NEW ACRIMARINES FROM CITRUS PLANTS[1]

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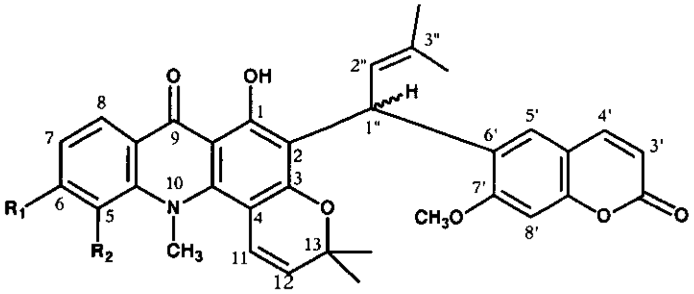
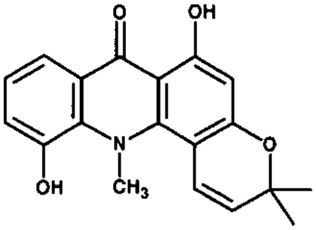
Abstracts ——Five new acrimarines (acridone-coumarin dimers) were isolated from some Citrus plants (Rutaceae) and their structures were elucidated by spectroscopic method.

Recently, we reported the isolation and structure elucidation[2,3] of acrimarine-A ~ -H, dimeric compounds in which an acridone unit has been coupled to a coumarin. Further examination of the roots of "Yalaha" [several hybrid seedlings resulting from a cross of Duncan grapefruit (Citrus paradisi Macf.) x Dancy tangerine (C. tangerine Hort. ex Tanaka)] and "Funadoko" (C. funadoko Hort. ex Tanaka) afforded five new compounds, named acrimarine-I (1), -J (4), -K (5), -L (6) and -M (7) and in this paper we wish to report the isolation and structure elucidation of these new compounds.

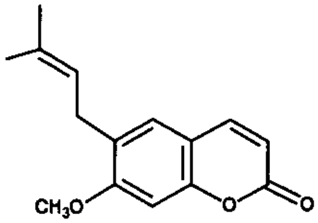
Acrimarine-I (1) was obtained as yellow oil, $[\alpha]_D$ +27.8°($CHCl_3$). The high resolution ms showed molecular formula $C_{34}H_{31}NO_7$, which was considered to give rise to two characteristic ions from the halves of the molecule at m/z 323 and 242 corresponding to formulae $C_{19}H_{17}NO_4$ and $C_{15}H_{14}O_3$, respectively, indicating the presence of acridone and coumarin structures. The ir (3400, 1720, 1620, 1560 cm^{-1}) and uv [205, 223 (sh), 270 (sh), 296, 328, 348 (sh) nm] spectra suggested the presence of 1-hydroxy-9-acridone moiety.[4] The ^1H nmr spectrum showed the presence of hydrogen bonded hydroxyl group (δ 14.63), ABC type aromatic protons [δ 7.94 (1H, dd, J= 7.9, 1.8 Hz), 7.15 (1H, t, J= 7.9 Hz), 7.09 (1H, dd, J= 7.9, 1.8 Hz)], 1,1-dimethylpyran ring [δ 6.61 (1H, d, J= 9.8 Hz), 5.48 (1H, d, J= 9.8 Hz), 1.14, 1.46 (each 3H, s)], characteristic H-4', H-3', H-5' and H-8' of coumarin nucleus [δ 7.67, 6.22 (each 1H, d, J= 9.2 Hz), 7.62, 6.69 (each 1H, s)], and a prenyl group connected with two aryl moieties [δ 5.94 (1H, d, J= 9.8 Hz), 5.74 (1H, d, J= 9.8 Hz), 1.80 (3H, s), 1.75 (3H, s)]. Two singlets at δ 3.75 and 3.72 in the ^1H nmr and two quartets at δ 55.9 and 48.5 in the ^{13}C nmr spectra showed the presence of each one methoxy and N-methyl group. In nOe experiments, irradiation of the methoxy signal at δ 3.75 gave 20% increments of the signal at δ 6.69 (H-8'). On the other hand, when the N-methyl signal at δ 3.72 was irradiated, 13% increments were observed on the signal at δ 6.61 (H-11). From these results, the location of methoxy group was assigned to C-7' and the angular orientation of the dimethylpyran ring was confirmed. The similalities of signal patterns of ^1H nmr spectrum suggested the presence of 5-hydroxynoracronycine (2)[5] and suberosin (3)[6] moieties in the structure. The location of the suberosin unit was elucidated by ^1H detected heteronuclear multiple bond connectivity (HMBC) spectrum. The H-bonded proton signal at δ 14.63 showed long-range correlations with the



