A New Dimeric Acridone Alkaloid from *Citrus paradisi* MACF[1)]

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A new bisacridone alkaloid, named bis-5-hydroxynoracronycine, was isolated from the roots of *Citrus paradisi* MACF. On the basis of spectroscopic analysis and synthesis, the structure was elucidated as a dimer of 5-hydroxynoracronycine.

Key words *Citrus paradisi*; Rutaceae; acridone alkaloid; bis-5-hydroxynoracronycine

In the course of our phytochemical studies of *Citrus* plants, we have reported the isolation and structure elucidation of many new coumarins and acridone alkaloids.[2)] Dimeric compounds such as bicoumarins,[3)] bisacridone alkaloids[4)] and acridone coumarin dimers[2,5)] are particularly characteristic constituents. Among the constituents of Hirado-buntan (*Citrus paradisi* MACF.), we reported[6)] the isolation and structure elucidation of acrignine-A, the first naturally occurring acridone-lignan. In continuing studies on the constituents of this plant, we have now isolated a new bisacridone alkaloid named bis-5-hydroxynoracronycine (**1**). In this paper we report the isolation and structure elucidation of this new alkaloid.

Bis-5-hydroxynoracronycine (**1**) was obtained as a racemate, yellow cubes, mp 207—209 °C, from the acetone extract of the roots of Hirado-buntan. The molecular formula $C_{38}H_{34}N_2O_8$ was deduced from the HR-MS (*m/z* 646.2297). This was confirmed by EI-MS, which showed *m/z* 646 (M^+). The IR (1635, 1620, 1563 cm^{-1}) and UV [202, 231, 271, 290 (sh), 345 (sh) nm] spectra suggested the presence of a 9-acridone moiety.[7)] The characteristic signals of two chelated hydroxy protons of the 1-hydroxy-9-acridone skeleton at δ 15.14 and 13.78 (each 1H, s) in the ^{1}H-NMR spectrum, coupled with its molecular ion, suggested the dimeric structure. The signals due to two sets of ABC-type aromatic protons [δ 7.50, 7.00 (each 1H, dd, J=1.83, 7.69 Hz), 6.97 (1H, t, J=7.69 Hz); 7.18, 6.58 (each 1H, dd, J=1.10, 7.70 Hz), 6.70 (1H, t, J=7.70 Hz)], an isolated aromatic proton [δ 5.87 (1H, s)], and a 2,2-dimethylpyran ring [δ 6.07, 4.94 (each 1H, d, J=9.90 Hz), 0.86, 0.74 (each 3H, s)] were observed. Two lower-field signals at δ 7.50 and 7.18 of the ABC-type aromatic protons were deshielded by a carbonyl group and the presence of H-8, H-7 and H-6 of both acridone skeletons was revealed. The existence of two *N*-methyl groups was revealed by the signals at δ_H 3.28 and 3.20 (each 3H, s) in the ^{1}H-NMR and δ_C 45.2 and 48.2 in the ^{13}C-NMR spectra. The remaining signals at δ 4.66 (1H, dd, J=6.96, 12.46 Hz), 1.79 (1H, dd, J=6.96, 13.55 Hz), 1.53 (1H, dd, J=12.46, 13.55 Hz), and 1.22, 1.15 (each 3H, s) suggested the presence of the partial structure $-CH-CH_2-C(CH_3)_2-$. From the above results, bis-5-hydroxynoracronycine was assumed to be a dimer of 5-hydroxynoracronycine (**2**)[8)] linked between C-2′ and C-11. Because of the small quantity obtained as a natural product, we carried out the dimerization of 5-hydroxynoracronycine (**2**) according to the procedure reported by Funayama *et al.*[9)] Treatment of **2** with concentrated H_2SO_4 MeOH (1 : 1) solution under a nitrogen atmosphere for 24 h afforded two main products A (30%) and B (12%). The major product A was identical with the natural product and further structure investigation was performed using the synthetic compound. The heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC) spectra allowed assignments of the proton and carbon signals.

