TWO NOVEL ACRIDONE-COUMARIN DIMERS, NEOACRIMARINES -A AND -B, FROM CITRUS PLANTS[1]

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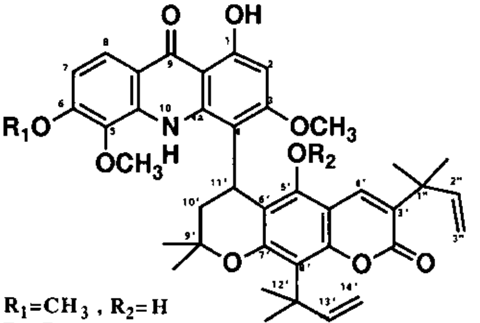
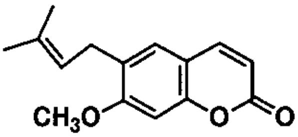
Abstracts —— Two novel acridone-coumarin dimers, named neoacrimarines -A (1) and -B (2), were isolated from the roots of *Citrus* plants and their structures were elucidated by spectroscopic method.

Recently, we reported the isolation and structure elucidation of many acrimarines, the first naturally occurring acridone-coumarin dimers from *Citrus* plants.[2] They are constructed by various acridone alkaloids coupled with suberosin (3)[3] as coumarin moiety. On continuing our phytochemical studies on the constituents of *Citrus* plants, we studied of the roots of "Yalaha"[4] [several hybrid seedlings resulting from a cross of Duncan grapefruit (*Citrus paradisi* Macf.) x Dancy tangerine (*C. tangerina* Hort. ex Tanaka)] and isolated two novel acrimarines. The newly isolated acrimarines were made up of acridone alkaloids and a clausarin[5] derivative as a coumarin moiety. In this communication, we describe the structure elucidations of the new alkaloids. The acetone extract of the root was subjected to column



(1) $R_1=CH_3$, $R_2=H$
(2) $R_1=R_2=H$
(4) $R_1=R_2=CH_3$



(3)

