

THREE NEW ACRIDONE-COUMARIN DIMERS FROM A *CITRUS* PLANT¹

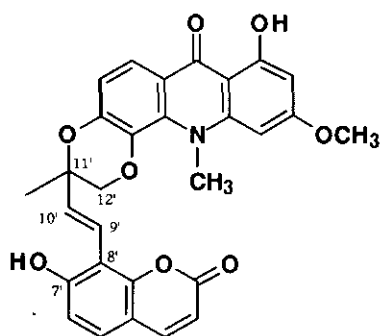
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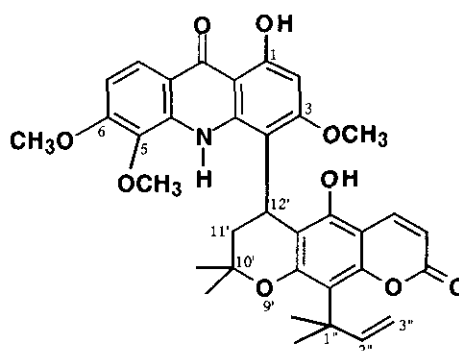
Abstracts——— Three new acridone-coumarin dimers, named dioxinoacrimarine-A (1), neoacrimarine-E (2) and acrimarine-N (3) were isolated from the roots of *Citrus* plant and their structures were elucidated on the basis of spectroscopic methods.

Our studies on the constituents of several species of genus *Citrus* plants (Rutaceae) have resulted in the isolation of many kinds of acridone alkaloids and coumarins.² Especially, acrimarines³ and neoacrimarines⁴ which were acridone-coumarin dimers constructed by various acridone alkaloids with suberosin or other coumarins are characteristic compounds. On continuing our phytochemical studies on the constituents of *Citrus* plants, we studied the roots of "Yalaha" [several hybrid seedlings resulting from a cross of Duncan grapefruit (*Citrus paradisi* Macf.) x Dancy tangerine (*C. tangerina* Hort. ex Tanaka)] and isolated three new acridone-coumarin dimers. The structural characteristic of one of the newly isolated dimeric compounds, named dioxinoacrimarine-A, is the presence of 1,4-dioxane ring connecting the acridone and coumarin nuclei. In this paper, we describe the structure elucidations of these new alkaloids.

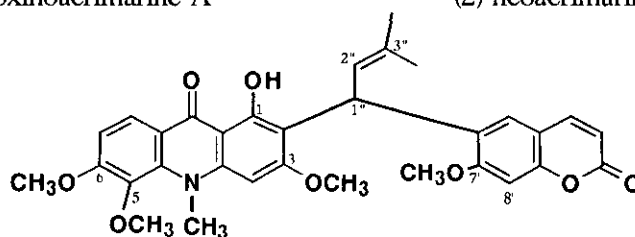
Dioxinoacrimarine-A (1) was isolated as yellow cubes, mp 179-181°C (acetone), $[\alpha]_D^{20} +18.0^\circ$ (CHCl₃). The molecular formula C₂₉H₂₃NO₈ was obtained by HRms (m/z 513.1422 [M⁺]). The ir (1725, 1630, 1600, 1560 cm⁻¹) and uv [220 (sh), 268, 300 (sh), 329, 383 nm] spectra indicated the presence of 1-hydroxy-9-acridone⁵ and coumarin⁶ skeletons. The ¹H-nmr spectrum showed the characteristic signals of hydrogen-bonded hydroxyl group (δ 14.59), *ortho*-coupled [δ 7.84, 7.04 (each 1H, d, J= 8.5 Hz)] and *meta*-coupled [δ 6.47, 6.24 (each 1H, d, J=2.4 Hz)] aromatic protons of 1,3,5,6-tetrasubstituted 9-acridone moiety, two pairs of AB-type signals [δ 7.89, 6.19 (each 1H, d, J= 9.8 Hz), δ 7.40, 6.84 (each 1H, d, J= 8.5 Hz)] of H-4, H-3, H-5, H-6 of coumarin skeleton. The signals of *trans* oriented olefinic protons [δ 7.03, 6.99 (each 1H, d, J=17.0 Hz)], isolated methylene [δ 4.44, 4.21 (each 1H, d, J=11.0 Hz)] and a methyl group (δ 1.58, 3H, s) in ¹H nmr, two doublets at δ



