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Oryzamutaic acid A, a novel yellow pigment from an *Oryza sativa* mutant with yellow endosperm

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ABSTRACT

Oryzamutaic acid A, a novel yellow pigment, was isolated from the endosperm (polished rice) of an *Oryza sativa* mutant. The structure and absolute configuration of oryzamutaic acid A were elucidated on the basis of spectroscopic analysis, single-crystal X-ray diffraction analysis, and biogenetic reason. Oryzamutaic acid A might be biogenetically derived from four L-amino acids because it contains three amino acid groups and one amine group.

Artificial mutation by means of radiation and chemicals has been used to improve the yield and quality of rice (*Oryza sativa*), wheat (*Triticum aestivum*), and barley (*Hordeum vulgare*) cultivars.[1] The rice cultivar Milky Queen, which has a low amylose content in its endosperm, was selected from the progeny obtained by treatment of the rice cultivar Koshihikari with the chemical mutagen, *N*-methyl-*N*-nitrosourea.[2] The *Wx-mq* gene for low amylose content in the endosperm of Milky Queen was cloned by RT-PCR, and a nearly full length cDNA sequence of the gene was determined.[3,4] Recently, the rare rice cultivar Hatsuyamabuki, which has yellow endosperm, has been selected from the progeny obtained by treatment of the rice cultivar Kinuhikari with γ-rays (Fig. 1). The agronomic traits of Hatsuyamabuki are almost the same as those of Kinuhikari except for the color of the endosperm. This cultivar is expected to be useful for the discrimination of forage rice from food rice and as a yellow cooking rice, as a brewing rice for yellow sake, and as a raw material for yellow pigment. However, the pigment or pigments responsible for the yellow endosperm of Hatsuyamabuki have not yet been isolated and identified. In this Letter, we report the isolation and absolute configuration of a yellow pigment, oryzamutaic acid A (Figs. 2 and 3).

The endosperm (polished rice) (20 kg) of Hatsuyamabuki was extracted with 100 L of aq MeOH (MeOH/H_2O, 1:9) for 1 day at 25 °C. Then, 900 mL of aq MeOH (MeOH/H_2O, 5:1) was added to the extract (324 g), and the solution was centrifuged at 15,000 *g* for 10 min at 25 °C. The supernatant (184 g) was subjected to chromatography on a C18 column with aq MeOH (MeOH/H_2O, 1:9) as the eluent. The yellow pigment fraction (2 g) was further purified by HPLC on a C18 column with aq MeOH (MeOH/H_2O, 1:9) as the

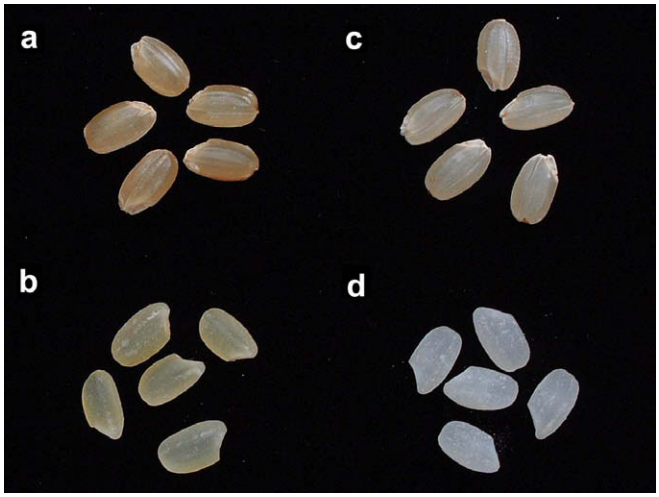

Figure 1. Brown rice (husked rice) and endosperm (polished rice) of Hatsuyamabuki (a and b, respectively) and Kinuhikari (c and d, respectively).

