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Oryzamutaic acids H–J, new alkaloids from an *Oryza sativa* mutant with yellow endosperm

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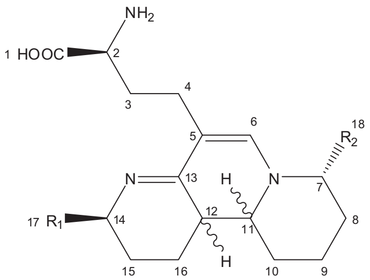
ABSTRACT

Three new nitrogen-containing heterocyclic alkaloids, oryzamutaic acids H–J (**1**–**3**), were isolated from the endosperm (polished rice) of an *Oryza sativa* mutant. The structures and relative stereochemistries of **1**–**3** were elucidated on the basis of spectroscopic analyses. The presence of three carbons, each bound to a carboxyl group and a nitrogen atom in the structure of **1**, suggests that oryzamutaic acids H–J (**1**–**3**) are generated from three molecules of a single amino acid.

Artificial mutation by means of radiation and chemicals has been used to improve the yield and quality of cultivars of rice (*Oryza sativa*),[1] which is a major global cereal crop in the world. The rare rice cultivar Hatsuyamabuki, which has yellow endosperm, was selected from the progeny obtained by the treatment of the rice cultivar Kinuhikari with γ-rays.[2] Although the agronomic traits of Hatsuyamabuki are almost the same as those of Kinuhikari (except for the color of the endosperm), Hatsuyamabuki is nevertheless of interest because its yellow endosperm has been found to contain oryzamutaic acids A–G, which are alkaloids with a novel type of nitrogen-containing heterocyclic ring system.[3,4] Oryzamutaic acids A–C are yellow, whereas oryzamutaic acids D–G are colorless. Oryzamutaic acids A–G have attracted great interest from a biogenetic point of view. In our continuing study of the alkaloids in the endosperm of Hatsuyamabuki, we isolated oryzamutaic acids H–J (**1**–**3**). In this Letter, we describe the isolation and structure elucidation of **1**–**3**.



1 R_1=COOH, R_2=COOH, ⋯ H-11, ◀ H-12
2 R_1=COOH, R_2=OH, ⋯ H-11, ◀ H-12
3 R_1=H, R_2=COOH, ⋯ H-11, ⋯ H-12

The endosperm (polished rice) (40 kg) of Hatsuyamabuki was extracted with 200 L of aq MeOH (MeOH/H_2O, 1:9) for 1 day at 25 °C. Then, 1.8 L of aq MeOH (MeOH/H_2O, 5:1) was added to the extract (648 g), and the solution was centrifuged at 3500g for 10 min at 25 °C. The supernatant (368 g) was subjected to C_{18} column chromatography (MeOH/H_2O, 0:1→1:0) followed by C_{18} HPLC to yield oryzamutaic acids H (**1**, 1.3 mg, 0.000003% yield), I (**2**, 0.4 mg, 0.000001% yield), and J (**3**, 1.3 mg, 0.000003% yield) as colorless powders.

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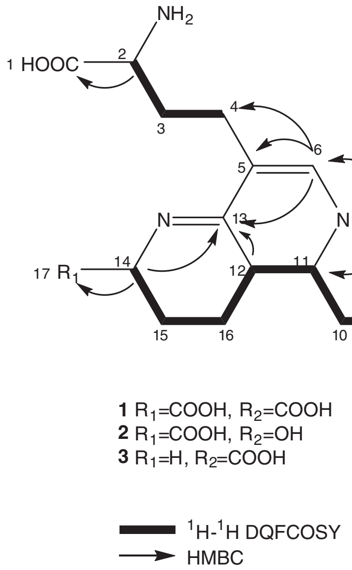
Table 1
NMR spectroscopic data (800 MHz for ^1H NMR, 125 MHz for ^{13}C NMR, D_2O) for oryzamutaic acids H–J (**1**–**3**)

