TWO NEW BISACRIDONE ALKALOIDS FROM *CITRUS* PLANTS[1]

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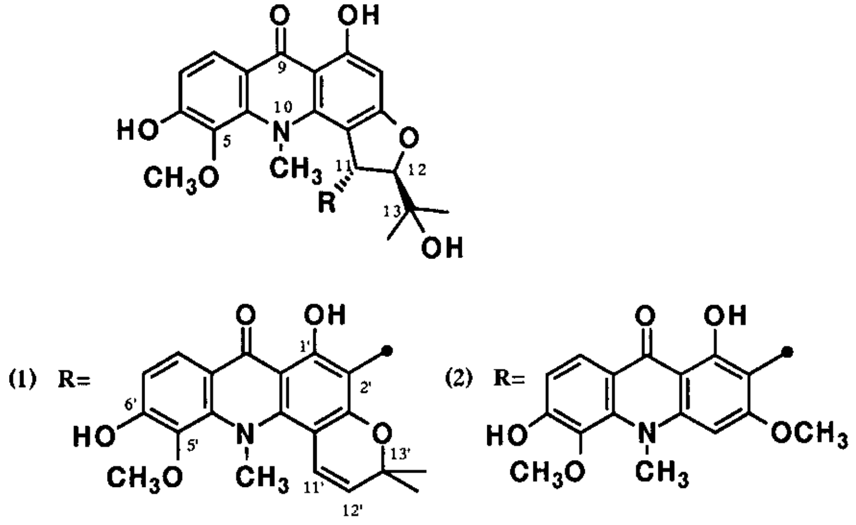
Abstract ——Two new binary acridone alkaloids named citbismine-D (1) and -E (2) were isolated from the roots of Marsh grapefruit (*Citrus paradisi*). The structures were elucidated by spectroscopic studies as **1** and **2**. Citbismine-E (2) was also obtained from the roots of Hirado-Buntan (*C. grandis*).

Previous chemical studies in our laboratory of the constituents of *Citrus* plants (Rutaceae) have resulted in the isolation of many acridone alkaloids,[2] coumarins[3] and acridone-coumarin dimers.[4] Recently, we reported the isolation and structure elucidation of new type bisacridone alkaloid citbismine-A[5] from the roots of Marsh grapefruit (*C. paradisi* Macf.) and Hirado-Buntan (*C. grandis* Osbeck f. Hirado). Further study of these plants resulted in the isolation of two additional bisacridone alkaloids named citbismine-D (1) and -E (2). This paper deals with the structure elucidation of these alkaloids.

Citbismine-D (1) was obtained from the roots of Marsh grapefruit as a pale yellow oil, $[\alpha]_D^{25} \pm 0°$ ($CHCl_3$). The molecular formula $C_{40}H_{38}N_2O_{11}$ was obtained by HRms (m/z 722.2473). The ir (1620, 1595 cm^{-1}) and uv [224 (sh), 267, 298 (sh), 338, 387 nm)] absorptions indicated the presence of a 9-acridone skeleton.[6] The ^1H nmr spectrum had signals due to two chelated hydroxyl groups (δ 15.84, 14.82), two *ortho*-coupled aromatic protons [δ 7.86, 6.81 (each 1H, d, J= 8.5 Hz), 8.04, 7.05 (each 1H, d, J= 9.2 Hz)], a lone aromatic proton [δ 6.18 (1H, s)], and a 2,2-dimethylpyran ring [δ 6.58, 5.51 (each 1H, d, J= 9.8 Hz), 1.30 (6H, s)]. The lowest signals of aromatic proton at δ 7.86 and 8.04 were deshielded by the 9- and 9'-carbonyl group, and thus were assigned as signals of H-8 and H-8'. The presence of 2-hydroxyisopropyl substituted dihydrofuran moiety was assumed based on the signals at δ 5.70, 4.56 (each 1H, d, J= 5.5 Hz), 1.38, 1.43 (each 3H, s) and 4.05 (1H, br s). The characteristic high field signal at δ 2.91 (3H, s) was assigned to the 5-methoxyl group, shielded by another acridone nucleus[7] and resonating at an unusually high field. The remaining signals at δ 3.77, 3.64 and 3.88 indicated the presence of methoxyl and *N*-methyl groups. The assignment of these