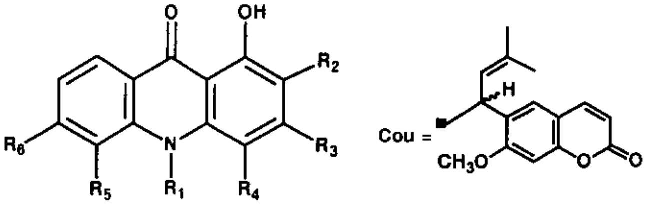
(1) R_1=H , R_2=OH
(4) R_1=OH , R_2=OCH$_3$



(2)



(3)



(5) R_1=CH$_3$, R_2=Cou , R_3=OH , R_4=H , R_5=OCH$_3$, R_6=OH
(6) R_1=R_2=H , R_3=OCH$_3$, R_4=Cou , R_5=OCH$_3$, R_6=OH
(7) R_1=CH$_3$, R_2=Cou , R_3=OH , R_4=R_5=R_6=H

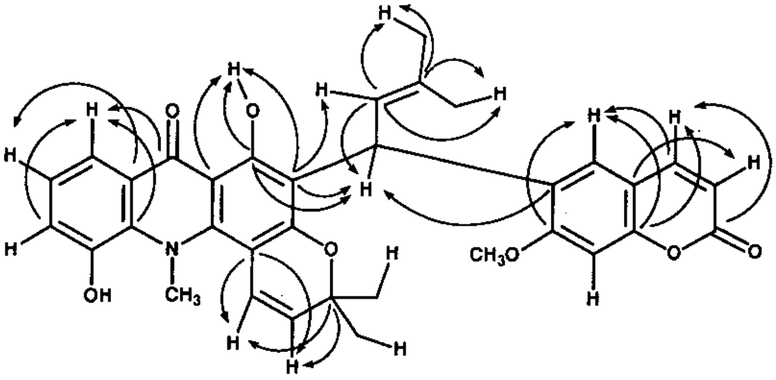


Figure 1

C-H Correlations in the HMBC spectrum (J=8 Hz) of acrimarine-I (1)

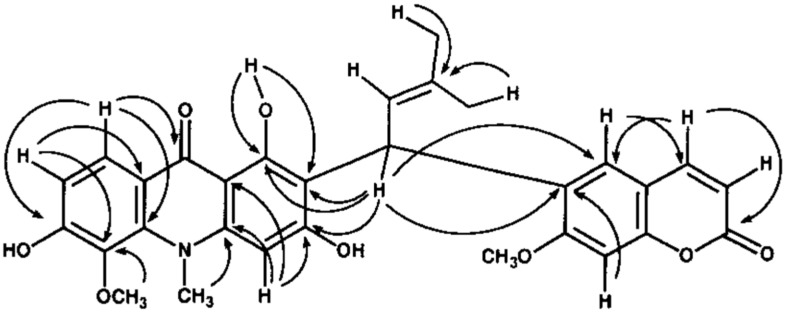


Figure 2

H-C Correlations in H-C long range COSY spectrum (J=5 Hz) of acrimarine-K (5)

carbon signals at δ 162.0 (C-1), 112.6 (C-2), and 107.1 (C-9a). Further, the proton signal at δ 5.74 (H-1") was correlated with carbon signals at δ 162.0 (C-1), 112.6 (C-2), 159.0 (C-3), 132.9 (C-3"), 128.6 (C-5'), and 130.6 (C-6'). Other CH long range correlations observed are shown by arrows in Figure 1. Based on these spectral data, the structure of acrimarine-I was concluded to 1.

Acrimarine-J (4) was obtained as yellow oil, $[\alpha]_D$ ±0°($CHCl_3$). The mass spectrum showed the molecular ion at m/z 595 which corresponded to $C_{35}H_{33}NO_8$, a difference of CH_2O compared with 1. The ^1H nmr features were similar to those of 1, except for appearances of one more methoxy (δ 3.90) signal and change of ABC type aromatic protons to AB type [δ 7.96, 6.97 (each 1H, d, J= 8.5 Hz). The signals at δ 3.90, 3.84, 3.75 in the ^1H nmr and δ 60.5, 56.5, 48.9 in the ^{13}C nmr spectra indicated the presence of two methoxy and one N-methyl group. The location of methoxy groups and the situation of 1,1-dimethylpyran ring were determined by nOe experiments. Irradiation of the signal at δ 3.75 and 3.84 showed 14% and 20% increments of the signals at δ 6.69 (H-11) and 6.83 (H-8'), respectively. When the signal at δ 3.90 was irradiated, no increments were observed on any signals. These results indicated the locations of methoxy groups at C-5 and C-7', phenolic hydroxyl group at C-6, and the angular situation of 1,1-dimethylpyran ring. On the basis of these results, structure **(4)** was assigned to acrimarine-J.

