# STRUCTURES OF NEOACRIMARINES -F AND -G, TWO NEW ACRIDONE-COUMARIN DIMERS FROM *CITRUS* PLANTS[1]

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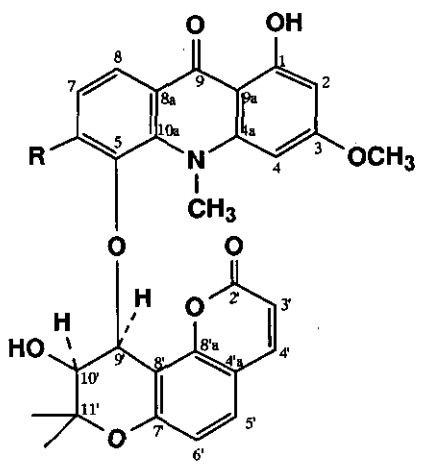
Abstracts——Two new neoacrimarines -F and -G were isolated from Yalaha (*Citrus paradisi* Macf. x *C. tangerina* Hort ex Tanaka) and Marsh grapefruit (*C. paradisi* Macf.), respectively. Their structures were determined through extensive use of NMR spectroscopy.

In the course of our phytochemical studies of the constituents of *Citrus* plants, we have reported the isolation and structure elucidation of many new coumarins,[2] acridone alkaloids[3] and acridone-coumarin dimers.[4] Acridone-coumarin dimer could be classified into two groups; one is acrimarines which were constructed by various acridone alkaloids and a sole coumarin unit, suberenol, the other is neoacrimarines which were composed by various acridone alkaloids and coumarins. Further investigation furnished to isolate two new neoacrimarine type acridone-coumarin dimers named neoacrimarine-F and -G from Yalaha and Marsh grapefruit, respectively. In this paper we report the isolation and structure elucidation of both compounds.

Neoacrimarine-F (1), yellow cubes, $[\alpha]_D$ +130.9° (MeOH), mp 218 - 220°, was obtained from acetone extracts of roots of Yalaha [Duncan grapefruit (*Citrus paradisi* Macf.) x Dancy tangerine (*C. tangerina* Hort ex Tanaka)]. The molecular formula $C_{29}H_{25}NO_9$ was established by HRMS ($M^+$ 531.1534). The IR (1730, 1630, 1605, 1590, 1565 $cm^{-1}$) and UV [220 (sh), 264, 295 (sh), 330, 369 nm] spectra suggested the presence of 9-acridone[5] and coumarin skeletons.[6] The $^1$H-NMR spectrum showed signals characteristic hydrogen-bonded hydroxyl group [δ 14.54 (1H, s)], *ortho*-coupled aromatic protons [δ 7.93, 6.95 (each 1H, d, J= 8.8 Hz)] and *meta*-coupled aromatic protons [δ 6.11, 5.57 (each 1H, d, J= 1.5 Hz)]. The lowest aromatic proton signal (δ 7.93) was deshielded by 9-carbonyl function and the 1,3,5,6-tetraoxygenated pattern of acridone moiety could be assigned. The other signals at δ 7.64, 5.54 (each 1H, d, J=9.5 Hz) and δ 7.64, 6.91 (each 1H, d, J=8.8 Hz) were assignable to H-4, H-3, H-5 and H-6 of coumarin skeleton, and the remaining methine signals at δ 5.13, 4.17 (each 1H, d, J=3.7 Hz) and two methyl signals at δ 1.64, 1.53 (each 3H, s) suggested the presence of 2,2-dimethyl-3,4-dioxygenated dihydropyran ring.



(1) R = OH
(2) R = H

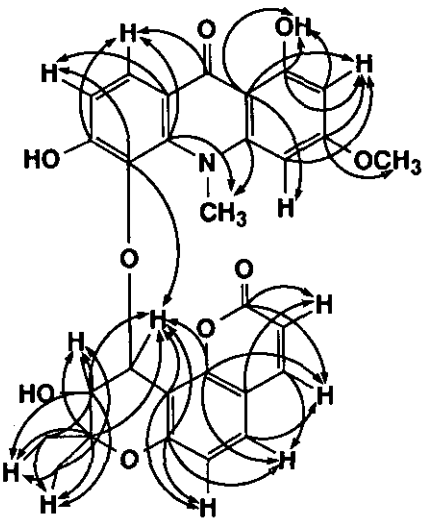


Figure 1 C-H Long-Range Correlations in the HMBC spectrum(J=5 and 8Hz) of neoacrimarine-F (1)

