The Structures of Neoacrimarines-C and -D, Two New Acridone-Coumarin Dimers from *Citrus hassaku*[1)]

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Two new acridone-coumarin dimers, named neoacrimarine-C (1) and -D (2), were isolated from the root of *Citrus hassaku*. Their structures were elucidated on the basis of spectroscopic data.

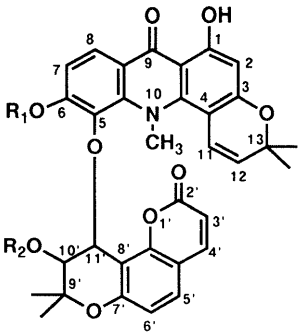
Keywords neoacrimarine; acridone-coumarin dimer; *Citrus*; Rutaceae; *Citrus hassaku*; neoacrimarine-C, -D

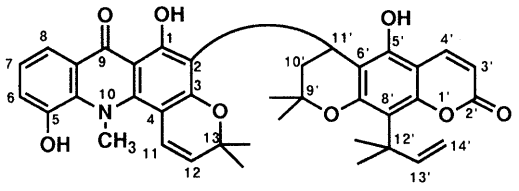
In our phytochemical studies of *Citrus* plants (Rutaceae), we have isolated many kinds of coumarins,[2)] acridone alkaloids,[3)] and flavanones.[4)] In particular, many acridone-coumarin dimers, named acrimarines,[5)] which are constructed from various acridone alkaloids and suberosin as a coumarin unit, are characteristic constituents. We recently reported the isolation and structural elucidation of two novel type acridone-coumarin dimers, named neoacrimarines-A and -B,[6)] which contain coumarin units other than suberosin. In further investigations of the constituents of *Citrus* plants, we isolated two novel neoacrimarines, namely neoacrimarine-C (**1**) and neoacrimarine-D (**2**), from the root of *Citrus hassaku* HORT. ex. TANAKA. In this paper, we wish to report the structural elucidations of these new compounds.

Structure of Neoacrimarine-C (1) Neoacrimarine-C (**1**) was isolated as yellow cubes, mp 111—113 °C, $[\alpha]_D$ +42.3° ($CHCl_3$). The molecular formula $C_{33}H_{29}NO_9$ was established from the high-resolution (HR) fast atom bombardment (FAB) mass spectrum (MS). The observation of a hydrogen-bonded hydroxyl signal at δ_H 14.67, an N-methyl signal at δ_H 3.42, and a carbonyl and an N-methyl signal at δ_C 182.79 and δ_C 51.35 ppm in the ^{1}H- and ^{13}C-NMR spectra, together with UV absorptions at λ_{max} 270, 283 (sh), 400 nm and a strong IR band at ν_{max} 1620 cm^{-1} suggested the presence of a 1-hydroxy-*N*-methyl-9-acridone moiety.[7)] The existence of a 7-oxygenated-8-substituted coumarin nucleus was indicated by the observations of two pairs of characteristic AB-type signals at δ_H 7.55 (H-4′) and 5.57 (H-3′) (J=9.3 Hz), and δ_H 7.62 (H-5′) and 6.93 (H-6′) (J=8.8 Hz), together with a lactone carbonyl signal at δ_C 159.05 and an IR band at ν_{max} 1720 cm^{-1}.

In the aromatic proton region of the ^{1}H-NMR spectrum, *ortho*-coupled doublets [δ_H 8.01 and 7.00 (J=8.8 Hz)] and a singlet (δ_H 5.99) were observed. The doublet at δ_H 8.01 is characteristic of H-8 in the acridone moiety,[7)] and a singlet at δ_H 5.99 was assignable to H-2. The AB quartet at δ_H 5.29 and 5.06 (each 1H, d, J=9.8 Hz) and the singlets at δ_H 1.31 and 1.47 (each 3H, s) suggested the presence of a 2,2-dimethylpyran ring. The remaining signals at δ_H 5.36, 4.39 (each 1H, d, J=4.4 Hz) and δ_H 1.65, 1.80 (each 3H, s) could be assigned to H-4, H-3 and *gem*-dimethyl of the other 2,2-dimethyldihydropyran ring, respectively. In the nuclear Overhauser effect (NOE) experiment, irradiation of the N-methyl signal at δ_H 3.42 induced an 11% increment of the signal of δ_H 5.06 (H-11). The result indicates the angular orientation of 2,2-dimethylpyran ring in the acridone moiety. The unusual high-field shift of the H-11 signal (δ_H 5.06) was attributed to the shielding effect of the coumarin ring. The above data suggested the presence of citracridone-III[8)] as an acridone unit and khellactone[9)] as a coumarin unit. The electron impact (EI)-MS supported these structures, showing prominent ions at m/z 244 ($C_{14}H_{12}O_4$, 37%) and 339 ($C_{19}H_{17}NO_5$, 39%) corresponding to coumarin and acridone units, respectively.