Position	Oryzamutaic acid H (**1**)		Oryzamutaic acid I (**2**)		Oryzamutaic acid J (**3**)	
	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)
1	178.0, C		178.0, C		178.0, C	
2	57.0, CH	3.81, m	57.3, CH	3.79, t (6.2)	57.6, CH	3.82, t (6.0)
3a	33.8, CH_2	2.15, m	33.2, CH_2	2.14, m	33.0, CH_2	2.06, m
3b		2.02, m		2.06, m		2.01, m
4a	26.1, CH_2	2.56, m	25.9, CH_2	2.60, ddd (15.1, 9.6, 5.2)	25.9, CH_2	2.37, m
4b		2.50, m		2.43, m		2.37, m
5	104.7, C		103.5, C		101.2, C	
6	164.5, CH	7.46, s	162.1, CH	7.60, s	159.7, CH	7.40, s
7	69.3, CH	4.35, d (5.0)	86.3, CH	5.35, t (2.4)	72.1, CH	4.35, d (5.6)
8a	29.9, CH_2	2.35, m	33.2, CH_2	2.02, m	32.9, CH_2	2.31, m
8b		1.92, m		1.91, m		1.82, m
9a	22.6, CH_2	1.89, m	19.0, CH_2	1.84, m	24.7, CH_2	1.86, m
9b		1.36, m		1.84, m		1.62, m
10a	32.6, CH_2	2.28, m	32.4, CH_2	2.33, m	25.5, CH_2	1.67, m
10b		1.47, m		1.51, m		1.67, m
11	60.1, CH	3.52, ddd (15.9, 11.7, 4.0)	57.3, CH	3.56, ddd (15.7, 11.7, 3.7)	61.8, CH	3.97, ddd (11.2, 7.8, 5.0)
12	42.8, CH	2.77, ddd (15.9, 11.3, 5.2)	46.2, CH	2.82, ddd (15.7, 11.2, 5.2)	40.4, CH	3.48, ddd (11.8, 7.1, 5.0)
13	168.8, C		170.5, C		167.8, C	
14a	60.6, CH	4.18, dd (11.4, 5.4)	60.9, CH	4.20, dd (11.4, 5.4)	46.4, CH_2	3.66, m
14b						3.54, m
15a	28.9, CH_2	2.53, m	28.7, CH_2	2.54, m	24.6, CH_2	2.12, m
15b		1.78, m		1.80, m		1.97, m
16a	24.9, CH_2	2.22, m	24.7, CH_2	2.26, m	23.3, CH_2	1.92, m
16b		1.50, m		1.53, m		1.71, m
17	181.3, C		180.7, C			
18	181.1, C				180.1, C	



1 R_1=COOH, R_2=COOH
2 R_1=COOH, R_2=OH
3 R_1=H, R_2=COOH

Figure 1. Selected 2D NMR correlations for oryzamutaic acids H–J (**1**–**3**).

Oryzamutaic acid H (**1**)[5] had the molecular formula, $C_{18}H_{25}N_3O_6$ established by HRESIMS [*m*/*z* 380.1911 $(M+H)^+$, Δ +9.5 mmu] indicating 8 degrees of unsaturation. The ^{13}C NMR and DEPT 135 spectra resolved 18 carbon signals comprising five quaternary carbons, including three carbonyls, six methine carbons, and seven methylene carbons. Oryzamutaic acid I (**2**)[6] had the molecular formula, $C_{17}H_{25}N_3O_5$ established by HRESIMS [*m*/*z* 352.1867 $(M+H)^+$, Δ −0.0 mmu] indicating 7 degrees of unsaturation. The ^{13}C NMR and DEPT 135 spectra resolved 17 carbon signals comprising four quaternary carbons, including two carbonyls, six methine carbons, and seven methylene carbons. Oryzamutaic acid J (**3**)[7] had the molecular formula, $C_{17}H_{25}N_3O_4$ established by HRESIMS [*m*/*z* 336.1908 $(M+H)^+$, Δ −1.0 mmu] indicating 7 degrees of unsaturation. The ^{13}C NMR and DEPT 135 spectra resolved 17 carbon signals comprising four quaternary carbons, including two carbonyls, five methine carbons, and eight methylene carbons.