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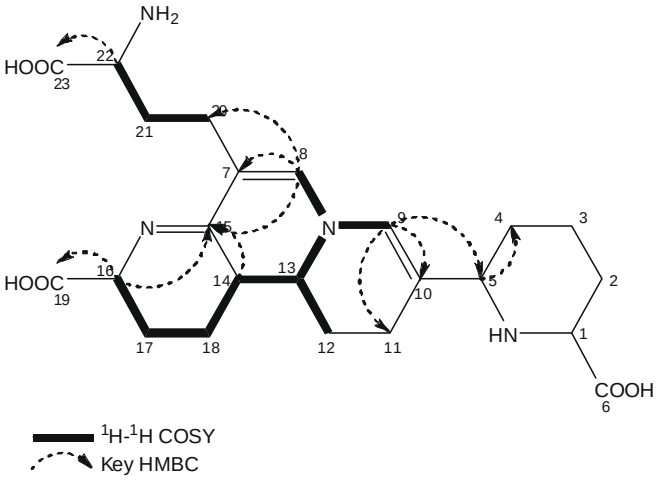

Figure 2. Structure of oryzamutaic acid A.

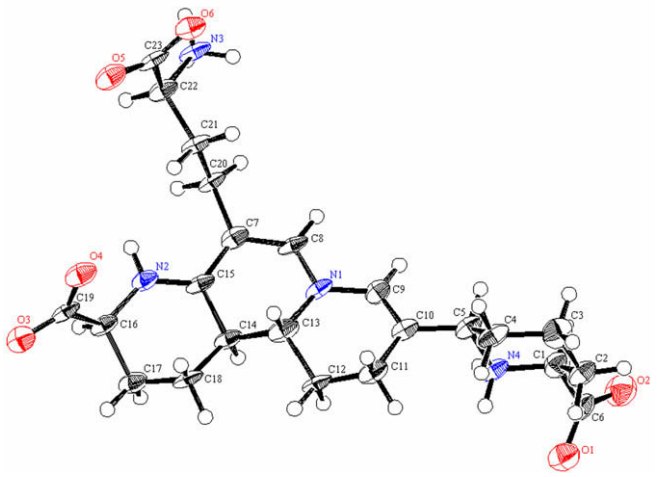

Figure 3. ORTEP drawing of oryzamutaic acid A.

eluent. Concentration of the HPLC fractions afforded a yellow powder, which was recrystallized from aqueous acetone (acetone/H_2O, 4:1) to afford oryzamutaic acid A as light yellow plates (35.0 mg).

The molecular formula, $C_{23}H_{32}N_4O_6$, of oryzamutaic acid A, $[\alpha]_D^{20}$ +403 (*c* 0.118, H_2O), UV (H_2O) λ_{max} 395 nm (ε 17200), was established by HRESIMS (microTOF) [*m/z* 461.2388 $(M+H)^+$, Δ −0.6 mmu] indicating 10 degrees of unsaturation. The IR absorption spectrum showed the presence of hydroxyl (3398 and 2953 cm^{-1}) and carbonyl (1628 and 1587 cm^{-1}) groups. The ^{13}C NMR and DEPT 135 spectra resolved 23 carbon signals comprising six quaternary carbons, including three carbonyls, eight methine carbons, and nine methylene carbons. A possible structure for oryzamutaic acid A was deduced from 1D and 2D NMR spectra. The ^1H–^1H COSY spectrum of oryzamutaic acid A indicated two partial structural units (bold lines in Fig. 2). The HMBC correlations of H-8 to C-7, C-15, and C-20 indicated that C-8, C-15, and C-20 were attached to C-7, and the correlation of H-22 to C-23 indicated the connection between C-22 and C-23 (Fig. 2, Table 1). The HMBC correlation of H-16 to C-19 indicated the connection between C-16 and C-19, the correlation of H-16 to C-15 and their chemical shifts indicated the connection of C-15 and C-16 through a nitrogen atom, and the correlation of H-14 to C-15 indicated the connection between C14 and C15. The HMBC correlations of H-9 to C-5, C-10, and C-11 indicated that C-5, C-9, and C-11 were attached to C-10, and the correlation of H-5 to C-4 indicated the connection between C-4 and C-5. The remaining structural details were elucidated by single-crystal X-ray diffraction analysis (Fig. 3).[5] Oryzamutaic acid A contains three amino acid groups and one amine group, which indicate that oryzamutaic acid A may be biogenetically derived from four L-amino acids. The absolute configuration was selected on the basis of biogenetic reason. The interatomic distances between N-2 and C-15, C-15 and C-7, C-8 and N-1, N-1 and C-9, and C-9 and C-10 indicated the resonance structure from N-2 to C-10 (through C-15, C-7, C-8, N-1, and C-9). This resonance structure may be the chromophore of oryzamutaic acid A. Compounds with a similar central $C_{12}N_2$ skeleton have been reported, but the bonds between N-2 and C-15, C-7 and C-8, and C-9 and C-10 in these compounds are reduced.[6–11]