(1) dioxinoacrimarine-A



(2) neoacrimarine-E



(3) acrimarine-N

119.33, 133.05, one singlet at δ 75.95, one triplet at δ 70.18, and one quartet at δ 22.24 in ^{13}C nmr led us to presume that **1** had a 2-methyl-2-substituted 1,4-benzodioxane moiety. Two singlets at δ 3.93, 3.89 in ^1H -nmr and δ 41.49, 55.59 in ^{13}C -nmr spectra showed the presence of an *N*-methyl and a methoxy group. In nOe experiment, on irradiation of the *N*-methyl signal at δ 3.93 showed 8.7% increments on the signal at δ 6.47. When the methoxy signal at δ 3.89 was irradiated, each 6.3% and 11.5% increments were observed on the signal at δ 6.47 and 6.24, respectively, indicating the location of methoxy group at C-3. The above data suggested the structure of dioxinoacrimarine-A was represented as **1**. The structure of dioxinoacrimarine-A was further confirmed through the use of HMBC experiments. Figure 1 shows the ^2J and ^3J correlations found by this technique. Particularly important observations arose from this study were the correlations between methylene protons (H-12') (δ 4.44 and 4.21) with C-5 (δ 131.33), olefinic proton (H-10') (δ 6.99) with C-11' (δ 75.95), C-8' (δ 109.68), C-9' (δ 119.33) and olefinic proton (H-9') (δ 7.03) with C-11' (δ 75.95), C-8' (δ 109.68), C-8'a (δ 152.84), C-7' (δ 160.00) establishing the structure as **1**.

Neoacrimarine-E (**2**) was obtained as yellow cubes, mp 212-215°C (acetone), $[\alpha]_{\text{D}} -21.6^\circ$ (CHCl_3). The HRms of **2** suggested the molecular formula to be $\text{C}_{35}\text{H}_{35}\text{NO}_9$. The ir and uv (see Experimental) spectra showed characteristic absorptions of 1-hydroxy-9-acridone⁵ and coumarin⁶ nuclei. The signals of hydrogen-bonded hydroxyl (δ 14.42), a lone [δ 6.47 (1H, s)] and *ortho*-coupled aromatic protons [δ 7.86, 7.06 (each 1H, d, $J=9.2$ Hz)] in the ^1H nmr spectrum suggested the presence of 1,3,5,6-tetraoxygenated 2- or 4-substituted 9-acridone moiety. The characteristic signals of H-4, H-3 of coumarin skeleton [δ 7.94, 6.02 (each 1H, d, $J=9.8$ Hz)], a 1,1-dimethylallyl group [δ 6.34 (1H,

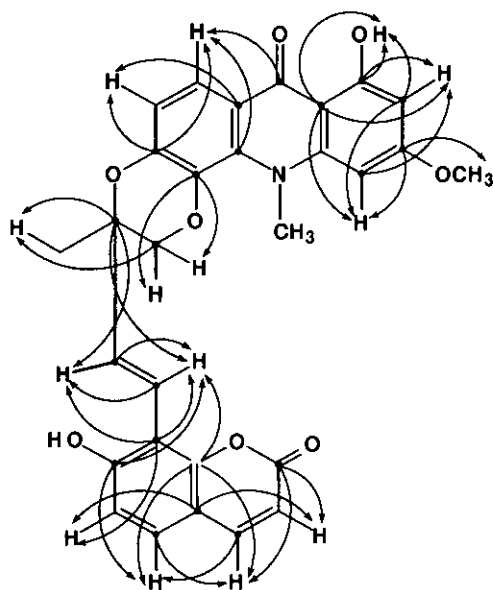


Figure 1 C-H Correlations in the HMBC spectrum ($J=8\text{Hz}$) of dioxinoacrimarine-A

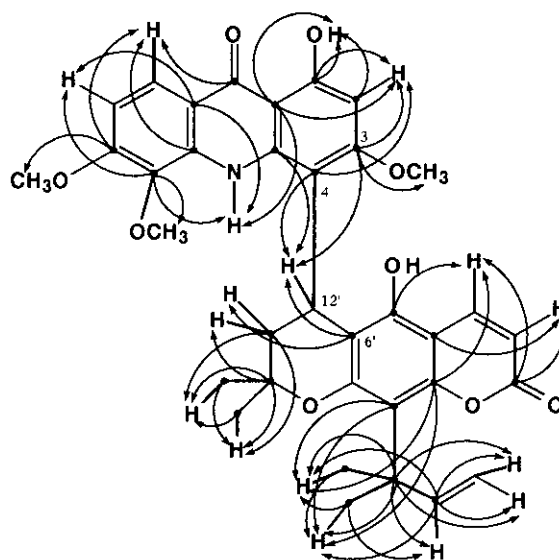


Figure 2 C-H Correlations in the HMBC spectrum ($J=8\text{Hz}$) of neoacrimarine-E