The gross structures of **1**–**3** were elucidated by analyses of 1D and 2D NMR spectra (Table 1, Fig. 1). The ^1H–^1H DQFCOSY spectra of **1**–**3** indicated two partial structural units. The HMBC correlation for H-7 to C-6 and C-11 and their chemical shifts indicated the connection of C-6, C-7, and C-11 through a nitrogen atom, and the correlation for H-6 to C-4, C-5, and C-13 indicated that C-4, C-6, and C-13 are attached to C-5. The HMBC correlation for H-14 to C13 and their chemical shifts indicated the connection of C-13 and C-14 through a nitrogen atom, and the correlation for H-12 to C13 indicated the connection of C-12 and C-13. The HMBC correlation for H-2 to C-1 indicated the connection of C-1 and C-2, and the correlation of H-14 to C-17 indicated the connection of C-14 and C-17.

The relative stereochemistries of **1** and **2** were deduced from the analyses of ^1H–^1H coupling constants (Table 1) and NOESY spectra (Fig. 2). The *J*(H-14/H-15b) and *J*(H-14/H-15a) values of **1** (11.4 and 5.4 Hz, respectively) and **2** (11.4 and 5.4 Hz, respectively) were similar to those of oryzamutaic acids C, E, and F.[4] The NOESY correlations for H-14/H-16b of **1** and **2** indicated that both H-14 and H-16b were axial, and the correlations for H-15b/H-12 of **1** and **2** indicated that both H-15b and H-12 were axial. Thus, the relative stereochemistries for C-14 to C-12 (through C-15 and C-16) of **1** and **2** were similar to those for oryzamutaic acids C, E, and F.[4] The *J*(H-12/H-11) values of **1** (15.9 Hz) and **2** (15.7 Hz) and the NOESY correlations for H-12/H-10b and H-11/H-16b of **1** and **2** indicated that the relative stereochemistries of H-12 to H-11 of **1** and **2** were *trans*, like that of oryzamutaic acids C and E.[4] The *J*(H-7/H-8b) and *J*(H-7/H-8a) values of **1** (5.0 and 0.0 Hz, respectively) and **2** (2.4 and 2.4 Hz, respectively), the NOESY correlations for H-8b/H-10b of **1** and **2**, which indicated that both H-8b and H-10b were axial, the correlations for H-9b/H-11 of **1** and **2**, which indicated that both H-9b and H-11 were axial, and the long range W coupling for H-8a/H-10a of **2** indicated the relative stereochemistries for C-7 to C-11 (through C-8, C-9, and C-10) of **1** and **2** as shown in Figure 2.