Acrimarine-K (5) was isolated as yellow oil, $[\alpha]_D$ ±0° ($CHCl_3$). The molecular formula $C_{30}H_{27}NO_8$ was established by high resolution ms. The ^1H nmr spectrum showed the signals of ortho coupled [δ 8.04, 6.94 (each 1H, d, J= 8.8 Hz)], isolated [δ 6.48 (1H, s)] aromatic protons, characteristic signals due to H-4', H-3', H-5' and H-8' of coumarin nucleus [δ 7.88, 6.16 (each 1H, d, J= 9.5 Hz), 7.73, 6.82 (each 1H, s)], and signals due to a prenyl group coupled to two aryl groups [δ 1.75 (3H, s), 1.72 (3H, s), 5.83 (1H, d, J= 9.5 Hz) and 6.08 (1H, d, J= 9.5 Hz)]. The signals at δ 3.98, 3.87, 3.77 in ^{1}H nmr and δ 40.2, 56.9, 62.2 in ^{13}C nmr spectra indicated the presence of two methoxy and one N-methyl group. The location of methoxy groups was determined by nOe experiments. Irradiation of the signal at δ 3.98 and 3.87 showed 14% and 16% increments of the signal at δ 6.48 (H-4) and 6.82 (H-8'), respectively. On the other hands, when the signal at δ 3.77 was irradiated, no increments were observed at any proton signals. Thus, the location of two methoxy groups was determined to C-5 and C-7'. From the above data, the location of suberosin unit was assigned at C-2 in the acridone skeleton, and the structure of acrimarine-K was determined to 5. The proposed structure was further confirmed by HC long range COSY shown by arrows in Figure **2**.

Acrimarine-L (6) was isolated as yellow oil, $[\alpha]_D$ ±0° ($CHCl_3$), and gave a molecular ion at m/z 529 which corresponded to $C_{30}H_{27}NO_8$, the same as that of acrimarine-K (5). The ^{1}H nmr spectrum showed a good similarity with that of 5, except for small differences of chemical shifts indicating the presence of suberosin and 1,3,5,6-oxygenated 9-acridone units. The signals at δ 3.97, 3.94 and 3.87 indicated the presence of methoxy groups. In the nOe experiments, irradiation of the signal at δ 3.97 and 3.87 showed 17% and 14% increments of the signal at δ 6.34 (H-2) and 6.92 (H-8'), respectively. On the other hands, when the signal at δ 3.94 was irradiated, no increments were observed. Meanwhile, when the signal at δ 9.14 assumed to NH signal was irradiated, 12% and 9% increments were observed on the signal at δ 5.78 (H-1") and 5.91 (H-2"), respectively. These results indicated the locations of methoxy groups were at C-3, C-5 and C-7', and the linking positions of acridone and coumarin rings were at C-4 and C-6', respectively. The above data led us to assign structure

6 to acrimarine-L.

Acrimarine-M (7) was isolated as yellow oil, $[\alpha]_D$ ±0° ($CHCl_3$). The molecular formula, $C_{29}H_{25}NO_6$, was confirmed by high resolution ms. The ^1H nmr spectrum showed, besides signals of suberosin unit, ABCD type [δ 8.38 (1H, d, J= 8.1 Hz), 7.76 (1H, t, J= 8.1 Hz), 7.73 (1H, d, J= 8.1 Hz), 7.30 (1H, t, J= 8.1 Hz)] and a lone [δ 6.54 (1H, s)] aromatic proton signal and two methyl singlets (δ 3.82 and 3.86). The location of two methyl signals at δ 3.86 and 3.82 was assigned by nOe experiments. When the signal at δ 3.82 was irradiated, each 14% and 10% increments were observed on the signals at δ 7.73 (H-5) and 6.54 (H-4), respectively. On the other hands, irradiation of the signal at δ 3.86 showed 11% increments of the signal at δ 6.82 (H-8'). The above data showed the presence of N-methyl group, the location of methoxy group at C-7', and the coupling position of the acridone and the coumarin nuclei was at C-2 and C-6'. From these results, the structure 7 was assigned to acrimarine-M. The absolute configurations of these new acrimarines remain to be determined.