dd, $J=17.7, 10.4$ Hz), 4.94 (1H, d, $J=17.7$ Hz), 4.85 (1H, d, $J=10.4$ Hz), 1.70, 1.69 (each 3H, s) were also observed. The signals at δ 5.03 (1H, dd, $J=12.2, 7.9$ Hz), 2.01 (1H, dd, $J=12.2, 7.9$ Hz), and 1.93 (1H, t, $J=12.2$ Hz) indicated the existence of the partial structure $-\text{CH}_2-\text{CH}-$ of dihydropyranoacoumarin. In nOe experiment, irradiation of methoxy signal at δ 3.97 and 3.91 induced 11.3% and 8.5% increments on the signal at δ 6.47 and 7.06, respectively, indicating the locations of these methoxyl groups at C-3 and C-6. When the methoxy signal at δ 3.61 was irradiated, no increments were observed on any aromatic proton signals, thus the location of this group was assigned to be C-5. From the above results, the structure of this compound was assumed to be that linear type dihydropyranoacoumarin have substituted to C-2 or C-4 of acridone skeleton. Finally, as shown in Figure 2, HMBC experiments allowed unequivocal assignment of the location of coumarin moiety on the acridone ring. Important 2J and 3J correlations were observed between H-12' (δ 5.03) and C-3 (δ 162.03), C-4 (δ 106.52), C-4a (δ 137.90) and C-6' (δ 106.81). The new compound must therefore be assigned the structure 2.

Acrimarine-N (3) was isolated as a yellow oil, $[\alpha]_D \pm 0^\circ (\text{CHCl}_3)$. The molecular formula $\text{C}_{32}\text{H}_{31}\text{NO}_8$ was obtained by HRms. The ir and uv (see Experimental) spectra indicated the presence of 1-hydroxy-9-acridone⁵ and coumarin⁶ skeletons. The ^1H and ^{13}C nmr spectrum of 3 revealed the presence of four methoxy (δ_{H} 4.01, 3.90, 3.77, 3.76; δ_{C} 61.34, 56.38, 55.94, 55.66), an *N*-methyl (δ_{H} 4.00; δ_{C} 40.44), and two vinyl methyl (δ_{H} 1.78, 1.71; δ_{C} 25.97, 18.08) groups. The observation of hydrogen-bonded proton signal at δ_{H} 14.70, carbonyl and an *N*-methyl carbon signals at δ_{C} 180.52 and δ_{C} 40.44 suggested the presence of 1-hydroxy-*N*-methyl-9-acridone system. The presence of a 7-

methoxy-6-substituted coumarin nucleus in the molecule was shown by the AB-type signals at δ 7.66, 6.20 (each 1H, d, $J=9.2$ Hz), two singlets at δ 7.58, 6.69 (each 1H) and a methoxy signal at δ 3.76 (3H, s). The presence of a prenyl group connected with two aryl entities was suggested by the signals at δ 5.96, 5.74 (each 1H, d, $J=9.2$ Hz) and two vinyl methyls (δ 1.78 and 1.71). In nOe experiments, irradiation of the methoxy signal at δ 4.01 gave a 15.9% enhancement of the doublet at δ 6.97 (H-7), irradiation of the methoxy signal at δ 3.76 showed 21.1% increment of the singlet at δ 6.69 (H-8'), and irradiation the *N*-methyl (δ 4.00) and *O*-methyl (δ 3.90) gave 17.6 and 14.2% enhancements of the singlet at δ 6.24, respectively. No nOe enhancement at any aromatic protons was observed on irradiation of the methoxy signal at δ 3.77. The above results indicated the location of the prenyl group in the acridone skeleton at C-2 and four methoxy groups at C-3, C-5, C-6 and C-7'. On the basis of the results stated above, we assigned the structure 3 to acrimarine-N leaving the absolute stereochemistry undetermined.

EXPERIMENTAL

Extraction and Isolation The roots of "Yalaha" [several hybrid seedlings resulting from a cross of Duncan grapefruit (*C. paradisi* Macf.) x Dancy tangerine (*C. tangerina* Hort. ex. Tanaka)] (750 g) collected at the orchard of Okitsu Branch, Fruit Tree Research Station was extracted with acetone (2 x 2l) under reflux for 8 h. The extract (103.1 g) was subjected to column chromatography using silica gel and eluted with toluene, toluene-CH₂Cl₂, CH₂Cl₂, acetone-CH₂Cl₂, acetone and MeOH. The acetone-CH₂Cl₂ (2:8) eluate was further subjected to silica gel column, centrifugal chromatography, PTLC developed with isopropyl ether, AcOEt-benzene (1:9), MeOH-CHCl₃ (1:9), MeOH-CHCl₃ (1:19) to give dioxinoacrimarine-A (3.2 mg), neoacrimarine-E (6.2 mg) and acrimarine-N (3.0 mg) together with many other compounds.