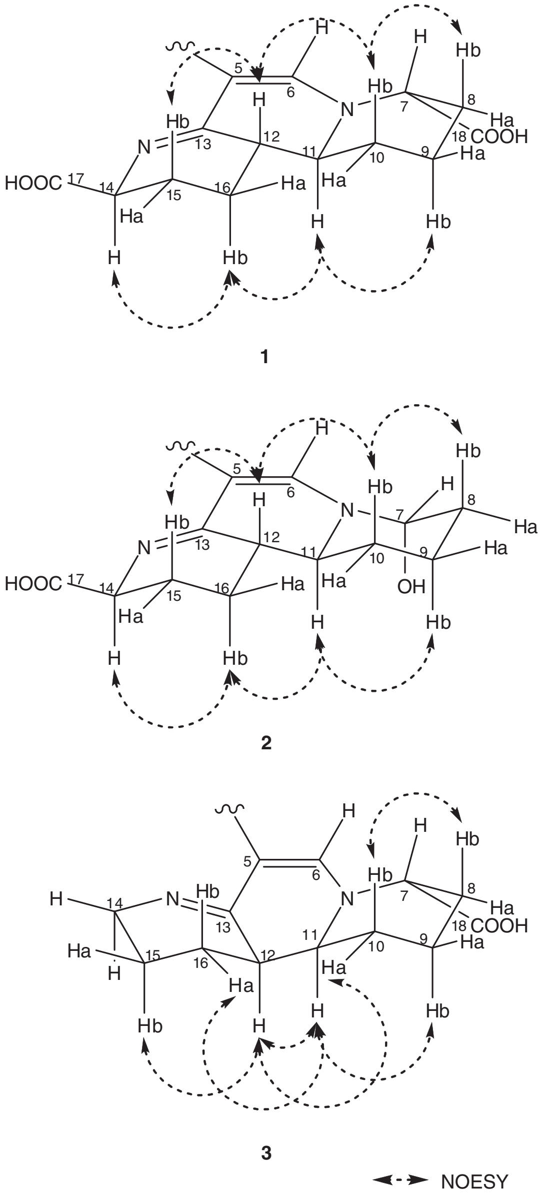
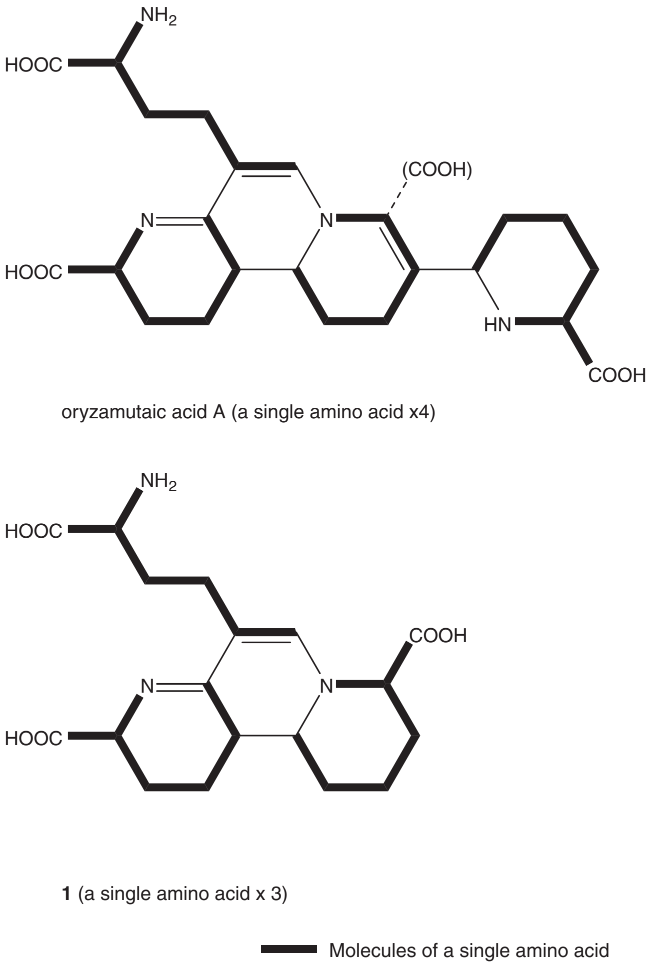


Figure 2. Selected NOESY correlations for oryzamutaic acids H–J (**1**–**3**).

Thus, the relative stereochemistries of oryzamutaic acids H and I were elucidated to be **1** and **2**, respectively.