signals was facilitated by the following NOE experiments. Irradiation of the signal at δ 3.77 showed 9.5% increments of the signal at δ 5.70 (H-11), suggesting the presence of a 10-*N*-methyl group and angular orientation of the dihydrofuran ring. When the signal at δ 3.64 was irradiated, 9.8% increment was observed of the signal at δ 6.58, indicating the presence of a 10'-*N*-methyl group and angular orientation of the 2,2-dimethylpyran ring. Irradiation of the signal at δ 3.88 showed no increments of any aromatic protons, leading to the conclusion that this group is located at C-5'. On the basis of these results, the linkage of two acridone moieties was assigned between C-11 and C-2'. From the above results, the structure of citbismine-D was concluded to be **1**.

Citbismine-E (**2**) was obtained from the roots of Marsh grapefruit and Hirado-Buntan as a pale yellow oil, $[\alpha]_D^{25}$ ±0° ($CHCl_3$). The HRms gave a molecular ion at m/z 670.2160, consistent with the molecular formula of $C_{36}H_{34}N_2O_{11}$. The presence of a 9-acridone skeleton[6] was assumed by ir (1620, 1595 cm^{-1}) and uv (221, 264, 275 (sh), 298 (sh), 335, 379 nm) absorptions. The ^{1}H-nmr spectrum showed similar signal patterns with that of **1**: the signals of two chelated hydroxyl groups (δ 15.92, 14.78), two *ortho*-coupled [δ 8.14, 7.03 (each 1H, d, J= 9.2 Hz), 7.86, 6.80 (each 1H, d, J= 8.5 Hz)] aromatic protons and a 2-hydroxyisopropyl substituted dihydrofuran ring [δ 5.72, 4.54 (each 1H, d, J= 5.5 Hz), 3.9 (1H, br s), 1.43, 1.38 (each 3H, s)], and methoxyl and *N*-methyl [δ 4.02, 3.88, 3.78, 3.57 and 2.80 (each 3H, s)] groups. The differences were the presence of signals of one additional methoxyl group (δ 3.57) and a lone aromatic proton (δ 6.46) instead of the disappearance of the signals of the 2,2-dimethylpyran moiety. The location of methoxyl and *N*-methyl groups was elucidated by the following NOE experiments. Irradiation of the signals at δ 4.02 and 3.57 showed 12 and 11% enhancements of the signal at δ 6.46, respectively. The result led us to conclude that the signals at δ 4.02 and 3.57 were due to the 10' *N*-methyl and 3'-methoxyl group, respectively. Irradiation of the signal at δ 3.78 showed 8.7% increments of the signal at δ 5.72, providing evidence for the presence of the 10-*N*-methyl group. Irradiation of signals at δ 2.80 and 3.88 showed no increments of aromatic protons, suggesting the location of these methoxyl groups to be C-5 and C-5', respectively. Thus, the structure of citbismine-E was concluded to be 2.

Both citbismines -D (1) and -E (2), in analogy with other citbismines, were obtained in the optically inactive form and we assumed that they may be artifacts or were formed without participation of enzymes in the plant cells.[8]

EXPERIMENTAL

Isolation : The extraction procedures for the roots of Marsh grapefruit and Hirado-Buntan are described in the previous paper.[9] The CH_2Cl_2 eluate (43.11 g), obtained from silica gel column chromatography of the acetone extract of Marsh grapefruit, was separated with a silica gel column, centrifugal chromatography and finally repeated PTLC [solvents: $CHCl_3$-MeOH (19:1), acetone-hexane (1:1), benzene-MeOH (9:1)] to give citbismine-D (1)(2.5 mg) and citbismine-E (2)(1.4 mg). The CH_2Cl_2 eluate (29.394 g), obtained from the silica gel column chromatography of the acetone extract of Hirado-Buntan, was treated in a similar manner to give citbismine-E (2)(4.6 mg).