In the HMBC spectrum (Fig. 1), the ^2J and ^3J correlations of the methine signal at δ 4.66 (H-11) with the carbon signals at δ 38.6 (C-12), 104.9 (C-4), 109.8 (C-2′), 158.3 (C-3′), and 161.1 (C-1′) supported the location of the linkage between C-11 and C-2′. Because the isomer-

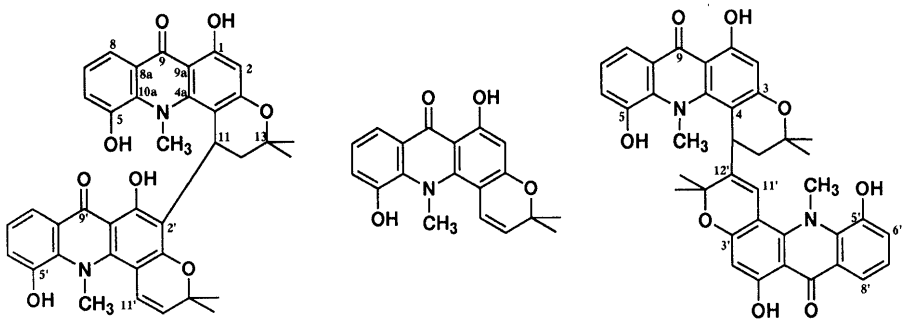

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Chart 1

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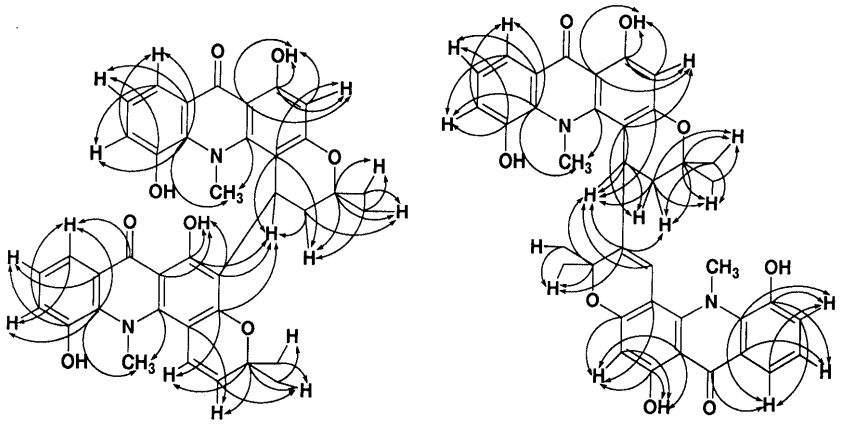

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Fig. 1. C–H Long-Range Correlations Observed in the HMBC Spectra of **1** and **3**

Table 1. ^{1}H- and ^{13}C-NMR Data for **1** and **3**

	1		**3**	
	δ_C	δ_H	δ_C	δ_H
1	161.7		161.8	
1-OH		13.78 (1H, s)		14.20 (1H, s)
2	97.6	5.87 (1H, s)	97.9	6.18 (1H, s)
3	161.2		162.3	
4	104.9		102.9	
4a	146.2		150.2	
5	148.8		147.9	
5-OH		9.24 (1H, br s)[b]		9.81 (1H, br s)[c]
6	119.8	7.00 (1H, dd, J=1.8, 7.7 Hz)	119.2	7.09 (1H, dd, J=2.2, 7.7 Hz)
7	123.3	6.97 (1H, t, J=7.7 Hz)	122.6	7.07 (1H, t, J=7.7 Hz)
8	115.5	7.50 (1H, dd, J=1.8, 7.7 Hz)	114.8	7.63 (1H, dd, J=2.2, 7.7 Hz)
8a	124.2[a]		123.7	
9	181.1		180.9	
9a	107.5		107.2	
10a	136.8		136.8	
10-NCH_3	48.2	3.28 (3H, s)	46.1	3.72 (3H, s)
11	26.9	4.66 (1H, dd, J=7.0, 12.5 Hz)	35.7	4.32 (1H, dd, J=8.1, 10.3 Hz)
12	38.6	1.79 (1H, dd, J=7.0, 13.6 Hz)	42.8	2.38 (1H, dd, J=8.1, 13.9 Hz)
		1.53 (1H, dd, J=12.5, 13.6 Hz)		1.90 (1H, dd, J=10.3, 13.9 Hz)
13	75.1		74.9	
13-CH_3	22.5	1.22 (3H, s)	28.7	1.44 (3H, s)
	29.4	1.15 (3H, s)	22.4	1.43 (3H, s)
1′	161.1		163.0	
1′-OH		15.14 (1H, s)		14.29 (1H, s)
2′	109.8		96.1	6.06 (1H, s)
3′	158.3		159.1	
4′	101.0		103.4	
4a′	150.1		146.2	
5′	148.5		147.9	
5′-OH		10.14 (1H, br s)[b]		10.01 (1H, br s)[c]
6′	118.3	6.58 (1H, dd, J=1.1, 7.7 Hz)	119.7	7.25 (1H, dd, J=1.5, 7.7 Hz)
7′	122.7	6.70 (1H, t, J=7.7 Hz)	122.8	7.16 (1H, t, J=7.7 Hz)
8′	114.3	7.18 (1H, dd, J=1.1, 7.7 Hz)	115.0	7.66 (1H, dd, J=1.5, 7.7 Hz)
8a′	124.3[a]		124.0	
9′	181.9		181.3	
9a′	106.2		105.8	
10a′	137.0		136.0	
10′-NCH_3	45.2	3.20 (3H, s)	47.0	3.43 (3H, s)
11′	120.3	6.07 (1H, d, J=9.9 Hz)	118.1	6.27 (1H, br s)
12′	122.4	4.94 (1H, d, J=9.9 Hz)	135.0	
13′	75.6		78.9	
13′-CH_3	29.6	0.86 (3H, s)	24.4	1.40 (3H, s)
	26.7	0.74 (3H, s)	25.4	1.20 (3H, s)