The relative stereochemistry of **3** was deduced from the analyses of ^{1}H–^{1}H coupling constants (Table 1) and NOESY spectra (Fig. 2). The *J*(H-7/H-8b) and *J*(H-7/H-8a) values of **3** (5.6 and 0.0 Hz, respectively) were similar to those of **1**. The NOESY correlation for H-8b/H-10b of **3** indicated that both H-8b and H-10b were axial, and the correlation for H-9b/H-11 of **3** indicated that both H-9b and H-11 were axial. Thus, the relative stereochemistry for C-7 to C-11 (through C-8, C-9, and C-10) of **3** was similar to that of **1**. The *J*(H-11/H-12) values of **3** (5.0 Hz) and the NOESY correlations for H-11/H-12, H-11/H-16a, and H-10a/H-12 of **3** indicated that the relative stereochemistries of H-11 to H-12 of **3** were *cis*, like that of oryzamutaic acid G.[4] Thus, the relative stereochemistry of oryzamutaic acid J was elucidated to be **3**.



oryzamutaic acid A (a single amino acid x4)

1 (a single amino acid x 3)

Molecules of a single amino acid

Figure 3. Molecules of a single amino acid in oryzamutaic acids A and H (**1**).

The structures of oryzamutaic acids H (**1**) and J (**3**) suggest possible biogenetic path for oryzamutaic acids A–G and H–J (**1**–**3**), which are alkaloids with a novel type of nitrogen-containing heterocyclic ring system.[3,4] Oryzamutaic acids A–G do not possess a carbonyl group attached to C-7,[3,4] whereas oryzamutaic acids H (**1**) and J (**3**) do (C-18) attached to C-7. The structure of oryzamutaic acid A suggests that it could be generated from four molecules of a single amino acid as shown in Figure 3 in bold line, and likewise oryzamutaic acids B–G and H–J (**1**–**3**) could be generated from three molecules of a single amino acid. Changes in biogenetic path have been observed in various plant mutants. For example, the grains of proanthocyanidin-free mutants *ant 17* and *ant 22* accumulate homoeriodictyol and chrysoeriol rather than proanthocyanidins.[8] Detailed study of the biogenetic path for oryzamutaic acids A–G and H–J (**1**–**3**) is in progress.

Oryzamutaic acids A–C, which are yellow, possess double bonds between C-7 and C-8, between N-3 and C-13, and between C-5 and C-6.[3,4] In contrast, oryzamutaic acids D–G and H–J (**1**–**3**) lack the double bond between C-7 and C-8 and are colorless. This fact suggests that the resonance structure extending from N-3 to C-8 (through C-13, C-5, C-6, N-2, C-7, and C-8) is responsible for the yellow color of oryzamutaic acids A–C.

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5. Oryzamutaic acid H (**1**) colorless powder; $[\alpha]_D^{23}$ +155° (c 0.065, H_2O); UV (H_2O) λ_{max} 349 nm (ε 2300); ATR-FTIR ν 3790, 3226, 2927, 2869, 1584, 1400, 1351, 1310, 1272, 1237, 1215, 1193, 1177, 1117, 1103, 1061, 1032, 763, 574, and 553 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS m/z 380.1911 $(M+H)^+$ (calcd for $C_{18}H_{26}N_3O_6$, 380.1816).
6. Oryzamutaic acid I (**2**) colorless powder; $[\alpha]_D^{23}$ +213° (c 0.060, H_2O); UV (H_2O) λ_{max} 342 nm (ε 2700); ATR-FTIR ν 3789, 3226, 2927, 2869, 2440, 2322, 1590, 1400, 1348, 1282, 1238, 1193, 1069, 763, 582, and 553 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS m/z 352.1867 $(M+H)^+$ (calcd for $C_{17}H_{26}N_3O_5$, 352.1867).
7. Oryzamutaic acid J (**3**) colorless powder; $[\alpha]_D^{24}$ $-6°$ (c 0.070, H_2O); UV (H_2O) λ_{max} 349 nm (ε 2500); ATR-FTIR ν 3790, 3372, 3279, 2949, 2870, 1580, 1575, 1504, 1470, 1441, 1374, 1363, 1350, 1298, 1268, 1206, 1197, 1135, 1103, 1032, 968, 947, 812, 793, 763, 582, and 553 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS m/z 336.1908 $(M+H)^+$ (calcd for $C_{17}H_{26}N_3O_4$, 336.1918).
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