

STRUCTURES OF NEOACRIMARINES -F AND -G, TWO NEW ACRIDONE-COUMARIN DIMERS FROM *CITRUS* PLANTS¹

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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 alkaloids³ 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, $[\alpha]_D^{20} +130.9^\circ$ (MeOH), mp 218 - 220 $^\circ$, 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 C₂₉H₂₅NO₉ 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-acridone⁵ and coumarin skeletons.⁶ The ¹H-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.

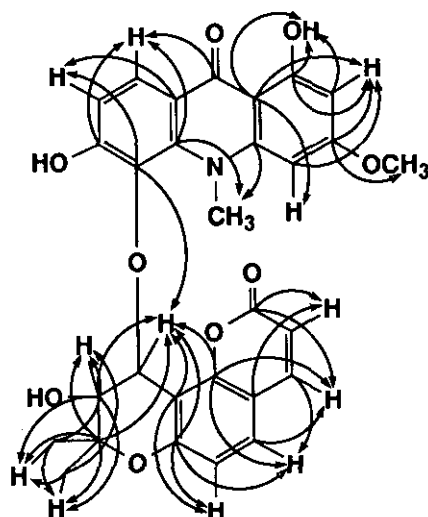
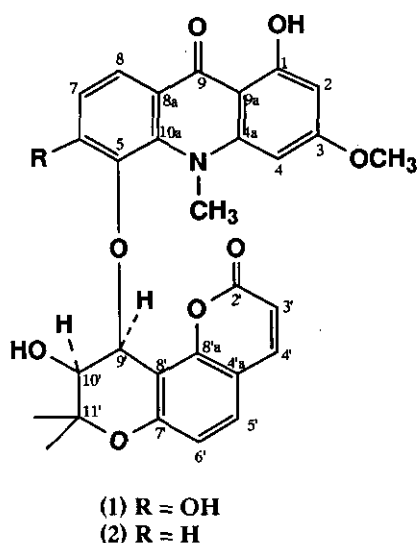


Figure 1 C-H Long-Range Correlations in the HMBC spectrum ($J=5$ and 8 Hz) of neoacrimarine-F (1)

Signals at δ_{H} 3.76, 3.18 (each 3H, s) and δ_{C} 55.4, 42.3 in the ^1H - and ^{13}C -NMR spectra indicated the presence of 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 ^1H -NMR⁷ 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 ^1H -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 $\text{C}_{29}\text{H}_{25}\text{NO}_8$. 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-acridone⁵ and coumarin skeletons.⁶ The ^1H - and ^{13}C -NMR spectra of **2** showed

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

Neocrimarine-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 neocrimarine-G was concluded as 2. The absolute stereochemistry of neocrimarine-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 neocrimarine-F (1) (21.3 mg). The CH_2Cl_2 eluate obtained from silica gel column chromatography of the acetone extract (138 g) of Marsh grapefruit¹⁰ was separated with a silica gel centrifugal

chromatography and finally repeated PTLTLC [solvents: benzene-acetone (8:2), hexane-acetone (7:3), CHCl₃:MeOH (19:1), CHCl₃:acetone (9:1)] to give neoacrimarine-G (2) (4.3 mg).

Neoacrimarine-F (1) Yellow cubes, mp 218 - 220°, [α]_D +130.9° (c=0.22, MeOH); HRMS m/z: 531.1534 (M⁺, found), 531.1529 (calcd for C₂₉H₂₅NO₉); EIMS m/z: 531, 287 (base peak), 288, 287, 286, 272, 258, 244, 201, 188, 160; IR ν_{\max} (CHCl₃, cm⁻¹): 1730, 1630, 1605, 1590, 1565; UV λ_{\max} (EtOH, nm): 220 (sh), 264, 295(sh), 330, 369; ¹H- and ¹³C-NMR (DMSO-d₆, δ): Table 1; NOE: irradiation at δ 3.18 (N-CH₃) - 10% enhancement at δ 5.57 (H-4); irradiation at δ 3.76 (OCH₃) - 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, [α]_D +130.2° (c= 0.0305, CHCl₃), HRMS m/z: 515.1612 (M⁺, found), 515.1580 (calcd for C₂₉H₂₅NO₈); EIMS m/z: 515 (M⁺), 272, 271, 244, 242, 201, 189, 188 (base peak), 187, 160; IR ν_{\max} (KBr, cm⁻¹): 3566, 1734, 1699, 1606, 1558; UV λ_{\max} (MeOH, nm): 220 (sh), 262, 280 (sh), 315, 324; ¹H- and ¹³C-NMR (CDCl₃, δ): Table 1; NOE: irradiation at δ 3.84 (OCH₃) - 26% and 6% enhancement at δ 6.26 (H-2) and 5.90 (H-4); irradiation at δ 3.56 (N-CH₃) - 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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