Signals at $\delta_H$ 3.76, 3.18 (each 3H, s) and $\delta_C$ 55.4, 42.3 in the $^1$H- and $^{13}$C-NMR spectra indicated the presence an *O*-methyl and an *N*-methyl groups. In the NOE experiments, irradiation of the methoxy signal at δ 3.76 showed 6% and 8% increments of the signals at δ 5.57 (H-4) and 6.11 (H-2). When the *N*-methyl signal at δ 3.18 was irradiated, 10% increment was observed on the signal at δ 5.57. These results suggested the location of *O*-methyl group at C-3. In order to establish the linking position between acridone and coumarin skeleton and to assign the structure unambiguously, a series of NMR experiments including H-H COSY, HMQC and HMBC was performed. In the HMBC spectrum (Figure 1), H-9' (δ 5.13) showed $^2J$ and $^3J$ correlations with six carbon resonances at δ 135.3 (C-5), 108.2 (C-8'), 155.9 (C-7'), 153.0 (C-8'a), 70.1 (C-10') and 79.1 (C-11'). Due to the results, the linkage of the acridone and coumarin units between C-5 and C-9' was established. The relative configuration of the two chiral carbons of khellactones is generally determined by *J* values of two methine protons and the difference of chemical shifts of two C-methyl signals in $^1$H-NMR[7] and $^{13}$C-NMR spectra.[8] The *J* values (3.7 Hz) of two methine protons, the appearance of two C-methyl singlets having little difference in chemical shift (Δ 0.11 ppm) in the $^1$H-NMR spectrum and large difference in chemical shift (6.7 ppm) of two C-methyl signals in the $^{13}$C-NMR spectrum suggested the presence of a *cis*-oriented methine protons. This orientation was confirmed by difference NOE experiments. Irradiation of the methine signal at δ 4.17 (H-10') gave 7% enhancement of the signal at δ 5.13 (H-9'), and irradiation of the H-9' signal showed 3% enhancement of H-10' signal. These results established the relative stereochemistry of the methine protons in **1** as *cis*. Consequently, the structure of the new compound was represented by **1**, except for the absolute stereochemistry.

Neoacrimarine-G (2) was obtained from the roots of Marsh grapefruit (*C. paradisi* Macf.) as a yellow oil. The HRMS gave a molecular ion at m/z 515.1612 consistent with a molecular formula $C_{29}H_{25}NO_8$. The IR (3566, 1734, 1699, 1606, 1558 cm$^{-1}$) and UV spectra [220 (sh), 262, 280 (sh), 315, 324 nm] showed the presence of 9-acridone[5] and coumarin skeletons.[6] The $^1$H- and $^{13}$C-NMR spectra of 2 showed

Table 1. $^{1}H$- and $^{13}C$-NMR spectral data of 1 and 2

| | 1 | 2 | | 1 | 2 |
|---|---|---|---|---|---|
| 1-OH | 14.54 | 14.44 | C-1 | 163.7 | 165.3 |
| H-2 | 6.11 (d, 1.5) | 6.26 (d, 2.2) | C-2 | 94.3 | 94.1 |
| | | | C-3 | 164.8 | 165.8 |
| 3-$OCH_3$ | 3.76 | 3.84 | 3-$OCH_3$ | 55.4 | 55.5 |
| H-4 | 5.57 (d, 1.5) | 5.90 (d, 2.2) | C-4 | 89.6 | 90.7 |
| | | | C-4a | 146.2 | 147.3 |
| | | | C-5 | 135.3 | 149.4 |
| H-6 | | 7.70 (br d, 8.1) | C-6 | 155.4 | 123.0 |
| 6-OH | 10.30 | | | | |
| H-7 | 6.95 (d, 8.8) | 7.31 (t, 8.1) | C-7 | 112.7 | 122.3 |
| H-8 | 7.93 (d, 8.8) | 8.22 (dd, 8.1, 1.1) | C-8 | 122.8 | 121.4 |
| | | | C-8a | 116.4 | 124.7 |
| | | | C-9 | 179.6 | 181.1 |
| | | | C-9a | 104.2 | 105.7 |
| | | | C-10a | 140.7 | 137.7 |
| 10-$NCH_3$ | 3.18 | 3.56 | 10-$NCH_3$ | 42.3 | 42.6 |
| | | | C-2' | 157.8 | 158.5 |
| H-3' | 5.54 (d, 9.5) | 5.88 (d, 9.5) | C-3' | 111.3 | 112.8 |
| H-4' | 7.64 (d, 9.5) | 7.44 (d, 9.5) | C-4' | 143.2 | 142.6 |
| | | | C-4'a | 111.8 | 112.1 |
| H-5' | 7.64 (d, 8.8) | 7.35 (d, 8.8) | C-5' | 130.2 | 129.6 |
| H-6' | 6.91 (d, 8.8) | 6.84 (d, 8.8) | C-6' | 113.9 | 114.6 |
| | | | C-7' | 155.9 | 156.3 |
| | | | C-8' | 108.2 | 108.0 |
| | | | C-8'a | 153.0 | 154.1 |
| H-9' | 5.13 (d, 3.7) | 5.65 (d, 4.8) | C-9' | 75.7 | 74.5 |
| H-10' | 4.17 (d, 3.7) | 4.11 (dd, 4.8, 9.2) | C-10' | 70.1 | 71.2 |
| 10'-OH | | 2.66 (d, 9.2) | | | |
| | | | C-11' | 79.1 | 78.9 |
| 11'-$CH_3$ | 1.53 | 1.57 | 11-$CH_3$ | 21.5 | 21.4 |
| | 1.64 | 1.59 | | 28.2 | 26.3 |