The linking position of the acridone and coumarin moieties was established with the aid of ^{1}H–^{1}H correlation spectroscopy (COSY), ^{1}H–^{13}C COSY, and heteronuclear





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1 : R_1= R_2= H

3 : R_1= Me, R_2= H

4 : R_1= R_2= Ac

multiple-bond connectivity (HMBC) spectroscopy. Figure 1 shows the qualitative multiple-bond interactions found by HMBC experiments. The key correlations included those between the methine proton (H-11′) and C-7′, C-8′a, C-5, C-8′, C-9′ and C-10′, which enabled the assignment of the ether linkage between C-5 and C-11′. Further confirmation of the structure was obtained through the spectroscopic examination of the mono-methyl ether (**3**), which was prepared by the treatment of neoacrimarine-C (**1**) with diazomethane. In the NOE experiment on **3**, irradiation of the newly generated methoxy signal at δ_H 4.02 showed an 11% increment of the signal at δ_H 7.19 (H-7), suggesting the location of the free hydroxyl group at C-6 and linkage at C-5. In the ^{1}H-NMR spectrum of the diacetate (**4**), which was obtained by the treatment of **1** with acetic anhydride–pyridine, the proton signal of C-10′ at δ_H 4.39 in **1** showed a downfield shift to δ_H 6.12, suggesting the location of the ether linkage at C-11′ of the coumarin moiety. The relative stereochemistry of the hydroxyl group (C-10′) and the ether linkage (C-11′) was determined to be *cis* from the coupling constants (J=4.4 Hz) between H-11′ (δ_H 5.36) and H-10′ (δ_H 4.39), and the observation of 6 and 9% NOE increments between these protons in the ^{1}H-NMR spectrum. From the results mentioned above, the structure of neoacrimarine-C was established to be **1**. Neoacrimarine-C (**1**) is considered biogenetically to be derived from citracridone-III and *cis*-khellactone.

Structure of Neoacrimarine-D (2) Neoacrimarine-D was obtained as an optically inactive yellow oil. The molecular formula $C_{38}H_{37}NO_8$ was established by HR FAB-MS. The fragment ions at m/z 323 ($C_{19}H_{17}NO_4$, 53%) and 312 ($C_{19}H_{20}O_4$, 31%) in the EI-MS suggested the presence of acridone and coumarin units, respectively. The IR and UV spectra also indicated the presence of acridone and coumarin moieties.[7,10] The ^{1}H-NMR spectrum showed the characteristic hydrogen-bonded hydroxyl group (δ_H 15.59) and ABC-type aromatic protons [δ_H 7.84 (1H, d, J=8.1 Hz), 7.49 (1H, d, J=8.1 Hz), 7.35 (1H, t, J=8.1 Hz)] attributed to H-8, H-6 and H-7, respectively, the lowest-field proton being deshielded by the 9-carbonyl group. The presence of the 2,2-dimethylpyran ring was suggested by the AB signals at δ_H 6.69 and 5.57 (each 1H, d, J=8.8 Hz), and two singlets at δ_H 1.30 and 1.42 (each 3H, s). The signals at δ_H 3.83 in the ^{1}H-NMR and δ_C 49.58 in the ^{13}C-NMR spectra indicated the presence of an N-methyl group. Irradiation of the N-methyl signal induced a 9% increment in the signal at δ_H 6.69 (H-11), suggesting the angular orientation of the 2,2-dimethylpyran ring fused to the acridone nucleus. Thus, the acridone moiety was considered to be 2-substituted 5-hydroxynoracronycine.[11] The signals at δ_H 7.98 and 5.98 (each 1H, d, J=9.8 Hz) are characteristic of H-4 and H-3 of the coumarin nucleus, and the signals at δ_H 6.37 (1H, dd, J=10.3, 17.6 Hz), 4.94 (1H, dd, J=1.5, 17.6 Hz), 4.83 (1H, dd, J=1.5, 10.3 Hz), 1.71 and 1.73 (each 3H, s) suggested the presence of a 1,1-dimethylallyl group. The remaining signals at δ_H 4.87 (1H, dd, J=11.0, 1.5 Hz), 2.26 (1H, t, J=11.0 Hz), and 2.00 (1H, m) indicated the existence of a –CH–CH_2– moiety. From the above data, the coumarin moiety was considered to contain a 2,2-dimethyldihydropyran ring.