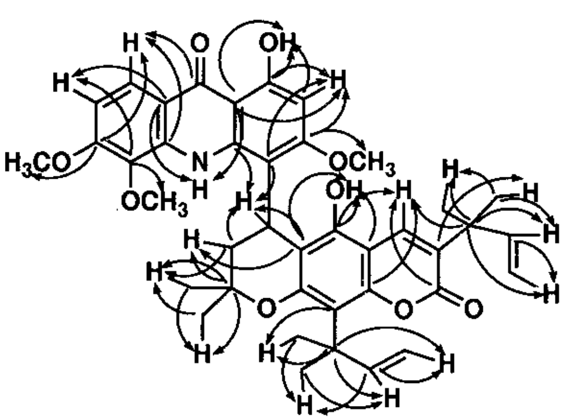


Figure 1 C-H Correlations in the HMBC spectrum (J=8 Hz) of neoacrimarine-A

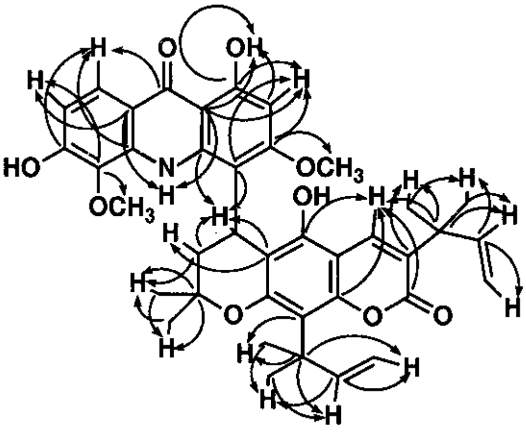


Figure 2 C-H Correlations in the HMBC spectrum (J=8 Hz) of neoacrimarine-B

and centrifugal chromatographies using silica gel. Each fractions were further separated and purified by ptlc to give two new alkaloids, neoacrimarine-A (**1**, 0.000044%) and neoacrimarine-B (**2**, 0.0000256%) along with many known coumarins and acridone alkaloids. Neoacrimarine-A (1) was isolated as yellow cubes, mp 225-230 °C (acetone) , $[\alpha]_D$ ±0° (CHCl3). The molecular formula $C_{40}H_{43}NO_9$ was established by hr fab-ms [m/z 682.3000 $(M+H)^+$]. The uv [216, 256, 266, 292(sh), 329, 396 nm] and ir (3400, 1720, 1635, 1605, 1570 cm^{-1}) spectra showed characteristic absorptions of 1-hydroxy-9-acridone[6] and coumarin[7] moieties. The ^1H nmr spectrum indicated the presence of hydrogen-bonded hydroxy group (δ 14.76), *ortho* coupled protons [δ 8.00 (1H, d, J=8.8 Hz), 6.88 (1H, d, J=8.8 Hz)] and a lone [δ 6.41 (1H, s)] aromatic proton. The lowest signal at δ 8.00 is deshielded by carbonyl function and could be assigned to H-8. Therefore, *ortho* coupled protons were concluded to be located at C-8 and C-7. The presence of two 1,1-dimethylallyl groups was suggested by the signals at δ 6.40 (1H, dd, J=10.8, 17.6 Hz), 6.08 (1H, dd, J=10.8, 17.6 Hz), 5.01 (1H, d, J=17.6 Hz), 4.99 (1H, d, J=17.6 Hz), 4.98 (1H, d, J=10.8 Hz), 4.90 (1H, d, J=10.8 Hz), 1.77 and 1.37 (each 6H, s). The presence of -CH-CH_2- moiety was assumed by the signals at δ 5.10 (1H, dd, J=12.7, 7.8 Hz), 2.08 (1H, t, J=12.7 Hz) and 1.94 (1H, dd, J=12.7, 7.8 Hz). Additional signals were observed at δ 1.49, 1.39 (each 3H, s), 7.69 (1H, s) and 5.90 (1H, s). These spectra indicated the presence of 1,3,5,6-tetraoxygenated 9-acridone and dihydroclausarin moieties in this compound. Three singlets at δ 4.02, 3.94, and 3.61 in ^{1}H nmr and at δ 61.02, 56.54, and 56.19 in ^{13}C nmr spectra indicated the presence of three methoxy groups. The locations of three methoxy groups were determined by nOe experiments. Irradiation of the signals at δ 3.94 and 4.02 showed 14% and 17% increments of the signals at δ 6.88 (H-7) and 6.41 (H-2), respectively. On the other hands, when the signal at δ 3.61 was irradiated, no increments were observed on any signals. The linked position of the acridone and the coumarin nuclei was determined by use of ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY and HMBC experiments. The ^{2}J and ^{3}J interactions were represented by the arrows in Figure 1. The particularly important observations were that the signal of H-11' (δ 5.10) correlated to C-4 (δ 99.00), C-4a (δ 140.48), C-6' (δ 103.17) and C-10' (δ 37.94). From these results, the linkage of two nuclei was determined between C-4 and C-11' of acridone and coumarin nuclei. Thus, the structure of neoacrimarine-A was established to be **1**.

Neoacrimarine-B (2)[8] was obtained as yellow cubes, mp 240-243°C (acetone), $[\alpha]_D$ ±0° (pyridine). The molecular formula $C_{39}H_{41}NO_9$ was obtained by hrms which was less CH_2 unit than that of neoacrimarine-A. The ^{1}H nmr spectrum (Table) was similar to that of (1) except for the small differences of chemical shifts and deficiency of one methoxy signal. Irradiation of the signal at δ 3.97 showed 8% increments of the signal at δ 6.45 (H-2), on the other hands, no increments were observed on any aromatic proton signals when the signal at δ 3.65 was irradiated. These results suggested the locations of two methoxy and two hydroxy groups were at C-3, C-5, C-6 and C-5'. Again HMBC was employed to assign the connectivities as shown in Figure 2. From these data, we concluded the structure of neoacrimarine-B as **2**. The structures of neoacrimarines -A and -B were further confirmed by

Table. ^{13}C and ^{1}H nmr spectral data of **1**, **2** and **4** in CDCl$_3$.