EXPERIMENTAL

Extraction and Isolation of Acrimarine-I (1) and -J (4) The roots of "Yalaha" [several hybrid seedlings resulting from a cross of Duncan grapefruit (*C. paradisi* Macf.) x Dancy tangerine (*C. tangerine* Hort. ex Tanaka)](750 g) collected at the orchard of Okitsu Branch, Fruit Tree Research Station was extracted with acetone (2 L x 2) under reflux for 8hr. The extract (103.1 g) was chromatographed over silica gel column and eluted with toluene, toluene-CH_2Cl_2, CH_2Cl_2, acetone-CH_2Cl_2, acetone and MeOH. The acetone-CH_2Cl_2 (1:9) eluate was further separated by silica gel column, centrifugal chromatography, PTLC developed with acetone-$CHCl_3$

(1:9), MeOH-$CHCl_3$ (1:19), acetone-benzene (2:8) to give acrimarine-I (2.3 mg) and -J (5.3 mg) together with many other compounds.

Isolation of Acrimarine-K (5), -L (6) and -M (7) The AcOEt eluate of silica gel column chromatography of acetone extracts of C. funadoko Hort. ex Tanaka reported before[2] was further separated by PTLC using appropriate solvent systems of isopropyl ether, acetone-$CHCl_3$(1:9), acetone-benzene (2:8), MeOH-$CHCl_3$ (1:19), AcOEt-benzene (3:7) to give acrimarine-K (13.7 mg), -L (3.2 mg) and -M (2.1 mg).

Acrimarine-I (1) Yellow oil, $[\alpha]_D^{26}$ $+27.8^{\circ}$ (c= 0.036, $CHCl_3$); uv λ_{max} (EtOH): 205, 223(sh), 270 (sh), 296, 328, 348 (sh) nm; ir ($CHCl_3$) 3400, 1720, 1620, 1560 cm^{-1}; hrms m/z 565.2115 $[M]^+$ ($C_{34}H_{31}NO_7$ calcd 565.2101), m/z 323.1135 $[(C_{19}H_{17}NO_4)^+$, calcd 323.1158], m/z 242.0950 $[(C_{15}H_{14}O_3)^+$, calcd 242.0943]; eims m/z: 565 (M^+, base peak), 550, 522, 496, 380, 365, 312, 297.

Acrimarine-J (4) Yellow oil, $[\alpha]_D^{28}$ $\pm 0^{\circ}$ (c= 0.106, $CHCl_3$); uv λ_{max} (EtOH) 205, 226 (sh), 271, 287, 304 (sh), 343 nm; ir ($CHCl_3$) 3440, 1720, 1620, 1560 cm^{-1}; hrms m/z 595.2209 $[M]^+$ ($C_{35}H_{33}NO_8$ calcd 595.2206); eims m/z 595 (M^+, base peak), 580, 552, 526, 484, 406, 354, 331, 278, 242.

Acrimarine-K (5) Yellow oil, $[\alpha]_D^{25}$ $\pm 0^{\circ}$ (c= 0.0695, $CHCl_3$); uv λ_{max} (MeOH) 205, 219, 263, 276, 300 (sh), 331 nm; ir ($CHCl_3$) 3500, 3400 (br), 1720, 1620, 1600, 1570 cm^{-1}; hrms m/z 529.1751 $[M]^+$ ($C_{30}H_{27}NO_8$ calcd 529.1734), m/z 243.0980 $[(C_{15}H_{15}O_3)^+$, calcd 243.1019], m/z 287.0826 $[(C_{15}H_{13}NO_5)^+$, calcd 287.0793]; eims m/z 529 (M^+), 486, 288, 287 (base peak), 273, 272, 244, 243, 242, 230, 228, 227, 214, 213, 212, 211.

