_D^{24}$ +2.6° (c=0.31, pyridine); HR-MS m/z: 290.0794 (M^+, found), 290.0790 (calcd for $C_{15}H_{14}O_6$); EI-MS m/z : 290, 229, 213, 205 (base peak), 204, 203, 201, 119, 118; UV λ_{max} (EtOH, nm) : 218 (sh), 251, 257, 322; IR ν_{max} ($CHCl_3$, cm^{-1}) : 3400, 1720, 1605, 1285, 1240, 1120; ^{1}H-NMR (DMSO-d_6, δ) : 7.97 (1H, d, J=9.4 Hz, H-4), 7.68 (1H, d, J=8.6 Hz, H-5), 7.10 (1H, d, J=8.6 Hz, H-6), 6.72 (1H, d, J=5.9 Hz, 12-OH), 6.27 (1H, d, J=9.4 Hz, H-3), 5.44 (1H, s, H-9), 5.09 (1H, d, J=5.9 Hz, H-12), 3.90 (1H, s, H-10), 3.87 (3H, s, CH_3O), 1.48 (3H, s, 11-CH_3); NOE: irradiation at δ 1.48 (11-CH_3) - 6% and 4% enhancement at δ 3.90 (H-10) and 5.09 (H-12);

irradiation at δ 3.87 (7-CH_3O) - 17% and 2% enhancement at δ 7.10 (H-6) and 5.44 (H-9); irradiation at δ 3.90 (H-10) - 9% enhancement at δ 5.44 (H-9); irradiation at δ 5.44 (H-9) - 9% enhancement at δ 3.90 (H-10); ^{13}C-NMR (DMSO-d_6, δ) : 160.73 (s, C-7), 159.87 (s, C-2), 153.29 (s, C-8a), 144.50 (d, C-4), 129.52 (d, C-5), 112.75 (s, C-4a), 112.37 (d, C-2), 111.99 (s, C-8), 108.52 (d, C-6), 96.26 (d, C-12), 69.75 (d, C-9), 63.49 (s, C-11), 61.21 (d, C-10), 56.56 (q, 7-CH_3O), 12.44 (q, 11-CH_3)

Citbismine-F (**2**): Yellow cubes; mp 330 - 341° (decomp); $[\alpha]_D^{24}$ 0° (*c*=0.11, $CHCl_3$); HR-MS m/z: 654.2220 (M^+, found), 654.2214 (calcd for $C_{36}H_{34}N_2O_{10}$); UV λ_{max} [EtOH, nm (log ε)]: 222 (3.87), 266 (4.28), 275 (4.27), 334 (3.77), 390 (3.41); IR ν_{max} ($CHCl_3$, cm^{-1}) : 1630, 1590; ^{1}H-NMR (DMSO-d_6, δ) : 15.62 (1H, s, 1'-OH), 14.86 (1H, s, 1-OH), 9.97 (1H, s, 5-OH), 8.12 (1H, d, J= 9.2 Hz, H-8'), 7.56 (1H, dd, J= 1.1, 7.7 Hz, H-8), 7.25 (1H, d, J= 9.2 Hz, H-7'), 7.03 (1H, t, J= 7.7 Hz, H-7), 6.94 (1H, br d, J= 7.7 Hz, H-6), 6.38 (1H, s, H-4'), 6.18 (1H, s, H-2), 5.57 (1H, d, J= 5.1 Hz, H-11), 4.73 (1H, s, 13-OH), 4.35 (1H, d, J= 5.1 Hz, H-12), 3.97 (3H, s, 6'-CH_3O), 3.91 (3H, s, 10'-NCH_3), 3.79 (3H, s, 10-NCH_3), 3.70 (3H, s, 5'-CH_3O), 3.52 (3H, s, 3'-CH_3O), 1.27, 1.22 (each 3H, s, 13-CH_3). NOE: irradiation at δ 3.91 (10'-NCH_3) - 7% enhancement at δ 6.38 (H-4'); irradiation at δ 3.79 (10-NCH_3) - 6% enhancement at δ 5.57 (H-11); irradiation at δ 3.52 (3'-CH_3O) - 12% enhancement at δ 6.38 (H-4'); irradiation at δ 3.97 (6'-CH_3O) - 18% enhancement at δ 7.25 (H-7'); irradiation at δ 3.70 (5'-CH_3O) - no increment was observed; ^{13}C-NMR (DMSO-d_6, δ) : 180.6 (s, C-9'), 179.6 (s, C-9), 169.4 (s, C-3), 164.8 (s, C-3'), 164.5 (s, C-1), 159.4 (C-1'), 157.7 (s, C-6'), 147.7 (s, C-5), 146.2 (s, C-4'a), 145.3 (s, C-4a), 137.9 (s, C-10'a), 136.7 (s, C-5'), 135.8 (s, C-10a), 123.5 (s, C-8a), 122.5 (d, C-7), 122.1 (d, C-8'), 119.2 (d, C-6), 116.5 (s, C-8'a), 115.0 (d, C-8), 109.7 (s, C-2'), 108.9 (d, C-7'), 106.6 (s, C-4), 105.4 (s, C-9a), 103.5 (s, C-9'a), 96.7 (d, C-12), 90.8 (d, C-2), 89.3 (d, C-4'), 70.8 (s, C-13), 61.0 (q, 5'-CH_3O), 56.4 (q, 6'-CH_3O), 55.8 (q, 3'-CH_3O), 44.4 (q, 10-NCH_3), 40.0 (q, 10'-NCH_3), 37.6 (d, C-11), 25.9 (q, 13-CH_3), 24.7 (q, 13-CH_3).

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