Dioxinoacrimarine-A (1) Yellow cubes, mp 179-181°C (acetone), $[\alpha]_D^{20} +18.0^\circ$ ($c=0.1$, CHCl₃), HRms m/z 513.1422 (M^+ , calcd for C₂₉H₂₃NO₈ 513.1424); Elms m/z 513, 298, 287, 286, 272, 271, 270, 244, 243, 242, 241, 228, 214, 213 (base peak), 199, 185, 162, 134; ir (CHCl₃) 1725, 1630, 1600, 1560 cm⁻¹; uv λ_{max} (EtOH) 220 (sh), 268, 300 (sh), 329, 383 nm; ¹H nmr (DMSO-d₆) δ 14.59 (1H, s, 1-OH), 7.89 (1H, d, $J=9.8$ Hz, H-4'), 7.84 (1H, d, $J=8.5$ Hz, H-8), 7.40 (1H, d, $J=8.5$ Hz, H-5'), 7.04 (1H, d, $J=8.5$ Hz, H-7), 7.03 (1H, d, $J=17.0$ Hz, H-9'), 6.99 (1H, d, $J=17.0$ Hz, H-10'), 6.84 (1H, d, $J=8.5$ Hz, H-6'), 6.47 (1H, d, $J=2.4$ Hz, H-4), 6.24 (1H, d, $J=2.4$ Hz, H-2), 6.19 (1H, d, $J=9.8$ Hz, H-3'), 4.44 (1H, d, $J=11.0$ Hz, H-12'), 4.21 (1H, d, $J=11.0$ Hz, H-12'), 3.93 (3H, s, N-Me), 3.89 (3H, s, 3-OMe), 1.58 (3H, s, 11-Me); NOE: irradiation at δ 3.93 (N-Me) - 8.7% enhancement at δ 6.47 (H-4); irradiation at δ 3.89 (3-MeO) - 6.3% and 11.5% enhancement at δ 6.47 (H-4) and δ 6.24 (H-2); ¹³C nmr (DMSO-d₆) δ 179.20 (C-9), 165.43 (C-3), 163.94 (C-1), 160.00 (x2, C-2', C-7'), 152.84 (C-8'a), 147.16 (x2, C-6, C-4a), 144.89 (C-4'), 135.25 (C-10a), 133.05 (C-10'), 131.33 (C-5), 128.28 (C-5'), 119.33 (C-9'), 118.54 (C-8), 116.23 (C-8a), 113.37 (C-6'), 113.00 (C-3'), 110.98 (C-4'a), 110.66 (C-7), 109.68 (C-8'), 104.12 (C-9a), 94.46 (C-2), 90.62 (C-4), 75.95 (C-11'), 70.18 (C-12'), 55.59 (MeO), 41.49 (NMe), 22.24 (11'-Me).