The coupling constants (J) in parentheses are in Hz. *a*—*c*) Assignments may be interchangeable.

ization of the angular 2,2-dimethylpyran ring of noracronycine to linear type was reported in the literature,[9] the retention of angular orientation of both acridone moieties was confirmed by difference nuclear Overhauser effect (NOE) experiments. Irradiation of the *N*-methyl signal at δ 3.20 caused a 9% enhancement of the signal at δ 4.66 (H-11), while irradiation of the *N*-methyl signal at δ 3.28 induced a 9.9% enhancement of the signal at δ 6.07 (H-11′). From the above results, the angular orientation of both acridone moieties was confirmed and the structure of bis-5-hydroxynoracronycine was concluded to be **1**.

The minor product B was obtained as a yellow amorphous powder. The molecular formula $C_{38}H_{34}N_2O_8$ was established by HR-MS and its IR and UV spectra were similar to those of product A. Comparison of the ^{1}H-NMR spectrum with that of **1** (Table 1) showed similar signal patterns of one acridone moiety. The differences were the absence of one olefinic proton signal of the 2,2-dimethylpyran ring and the appearance of one aromatic proton singlet of the other acridone moiety. Thus, a dimeric structure linked between C-11 and C-12′ was assumed. To confirm this speculation, HMBC and difference NOE experiments were performed. In the HMBC spectrum (Fig. 1), the signal of H-11 (δ 4.32) showed correlations with δ_C 42.8 (C-12), 78.9 (C-13′), 102.9 (C-4), 118.1 (C-11′), 135.0 (C-12′) and 162.3 (C-3). In the difference NOE experiments, irradiation of the *N*-methyl signal at δ 3.72 caused a 12.7% enhancement of the signal at δ 4.32 (H-11), and irradiation of the other *N*-methyl signal at δ 3.43 caused a 15.4% increment of the signal at δ 6.27 (H-11′). These results established the linking position between C-11 and C-12′ and the angular orientation of the two acridone moieties. From the above results, the structure of the product B was established as **3**. Though isomerization of the angular 2,2-dimethylpyran ring was reported in the reaction of noracronycine,[9] the angular orientation was maintained in the case of 5-hydroxynoracronycine.

Known compounds, glycocitrine-I (**4**),[10] grandisinine (**5**),[11] citpressine-I (**6**), -II (**7**),[12] natsucitrine-II (**8**),[13] citracridone-I (**9**),[12] osthenol (**10**),[14] xanthoxyletin (**11**),[15] xanthyletin (**12**),[16] crenulatin (**13**)[17] and citflavanone (**14**)[18] were also isolated and identified by comparison with authentic samples or literature data. Further investigation of other constituents is in progress.