The linking position of the acridone and coumarin moieties and the orientation of the 2,2-dimethylpyran ring of the coumarin moiety were resolved by means of an HMBC experiment. As shown in Fig. 2, H-11′ showed connectivities with C-2 and C-3. Therefore, the linkage could be placed between C-2 and C-11′. A cross peak was also observed between H-11′ and C-5′, so the linear orientation of the pyran ring of the coumarin moiety was established. From the above results coupled with the C-H long-range correlations shown by arrows in Fig. 2, the structure of neoacrimarine-D was assigned as **2**.

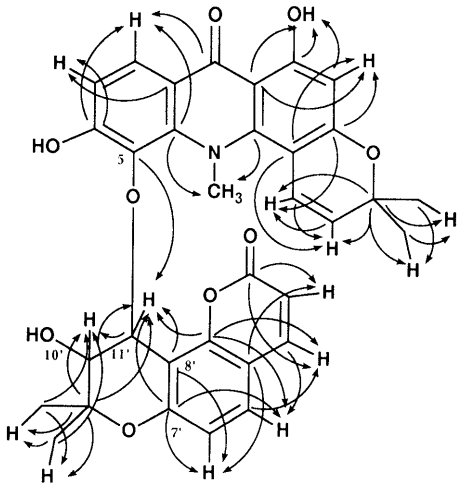

Fig. 1. C–H Long-Range Correlations in the HMBC Spectrum of Neoacrimarine-C (**1**)

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-360 polarimeter. UV spectra were measured on a Shimadzu UV-160A spectrometer and IR spectra were taken with a Shimadzu IR-435 spectrometer. NMR spectra were recorded on a JEOL 200 FX or JEOL-GSX 500 spectrometer and chemical shifts are given in a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. HMBC spectra

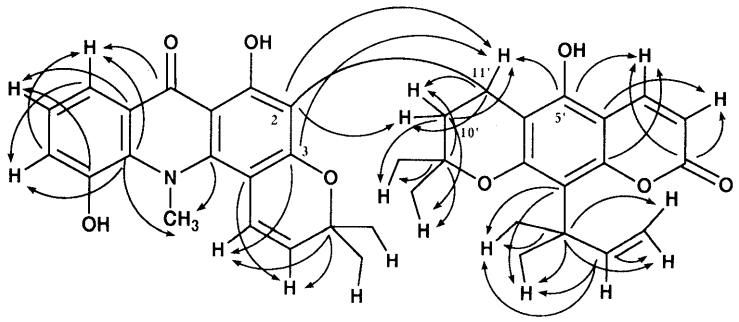

Fig. 2. C–H Long-Range Correlations in the HMBC Spectrum of Neoacrimarine-D (**2**)

TABLE I. ^{13}C- and ^{1}H-NMR Spectral Data for Neoacrimarine-C, -D in Acetone-d_6