	1		**2***		**4**	
	δ C	δ H	δ C	δ H	δ C	δ H
1	165.67	14.76 (s)	162.29	14.55 (s)	163.61	14.48 (s)
2	92.57	6.41 (s)	92.33	6.45 (s)	92.35	6.43 (s)
3	162.50		161.78		161.79	
3 OMe	56.54	4.02 (s)	60.24	3.97 (s)	56.32	4.00 (s)
4	99.00		106.58		108.35	
4a	140.48		137.64		138.80	
5	134.37		132.18		134.38	
5 OMe	61.02	3.61 (s)	56.41	3.65 (s)	60.98	3.62 (s)
6	154.98		153.37		154.75	
6 OMe	56.19	3.94 (s)			56.18	3.92 (s)
7	107.99	6.88 (d, 8.8)	112.66	6.80 (d, 8.5)	107.50	6.83 (d, 9.2)
8	121.82	8.00 (d, 8.8)	121.02	7.71 (d, 8.5)	121.86	7.98 (d, 9.2)
8a	114.39		111.96		114.41	
9	181.41		180.37		181.62	
9a	104.48		103.75		112.26	
10a	135.00		135.26		135.28	
NH		8.43 (s)		8.22 (s)		8.08 (s)
2'	159.61		158.32		159.15	
3'	129.31		127.48		130.82	
4'	132.97	7.69 (s)	133.71	7.82 (s)	132.96	7.53 (s)
4a'	103.53		103.07		105.68	
5'	150.57		151.74		154.47	
5' OMe					61.43	3.39 (s)
5' OH		5.90 (s)		9.62 (br s)		
6'	103.17		106.85		104.44	
7'	155.93		156.26		156.33	
8'	115.94		113.99		119.90	
8a'	152.88		151.88		152.76	
9'	77.46		76.33		77.53	
9' Me	23.28	1.39 (s)	22.50	1.32 (s)	23.24	1.49 (s)
	29.43	1.49 (s)	28.92	1.45 (s)	29.14	1.52 (s)
10'	37.94	1.94 (dd, 12.7, 7.8) 2.08 (t, 12.7)	37.55	2.00 (m)	38.12	1.94 (dd, 7.3, 12.8) 2.04 (t, 12.8)
11'	25.10	5.10 (dd, 12.7, 7.8)	25.12	5.04 (dd, 7.9, 12.2)	25.35	5.19 (dd, 7.3, 12.8)
12'	41.33		40.61		41.64	
12' Me	29.19	1.77 (s)	29.24	1.70 (s)	29.54	1.78 (s)
	30.03		29.62	1.69 (s)	29.94	
13'	150.55	6.40 (dd, 10.8, 17.6)	150.26	6.35 (dd, 10.4, 17.1)	150.36	6.42 (dd, 10.4, 18.9)
14'	107.53	4.90 (d, 10.8) 5.01 (d, 17.6)	107.36	4.90 (dd, 1.2, 17.1) 4.96 (dd, 1.2, 10.4)	107.75	5.00 (d, 10.4) 5.02 (dd, 1.2, 18.9)
1"	40.18		39.73		40.31	
1" Me	25.92	1.37 (s)	25.89	1.28 (s)	25.98	1.37 (s)
					26.03	
2"	145.55	6.08 (dd, 10.8, 17.6)	145.40	6.03 (dd, 17.7, 10.4)	145.50	6.07 (dd, 11, 17.7)
3"	111.80	4.98 (d, 10.8) 4.99 (d, 17.6)	111.47	4.96 (dd, 1.2, 17.7) 5.04 (dd, 1.2, 10.4)	112.02	4.92 (d, 11) 4.99 (d, 17.7)

Figures in parentheses are J in Hz. *Measured in DMSO-d$_6$.

chemical correlations. By treatment with diazomethane, both **1** and **2** gave the same tetramethoxy compound (4)[9]. The ^{1}H nmr spectrum of **4** showed four methoxy signals at δ 4.00, 3.92, 3.62 and 3.39. In the nOe experiments, when the signals at δ 4.00 (3-MeO) and 3.92 (6-MeO) were irradiated, 24% and 16% increments were observed on the signals at δ 6.43 (H-2) and 6.83 (H-7). No increments were observed on any signals on irradiation the signal at δ 3.62 (5-MeO). Irradiation the signal at δ 3.39 (5'-MeO), 8% and 6% increments were observed on the signals at δ 7.53 (H-4') and 5.19 (H-11'). The above results supported the linear structure of pyrano coumarin moiety and we confirmed the structure of neoacrimarines -A and -B as **1** and **2**.

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8 Neoacrimarine-B (2), hrms m/z 667.2784 (M^+, calcd for $C_{39}H_{41}NO_9$ 667.2781); ir (Nujol) 3350, 1690, 1640, 1605 cm^{-1}; uv λ_{max} (EtOH) 215, 256, 264, 280 (sh), 295 (sh), 332 nm.

9 Tetramethoxy compound (4), Yellow oil, hrms m/z 695.3096 (M^+, calcd for $C_{41}H_{45}NO_9$ 695.3094); ir ($CHCl_3$) 3400, 1710, 1630, 1600, 1580, 1560 cm^{-1}; uv λ_{max} (EtOH) 214, 256, 265, 299, 329, 389 nm.

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