THREE NEW ACRIDONE-COUMARIN DIMERS FROM A *CITRUS* PLANT[1]

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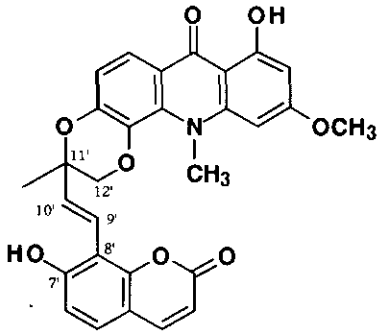
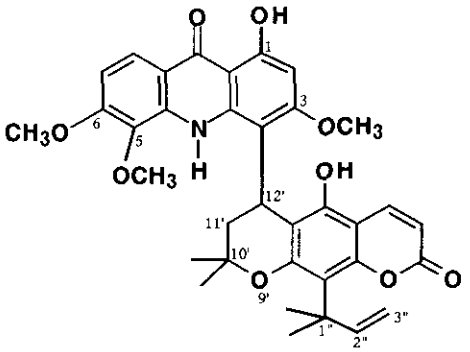
Abstracts——— Three new acridone-coumarin dimers, named dioxinoacrimarine-A (1), neoacrimarine-E (2) and acrimarine-N (3) were isolated from the roots of *Citrus* plant and their structures were elucidated on the basis of spectroscopic methods.

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, acrimarines[3] and neoacrimarines[4] 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.

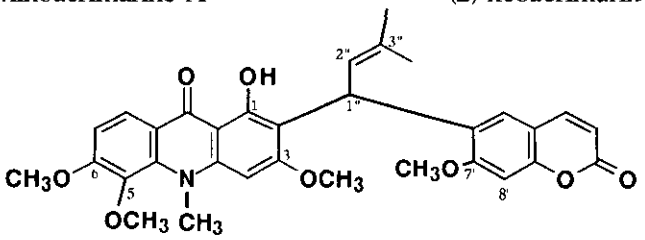
Dioxinoacrimarine-A (1) was isolated as yellow cubes, mp 179-181°C (acetone), $[\alpha]_D$ +18.0° ($CHCl_3$). The molecular formula $C_{29}H_{23}NO_8$ was obtained by HRms (m/z 513.1422 [M^+]). The ir (1725, 1630, 1600, 1560 cm^{-1}) and uv [220 (sh), 268, 300 (sh), 329, 383 nm] spectra indicated the presence of 1-hydroxy-9-acridone[5] and coumarin[6] skeletons. The ^{1}H-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 ^{1}H 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 ^{1}H-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 ^{2}J and ^{3}J 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), $[\alpha]_D$ -21.6°($CHCl_3$). The HRms of **2** suggested the molecular formula to be $C_{35}H_{35}NO_9$. The ir and uv (see Experimental) spectra showed characteristic absorptions of 1-hydroxy-9-acridone[5] and coumarin[6] 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 ^{1}H 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,

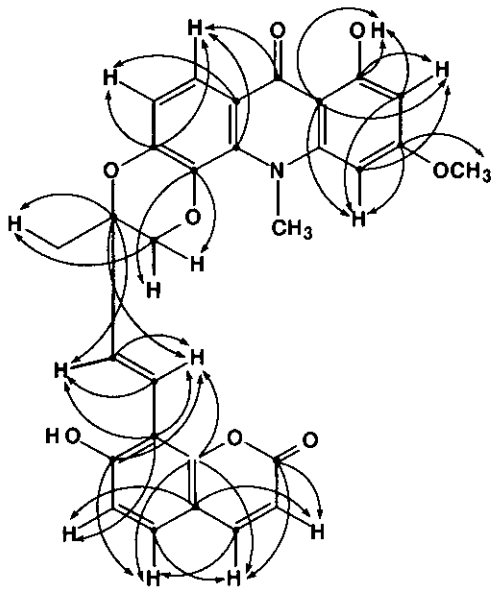


Figure 1 C-H Correlations in the HMBC spectrum (J=8Hz) of dioxinoacrimarine-A

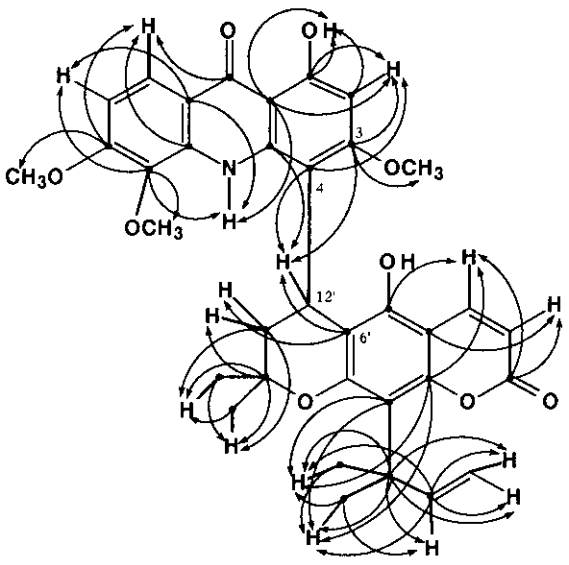


Figure 2 C-H Correlations in the HMBC spectrum (J=8Hz) of neoacrimarine-E

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 -CH_2-CH- of dihydropyranocoumarin. 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 dihydropyranocoumarin 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, $[\alpha]_D$ ±0°($CHCl_3$). The molecular formula $C_{32}H_{31}NO_8$ was obtained by HRms. The ir and uv (see Experimental) spectra indicated the presence of 1-hydroxy-9-acridone[5] and coumarin[6] 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-CH_2Cl_2, CH_2Cl_2, acetone-CH_2Cl_2, acetone and MeOH. The acetone-CH_2Cl_2 (2:8) eluate was further subjected to silica gel column, centrifugal chromatography, PTLC developed with isopropyl ether, AcOEt-benzene (1:9), MeOH-$CHCl_3$ (1:9), MeOH-$CHCl_3$ (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), $[\alpha]_D$ +18.0° (c=0.1, $CHCl_3$), HRms m/z 513.1422 (M^+, calcd for $C_{29}H_{23}NO_8$ 513.1424); EIms m/z 513, 298, 287, 286, 272, 271, 270, 244, 243, 242, 241, 228, 214, 213 (base peak), 199, 185, 162, 134; ir ($CHCl_3$) 1725, 1630, 1600, 1560 cm^{-1}; uv λ_{max} (EtOH) 220 (sh), 268, 300 (sh), 329, 383 nm; ^1H nmr (DMSO-d_6) 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);^{13}C nmr (DMSO-d_6) 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).

Neoacrimarine-E (2) Yellow cubes, mp 212-215°C (acetone), $[\alpha]_D$ -21.6° (c=0.3, $CHCl_3$), HRms m/z 613.2325 (M^+, calcd for $C_{35}H_{35}NO_9$ 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 (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 (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, $[\alpha]_D$ ±0° (c=0.3, $CHCl_3$), HRms m/z 557.2048 (M^+, calcd for $C_{32}H_{31}NO_8$ 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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