	Neoacrimarine-C (1)		Neoacrimarine-D (2)	
	δ_C	δ_H	δ_C	δ_H
1	166.01	14.67 (s)	160.10	15.59 (s)
2	98.75	5.99 (s)	111.48	
3	161.60		160.13	
4	103.15		104.00	
4a	148.03		147.89	
5	138.52		149.79	
6	156.87		121.40	7.49 (d, J=8.1 Hz)
7	114.75	7.00 (d, J=8.8 Hz)	124.75	7.35 (t, J=8.1 Hz)
8	124.31	8.01 (d, J=8.8 Hz)	117.65	7.84 (d, J=8.1 Hz)
8a	119.36		125.21	
9	182.79		183.43	
9a	107.48		112.03	
10a	146.06		138.42	
N Me	51.35	3.42 (s)	49.58	3.83 (s)
11	122.06	5.06 (d, J=9.8 Hz)	121.97	6.69 (d, J=8.8 Hz)
12	124.09	5.29 (d, J=9.8 Hz)	125.17	5.57 (d, J=8.8 Hz)
13	77.33		77.79	
13 Me	26.55	1.31 (s)	28.02	1.30 (s)
	29.16	1.47 (s)		1.42 (s)
2′	159.05		161.47	
3′	113.19	5.57 (d, J=9.3 Hz)	110.52	5.98 (d, J=9.8 Hz)
4′	144.44	7.55 (d, J=9.3 Hz)	140.57	7.98 (d, J=9.8 Hz)
4′a	114.00		104.69	
5′	131.61	7.62 (d, J=8.8 Hz)	151.51	
6′	115.39	6.93 (d, J=8.8 Hz)	107.47	
7′	157.66		159.31	
8′	109.90		115.87	
8′a	155.35		153.92	
9′	80.42		77.57	
9′Me	22.30	1.80 (s)	30.94	1.35 (s)
	29.34	1.65 (s)		1.51 (s)
10′	72.87	4.39 (d, J=4.4 Hz)	38.84	2.00 (m)
				2.26 (t, J=11.0 Hz)
11′	76.67	5.36 (d, J=4.4 Hz)	23.48	4.87 (dd, J=11.0, 1.5 Hz)
12′			42.22	
12′Me			31.32	1.71 (s)
				1.73 (s)
13′			152.19	6.37 (dd, J=10.3, 17.6 Hz)
14′			108.30	4.83 (dd, J=10.3, 1.5 Hz)
				4.94 (dd, J=17.6, 1.5 Hz)

were measured at J=3.3, 5, and 8 Hz on the JEOL GX-400 spectrometer. For column chromatography, Wakogel 60 was used. Preparative thin-layer chromatography (PTLC) was carried out on precoated Merck Kieselgel 60 plates.

Extraction and Isolation The acetone extract (485 g) of dried roots (3.2 kg) of *Citrus hassaku* collected at Innoshima (Hiroshima Prefecture) was subjected to silica gel column chromatography and eluted with hexane, benzene, CH_2Cl_2, acetone and MeOH. The acetone eluate was further separated by column, centrifugal, and preparative thin-layer chromatography (TLC) [solvents: isopropyl ether, *n*-hexane–acetone (1 : 1), MeOH–benzene (19 : 1), $CHCl_3$–acetone (9 : 1)] to give neoacrimarine-C (**1**) (14.3 mg) and neoacrimarine-D (**2**) (9.6 mg).

Neoacrimarine-C (1) Yellow cubes, mp 111—113 °C, $[\alpha]_D$ +42.3° (c=0.026, $CHCl_3$). HR FAB-MS Calcd for $C_{33}H_{30}NO_9$: 584.1921. Found: 584.1930 $[M+H]^+$. EI-MS m/z: 339, 337, 325, 324 (base peak), 322, 309, 294, 244, 201, 188, 160. UV λ_{max}^{MeOH} nm: 270, 283 (sh), 303 (sh), 337 (sh), 400. IR ($CHCl_3$): 1720, 1620, 1600 cm^{-1}. NOE: irradiation of the signal at δ_H 5.36 (H-11′)–6% enhancement of the signal at δ_H 4.39 (H-10′); irradiation at δ_H 4.39 (H-10′)–9% enhancement at δ_H 5.36 (H-11′); irradiation at δ_H 3.42 (N-Me)–11% enhancement at δ_H 5.06 (H-11). ^{1}H- and ^{13}C-NMR (acetone-d_6, δ): see Table I.