Experimental

Melting points were measured on a Yanagimoto melting point hot-stage apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-360 digital polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL GX-270, GX-400 and GSX-500 spectrometers. Chemical shifts are shown on the δ (ppm) scale with tetramethylsilane (TMS) as an internal reference. HMBC spectra were measured at J=5 and 8 Hz on the JEOL GX-400 spectrometer. EI- and HR-MS were taken with a Hitachi M-80 spectrometer having a direct inlet system. UV spectra were recorded on a Shimadzu UV-160A spectrometer and IR spectra on a Shimadzu IR-450 and JASCO IR-230 spectrometer. The preparative thin-layer chromatography (PTLC) was done on Kieselgel 60 F_{254} (Merck).

Extraction and Isolation The roots (1.1 kg) of Hirado-buntan (*Citrus paradisi* MACF.) cultivated and collected at the orchard of the Fruit Tree Research Station, Okitsu, Shizuoka prefecture, were extracted with acetone (3 l, 8 h × 5) under reflux. Evaporation of the solvent under reduced pressure gave the acetone extract (101.2 g), which was subjected to silica gel column chromatography using toluene, CH_2Cl_2, acetone and MeOH, successively. The CH_2Cl_2 eluate (29.77 g) was further subjected to a combination of column chromatography and PTLC [solvent : benzene acetone (8 : 2); diisopropyl ether; AcOEt benzene (1 : 1)] to give bis-5-hydroxynoracronycine (**1**) (0.5 mg), together with glycocitrine-I (**4**) (82.9 mg), grandisinine (**5**) (42.4 mg), citpressine-I (**6**) (18.9 mg), -II (**7**) (41.2 mg), natsucitrine-II (**8**) (2.7 mg), citracridone-I (**9**) (88.9 mg), osthenol (**10**) (5.4 mg), xanthoxyletin (**11**) (67.0 mg), xanthyletin (**12**) (1.403 g), crenulatin (**13**) (1.4 mg) and citflavanone (**14**) (6.5 mg).

Bis-5-hydroxynoracronycine (1) Yellow cubes, $[\alpha]_D$ ±0° (c=0.07, $CHCl_3$), mp 207—209 °C, UV λ_{max} (EtOH) nm: 202, 231, 271, 290 (sh), 345 (sh). IR ν_{max} $CHCl_3$ cm^{-1}: 3500, 1635, 1620, 1563. ^{1}H- and ^{13}C-NMR (DMSO-d_6, δ): Table 1. HR-MS m/z: Calcd for $C_{38}H_{34}N_2O_8$: 646.2312. Found 646.2297. EI-MS m/z: 646 (M^+), 631, 337, 323, 308, 294, 270, 202 (base peak), 174, 149.

Synthesis of Bis-5-hydroxynoracronycine (1) 5-Hydroxynoracronycine (**2**)[19] (50 mg) was stirred with the mixture of concentrated H_2SO_4 and MeOH (1 : 1) under a nitrogen atmosphere at room temperature. After 24 h, the reaction mixture was poured into ice-water and extracted with $CHCl_3$. The extract was washed with 5% $NaHCO_3$ and H_2O successively, dried over $MgSO_4$ and evaporated to give an oily residue (49.1 mg). The residue was subjected to PTLC [solvent : $CHCl_3$ acetone (9 : 1)] to give product A (**1**) (15 mg) and product B (**3**) (6 mg). Product A was identical with a natural sample of **1** on the basis of TLC and ^{1}H-NMR spectral comparisons. Product B (**3**):Yellow amorphous powder. UV λ_{max} (EtOH) nm: 202, 229 (sh), 268, 288 (sh), 325. IR ν_{max} ($CHCl_3$) cm^{-1}: 3228 (br), 1631, 1568, 1504. ^{1}H- and ^{13}C-NMR (DMSO-d_6, δ): Table 1. HR-MS m/z: Calcd for $C_{38}H_{34}N_2O_8$: 646.2312. Found 646.2296. EI-MS m/z: 646 (M^+), 631, 352, 338, 323, 308 (base peak), 293, 279, 268, 256, 236, 223, 213, 167, 154, 149.

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