Acrimarine-L (6) Yellow oil, $[\alpha]_D^{25}$ $\pm 0^{\circ}$ (c= 0.061, $CHCl_3$); uv λ_{max} (MeOH) 205, 221, 256, 266, 286 (sh), 295 (sh), 328 nm; ir ($CHCl_3$) 3380 (br), 1730, 1640, 1620, 1570 cm^{-1}; hrms m/z 529.1730 $[M]^+$ ($C_{30}H_{27}NO_8$ calcd 529.1734), m/z 242.0918 $[(C_{15}H_{14}O_3)^+$, calcd 242.0942], m/z 287.0751

Table. ^{1}H and ^{13}C nmr spectral data of acrimarines (acetone-d_6)

	1*		4		5		6	7
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_H
1-OH	14.63	162.0	15.07	162.8	15.26	164.1	14.65	15.42
2		112.6		112.3		105.5	6.34	
3		159.0		161.8		163.6		
3-OMe							3.97	
4		102.4		103.4	6.48	92.8		6.54
4a		146.2		146.8		147.0		
5		146.3		143.5		136.6		7.73 d (8.1)
5-OMe			3.90	60.5	3.77	62.2	3.94	
6	7.09 dd (7.9, 1.8)	120.0		159.4		157.3		7.76 t (8.1)
7	7.15 t (7.9)	123.0	6.97 d (8.5)	113.8	6.94 d (8.8)	113.3	6.88 d (8.7)	7.30 t (8.1)
8	7.94 dd (7.9, 1.8)	118.6	7.96 d (8.5)	124.9	8.04 d (8.8)	124.2	7.84 d (8.7)	8.38 d (8.1)
8a		125.6		118.6		117.9		
9		182.1		182.4		181.3		
9a		107.1		106.9		112.8		
10a		137.0		132.5		139.9		
N-Me	3.72	48.5	3.75	48.9	3.98	40.2		3.82
N-H							9.14	
11	6.61 d (9.8)	121.3	6.69 d (9.8)	121.9				
12	5.48 d (9.8)	123.6	5.81 d (9.8)	123.3				
13		76.4		77.3				
13-Me	1.14	27.3	1.50	26.1				
	1.46	32.7	1.50	27.4				
2'		161.9		161.3		161.8		
3'	6.22 d (9.2)	112.4	6.17 d (9.8)	113.2	6.16 d (9.5)	113.5	6.21 d (9.7)	6.15 d (9.5)
4'	7.67 d (9.2)	144.2	7.92 d (9.8)	145.0	7.88 d (9.5)	145.6	7.98 d (9.7)	7.89 d (9.5)
4a'		111.5		113.3		112.8		
5'	7.62	128.6	7.76	129.7	7.73	130.0	7.78	7.74
6'		130.6		131.0		111.7		
7'		160.9		159.4		162.3		
7'-OMe	3.75	55.9	3.84	56.5	3.87	56.9	3.87	3.86
8'	6.69	98.4	6.83	99.2	6.82	99.6	6.92	6.82
8'a		154.3		155.4		155.8		
1"	5.74 d (9.8)	26.4	5.63 d (9.8)	33.5	5.83 d (9.5)	34.1	5.78 d (8.4)	5.83 d (9.5)
2"	5.94 d (9.8)	124.1	6.01 d (9.8)	125.8	6.08 d (9.5)	126.7	5.91 d (8.4)	6.09 d (9.5)
3"		132.9		132.5		131.3		
3"-Me	1.75	18.1	1.74	18.2	1.72	18.7	1.66	1.73
	1.80	26.1	1.76	26.6	1.75	26.5	1.82	1.75

Values are given in δ ppm. Figures in parenthses are coupling constant (J) in Hz. * Measured in $CDCl_3$.

[$(C_{15}H_{13}NO_5)^+$, calcd 287.0792]; eims m/z 529 (M^+), 301, 288, 287, 272, 244, 243, 242, 229, 228, 227, 214, 213 (base peak), 212, 211. ^{13}C nmr (acetone-d_6) δ 57.0, 57.2, 61.5.[7]

Acrimarine-M (7) Yellow oil, $[\alpha]_D^{25}$ ±0^0 (c= 0.052, $CHCl_3$); uv λ_{max} (MeOH) 205, 224, 248, 277, 296 (sh), 331 nm; ir ($CHCl_3$) 3400 (br), 1725, 1620, 1600 cm^{-1}; hrms m/z 483.1707 $[M]^+$ ($C_{29}H_{25}NO_6$ calcd 483.1681), m/z 241.0735 [$(C_{14}H_{11}NO_3)^+$, calcd 241.0737], m/z 242.0979 [$(C_{15}H_{14}O_3)^+$, calcd 242.0942]; eims m/z 483 (M^+), 441, 440 (base peak), 396, 294, 292, 266, 254, 243, 242, 241, 228, 227, 226, 213, 212, 211.

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7. Because of the small quantity of the sample available, it was impossible to detect the other signals.

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