***O*-Methylation of Neoacrimarine-C** To a solution of neoacrimarine-C (**1**) (6.1 mg) in 5 ml of MeOH, 10 ml of ethereal diazomethane prepared in the usual manner was added and the mixture was allowed to stand overnight at room temperature. The solvent was evaporated, and the residue was subjected to PTLC [*n*-hexane–acetone (3 : 1)] to give **3** as a yellow oil. Yield 1.4 mg. EI-MS m/z: 597 (M^+), 354, 353, 352, 339, 338 (base peak), 322, 305, 244, 203, 201, 189, 188, 187, 175, 160. UV λ_{max}^{MeOH} nm: 269, 285, 338 (sh), 402. IR ($CHCl_3$): 3480 (br), 1740, 1630, 1605, 1585 cm^{-1}. ^{1}H-NMR (acetone-d_6) δ: 14.37 (1H, s), 8.06 (1H, d, J=8.8 Hz), 7.51 (1H, d, J=8.8 Hz), 7.50 (1H, d, J=9.8 Hz), 7.19 (1H, d, J=8.8 Hz), 6.87 (1H, d, J=8.8 Hz), 6.03 (1H, s), 5.59 (1H, d, J=9.8 Hz), 5.56 (1H, d, J=3.9 Hz), 5.40 (1H, br s), 5.39 (1H, d, J=9.8 Hz), 5.00 (1H, d, J=5.9 Hz), 4.11 (1H, t, J=4.9 Hz), 4.02, 3.57, 1.78, 1.60, 1.48, 1.37 (each 3H, s).

Acetylation of Neoacrimarine-C Acetic anhydride (0.3 ml) was added to a solution of neoacrimarine-C (0.6 mg) in pyridine (0.5 ml), and the mixture was stirred for 1 d at room temperature. After addition of MeOH (2 ml), the reaction mixture was evaporated under reduced pressure to give an oily residue. Purification by PTLC [*n*-hexane–acetone (3 : 1)] afforded 6,10′-diacetate (**4**) as a yellow oil (0.4 mg). EI-MS m/z: 667 (M^+), 625, 565, 550, 499, 423, 408, 381, 366, 324, 309, 287, 245, 244, 229 (base peak), 213, 201. UV λ_{max}^{MeOH} nm: 245, 264, 286, 294 (sh), 314 (sh), 342 (sh), 406. IR ($CHCl_3$): 3500 (br), 1740, 1720, 1630, 1605, 1560 cm^{-1}. ^{1}H-NMR (acetone-d_6) δ: 14.12 (1H, s), 7.89 (1H, d, J=8.8 Hz), 7.65 (1H, d, J=9.8 Hz), 7.53 (1H, d, J=8.8 Hz), 6.91 (1H, d, J=8.8 Hz), 6.87 (1H, d, J=8.8 Hz), 6.48 (1H, d, J=9.8 Hz), 6.16 (1H, s), 6.12 (1H, d, J=3.8 Hz), 5.76 (1H, d, J=9.8 Hz), 5.72 (1H, d, J=9.8 Hz), 5.58 (1H, d, J=3.8 Hz), 3.80, 2.38, 2.18, 1.89, 1.55, 1.53, 1.50 (each 3H, s).

Neoacrimarine-D (2) Yellow oil, $[\alpha]_D \pm 0°$ (c=0.21, $CHCl_3$). HR FAB-MS Calcd for $C_{38}H_{38}NO_8$: $[M+H]^+$ 636.2597. Found: 636.2561. EI-MS m/z: 635 (M^+), 324, 323, 312, 309, 308, 298, 297 (base peak), 294, 293, 229. UV λ_{max}^{MeOH} nm: 212, 237 (sh), 268, 295, 328, 346 (sh), 418. IR ($CHCl_3$): 3400 (br), 1710, 1620, 1600, 1565 cm^{-1}. ^{1}H- and ^{13}C-NMR (acetone-d_6, δ): see Table I.

Acknowledgement We thank Misses K. Suwa and S. Horiyama, Mukogawa Women's University, for measurements of MS and NMR spectra. Thanks are also due to Mr. K. Masuda, Meijo University, for measurements of HR FAB-MS.

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