Neoacrimarine-F (1) was measured in DMSO-$d_6$ and -G (2) in $CDCl_3$.
J values in parentheses are expressed in Hz.

similar signal patterns with those of **1** (Table 1). The prominent features distinguishing **2** from **1** were the replacement of the signals of AB type aromatic proton [δ 7.93, 6.95 (each 1H, d, J=8.8 Hz)] of acridone moiety seen in **1** by ABC type signals of aromatic proton [δ 8.22 (1H, dd, J= 8.1, 1.1 Hz), 7.70 (1H, br d, J= 8.1 Hz), 7.31 (1H, t, J= 8.1 Hz)] in **2**. The location of *O*-methyl group was determined by NOE experiments. Irradiation of the *N*-methyl signal (δ 3.56) resulted in 28% enhancement of the signal at δ 5.90 (H-4 ). When the methoxy signal at δ 3.84 was irradiated, 26% and 6% enhancement were observed of the signals at δ 6.26 (H-2) and 5.90 (H-4), indicating that an *O*-methyl group was located at C-3. The structure of coumarin and acridone moieties was confirmed by HMBC spectrum in analogy with **1**. The relative stereochemistry of two methine protons in **2** was established by difference NOE experiments. Irradiation of the signal at δ 5.65 (H-9') produced a 8% enhancement of the H-10' signal at δ 4.11 (*vice versa*). From the above results, the structure of neoacrimarine-G was concluded as **2**. The absolute stereochemistry of neoacrimarine-G remains to be solved.

## EXPERIMENTAL

Isolation The $CH_2Cl_2$-acetone eluate obtained through the separation process of the acetone extract (103.1 g) of Yalaha (*C. paradisi* Macf. x *C. tangerina* Hort. ex Tanaka)[9] was subjected to repeated PTLC using solvent systems [benzene-AcOEt (8:2), acetone-hexane (1:1), $CHCl_3$-acetone (9:1)] gave neoacrimarine-F (1)(21.3 mg). The $CH_2Cl_2$ eluate obtained from silica gel column chromatography of the acetone extract (138 g) of Marsh grapefruit[10] was separated with a silica gel centrifugal

chromatography and finally repeated PTLC [solvents: benzene-acetone (8:2), hexane-acetone (7:3), $CHCl_3$:MeOH (19:1), $CHCl_3$:acetone (9:1)] to give neoacrimarine-G (2)(4.3 mg).

Neoacrimarine-F (1) Yellow cubes, mp 218 - 220°, $[\alpha]_D$ +130.9° (c=0.22, MeOH); HRMS m/z: 531.1534 ($M^+$, found), 531.1529 (calcd for $C_{29}H_{25}NO_9$); EIMS m/z: 531, 287 (base peak), 288, 287, 286, 272, 258, 244, 201, 188, 160; IR $\nu_{max}$ ($CHCl_3$, $cm^{-1}$): 1730, 1630, 1605, 1590, 1565; UV $\lambda_{max}$ (EtOH, nm): 220 (sh), 264, 295(sh), 330, 369; $^1H$- and $^{13}C$-NMR (DMSO-$d_6$, δ): Table 1; NOE: irradiation at δ 3.18 ($N\text{-}CH_3$) - 10% enhancement at δ 5.57 (H-4); irradiation at δ 3.76 ($OCH_3$) - 6% and 8% enhancement at δ 5.57 (H-4) and 6.11 (H-2); irradiation at δ 4.17 (H-10') - 7% enhancement at δ 5.13 (H-9'); irradiation at δ 5.13 (H-9') - 3% enhancement at δ 4.17 (H-10').

Neoacrimarine-G (2) Yellow oil, $[\alpha]_D$ +130.2° (c= 0.0305, $CHCl_3$), HRMS m/z: 515.1612 ($M^+$, found), 515.1580 (calcd for $C_{29}H_{25}NO_8$); EIMS m/z: 515 ($M^+$), 272, 271, 244, 242, 201, 189, 188 (base peak), 187, 160; IR $\nu_{max}$ (KBr, $cm^{-1}$): 3566, 1734, 1699, 1606, 1558; UV $\lambda_{max}$ (MeOH, nm): 220 (sh), 262, 280 (sh), 315, 324; $^1H$- and $^{13}C$-NMR ($CDCl_3$, δ): Table 1; NOE; irradiation at δ 3.84 ($OCH_3$) - 26% and 6% enhancement at δ 6.26 (H-2) and 5.90 (H-4); irradiation at δ 3.56 ($N\text{-}CH_3$) - 28% enhancement at δ 5.90 (H-4); irradiation at δ 5.65 (H-9') - 8% and 5% enhancement at δ 7.70 (H-6) and 4.11 (H-10'); irradiation at δ 4.11 (H-10') - 8% enhancement at δ 5.65 (H-9').

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