Citbismine-D (1): Pale yellow oil, $[\alpha]_D^{25}$ ±0° ($CHCl_3$, c=0.140), ir ν_{max}($CHCl_3$) 1620, 1595 cm^{-1}; uv λ_{max} (EtOH) 224 (sh), 267, 298 (sh), 338, 387 nm; HRms m/z 722.2473 (M^+, calcd for $C_{40}H_{38}N_2O_{11}$ 722.2476); EIms m/z 722 (M^+), 663, 649 (base peak), 369, 353, 352, 338, 311; ^1H nmr (acetone-d_6) δ 15.84 (1H, s, 1'-OH), 14.82 (1H, s, 1-OH), 9.18 (1H, s, 6' or 6-OH), 8.62 (1H, s, 6 or 6'-OH), 8.04 (1H, d, J= 9.2 Hz, H-8'), 7.86 (1H, d, J= 8.5 Hz, H-8), 7.05 (1H, d, J= 9.2 Hz, H-7'), 6.81 (1H, d, J= 8.5 Hz, H-7), 6.58 (1H, d, J= 9.8 Hz, H-11'), 6.18 (1H, s, H-2), 5.70 (1H, d, J= 5.5 Hz, H-11), 5.51 (1H, d, J= 9.8 Hz, H-12'), 4.56 (1H, d, J= 5.5 Hz, H-12), 4.05 (1H, br s, 13-OH), 3.88 (3H, s, 5'-MeO), 3.77 (3H, s, 10-NMe), 3.64 (3H, s, 10'-NMe), 2.91 (3H, s, 5-MeO), 1.43, 1.38 (each 3H, s, 13-Me), 1.30 (6H, s, 13'-Me); NOE: irradiation at δ 3.77 (10-NMe) - 9.5% enhancement at δ 5.70 (H-11); irradiation at δ 3.64 (10'-NMe) - 9.8% enhancement at δ 6.58 (H-11'); irradiation at δ 5.70 (H-11) - 7.9% enhancement at δ 3.77 (10-NMe).

Citbismine-E (2): Pale yellow oil, $[\alpha]_D^{25}$ ±0° ($CHCl_3$, c=0.120), ir ν_{max}($CHCl_3$) 1620, 1595 cm^{-1}; uv λ_{max} (EtOH) 221, 264, 275 (sh), 298 (sh), 335, 379 nm; HRms m/z 670.2160 (M^+, calcd for $C_{36}H_{34}N_2O_{11}$ 670.2163); EIms m/z 670 (M^+), 612, 611, 599, 598, 597, 582, 369, 351, 336, 311, 301 (base peak), 296, 287, 286; ^1H-nmr (acetone-d_6) δ 15.92 (1H, s, 1'-OH), 14.78 (1H, s, 1-OH), 9.3 (1H, br s, 6' or 6-OH), 8.7 (1H, br s, 6 or 6'-OH), 8.14 (1H, d, J= 9.2 Hz, H-8'), 7.86 (1H, d, J= 8.5 Hz, H-8), 7.03 (1H, d, J= 9.2 Hz, H-7'), 6.80 (1H, d, J= 8.5 Hz, H-7), 6.46 (1H, s, H-4'), 6.14 (1H, s, H-2), 5.72 (1H, d, J= 5.5 Hz, H-11), 4.54 (1H, d, J= 5.5 Hz, H-12), 4.02 (3H, s, 10'-NMe), 3.9 (1H, br s, 13-OH), 3.88 (3H, s, 5'-MeO), 3.78 (3H, s, 10-NMe), 3.57 (3H, s, 3'-MeO), 2.80 (3H, s, 5-MeO), 1.43, 1.38 (each 3H, s, 13-Me); NOE: irradiation at δ 4.02 (10'-NMe) - 12.4% enhancement at δ 6.46 (H-4'); irradiation at δ 3.78 (10-NMe) - 8.7% enhancement at δ 5.72 (H-11); irradiation at δ 3.57 (3'-MeO) - 10.8% enhancement at δ 6.46 (H-4'); irradiation at δ 5.72 (H-11) - 7.2% enhancement at δ 3.78 (10-NMe).

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