Neocrimarine-E (2) Yellow cubes, mp 212-215°C (acetone), $[\alpha]_D -21.6^\circ$ ($c=0.3$, CHCl_3), HRms m/z 613.2325 (M^+ , calcd for $\text{C}_{35}\text{H}_{35}\text{NO}_9$ 613.2312); EIms m/z 613, 312, 302, 301, 298, 297 (base peak), 286, 269, 243, 241; ir (CHCl_3) 3400, 1720, 1620, 1600, 1560 cm^{-1} ; uv λ_{max} (EtOH) 205, 255, 326 nm; ^1H nmr ($\text{DMSO}-d_6$) δ 14.42 (1H, s, 1-OH), 9.65 (1H, br s, 5'-OH), 8.21 (1H, s, NH), 7.94 (1H, d, $J=9.8$ Hz, H-4'), 7.86 (1H, d, $J=9.2$ Hz, H-8), 7.06 (1H, d, $J=9.2$ Hz, H-7), 6.47 (1H, s, H-2), 6.34 (1H, dd, $J=17.7, 10.4$ Hz, H-2''), 6.02 (1H, d, $J=9.8$ Hz, H-3'), 5.03 (1H, dd, $J=12.2, 7.9$ Hz, H-12'), 4.94 (1H, d, $J=17.7$ Hz, H-3''), 4.85 (1H, d, $J=10.4$ Hz, H-3''), 3.97 (3H, s, 3-MeO), 3.91 (3H, s, 6-MeO), 3.61 (3H, s, 5-MeO), 2.01 (1H, dd, $J=12.2, 7.9$ Hz, H-11'), 1.93 (1H, t, $J=12.2$ Hz, H-11'), 1.70 (3H, s, 1''-Me), 1.69 (3H, s, 1''-Me), 1.45 (3H, s, 10'-Me), 1.33 (3H, s, 10'-Me); NOE: irradiation at δ 3.97 (3-MeO) - 11.3% enhancement at δ 6.47 (H-2); irradiation at δ 3.91 (6-MeO) - 8.5% enhancement at δ 7.06 (H-7); irradiation at δ 5.03 (H-12') - 5.1% enhancement at δ 2.01 (H-11'); ^{13}C nmr ($\text{DMSO}-d_6$) δ 180.59 (C-9), 162.38 (C-1), 162.03 (C-3), 159.52 (C-2'), 157.20 (C-7'), 154.72 (C-6), 152.11 (C-5'), 150.33 (C-2''), 139.68 (C-4'), 137.90 (C-4a), 134.50 (C-10a), 133.54 (C-5), 121.26 (C-8), 114.66 (C-8'), 113.11 (C-8a), 109.29 (C-3'), 108.23 (C-7), 107.40 (C-3''), 106.81 (C-6'), 106.52 (C-4), 103.63 (C-4'a), 103.14 (C-9a), 92.46 (C-2), 76.54 (C-10'), 60.69 (3-MeO), 56.49 (5-MeO), 56.29 (6-MeO), 37.58 (C-11'); 29.71 (1''-Me), 29.62 (1''-Me), 28.91 (10'-Me), 25.20 (C-12'), 22.62 (10'-Me).

Acrimarine-N (3) Yellow oil, $[\alpha]_D \pm 0^\circ$ ($c=0.3$, CHCl_3), HRms m/z 557.2048 (M^+ , calcd for $\text{C}_{32}\text{H}_{31}\text{NO}_8$ 557.2050); EIms m/z 557 (M^+), 526, 516, 515, 514 (base peak), 502, 484, 368, 354, 340, 328, 315, 242; ir (CHCl_3) 3410 (br), 1720, 1620, 1590, 1560 cm^{-1} ; uv λ_{max} (EtOH) 221 (sh), 255 (sh), 278, 300 (sh), 331; ^1H nmr (CDCl_3) δ 14.70 (1H, s, 1-OH), 8.22 (1H, d, $J=9.2$ Hz, H-8), 7.66 (1H, d, $J=9.2$ Hz, H-4'), 7.58 (1H, s, H-5'), 6.97 (1H, d, $J=9.2$ Hz, H-7), 6.69 (1H, s, H-8'), 6.24 (1H, s, H-4), 6.20 (1H, d, $J=9.2$ Hz, H-3'), 5.96 (1H, $J=9.2$ Hz, H-2''), 5.74 (1H, d, $J=9.2$ Hz, H-1''), 4.01 (3H, s, 6-MeO), 4.00 (3H, s, N-Me), 3.90 (3H, s, 3-MeO), 3.77 (3H, s, 5-MeO), 3.76 (3H, s, 7'-MeO), 1.78, 1.71 (each 3H, s, 3''-Me); NOE: irradiation at δ 4.01 (6-MeO) - 15.9% enhancement at δ 6.97 (H-7); irradiation at δ 4.00 (N-Me) - 17.6% enhancement at δ 6.24 (H-4); irradiation at δ 3.90 (3-MeO) - 14.2% enhancement at δ 6.24 (H-4); irradiation at δ 3.76 (7'-MeO) - 21.1% enhancement at δ 6.69 (H-8'); ^{13}C nmr (CDCl_3) δ 180.52 (C-9), 163.70 (C-3), 161.88 (C-1), 161.80 (C-2'), 160.92 (C-7'), 157.48 (C-6), 154.28 (C-8'a), 146.32 (C-4a), 144.31 (C-4'), 138.57 (C-10a), 137.05 (C-5), 132.76 (C-3''), 130.31 (C-6'), 128.37 (C-5'), 124.43 (C-2''), 123.11 (C-8), 118.02 (C-8a), 112.31 (C-3'), 111.77 (C-4'a), 111.58 (C-2), 107.60 (C-7), 105.31 (C-9a), 98.43 (C-8'), 87.47 (C-4), 61.34 (5-MeO), 56.38 (7'-MeO), 55.94 (3-MeO), 55.66 (6-MeO), 40.44 (N-Me), 32.60 (C-1''), 25.97 (3''-Me), 18.08 (3''-Me).

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