Table 1
NMR data for oryzamutaic acid A (D_2O, δ ppm)

No.	δ_H[a]	δ_C[b]	HMBC (^1H–^{13}C)
1	3.53–3.47 (1H, m)	61.5	C-6
2	2.13–2.08 (1H, m)	26.9	
	1.50–1.41 (1H, m)		
3	1.88–1.84 (1H, m)	23.6	
	1.54–1.47 (1H, m)		
4	1.81–1.73 (1H, m)	27.4	
	1.63–1.55 (1H, m)		
5	3.63–3.56 (1H, m)	61.0	C-4, C-9, C-10
6		175.1	
7		101.8	
8	7.30 (1H, s)	153.8	C-7, C-9, C-13, C-15, C-20
9	6.52 (1H, s)	130.7	C-5, C-8, C-10, C-11, C-13
10		118.6	
11	2.19–2.11 (1H, m)	21.7	
12	2.32–2.25 (1H, m)	26.0	
	1.58–1.52 (1H, m)		
13	3.55–3.47 (1H, m)	57.2	
14	2.86–2.78 (1H, m)	39.9	C-12, C-13, C-15, C-18
15		167.7	
16	4.11 (1H, d, 5.9)	57.6	C-15, C-17, C-18, C-19
17	2.22–2.14 (1H, m)	25.5	
	2.02–1.93 (1H, m)		
18	1.94–1.87 (1H, m)	19.4	
	1.26–1.17 (1H, m)		
19		178.0	
20	2.28–2.20 (2H, m)	23.6	C-7, C-8, C-15, C-21, C-22
21	1.93–1.85 (1H, m)	30.4	C-7, C-20, C-23
	1.82–1.73 (1H, m)		
22	3.61–3.55 (1H, m)	55.0	C-20, C-21, C-23
23		175.3	

[a] Spectra were recorded at 400 MHz.
[b] Spectra were recorded at 100 MHz.

To evaluate whether oryzamutaic acid A occurs only in Hatsuyamabuki, we determined oryzamutaic acid A content in the endosperm of Hatsuyamabuki and Kinuhikari. By subjecting the aq MeOH extracts of the endosperms of Hatsuyamabuki and Kinuhikari to HPLC, we estimated the oryzamutaic acid A content in Hatsuyamabuki to be 1.4 ± 0.0 μg/g; no oryzamutaic acid A content was detected in Kinuhikari (Fig. 4).[12] These results suggest that oryzamutaic acid A is unique to Hatsuyamabuki, that it plays an important role in the endosperm color of Hatsuyamabuki, and that it is accumulated by means of a change in some biosynthetic pathway, such as the gain or loss of an enzyme related to metabolism.

Changes in biosynthetic pathways have been observed in various plant mutants. In *Ospds* and *Oszds* rice mutants, carotenoids do not accumulate, although lycopene does accumulate in β-*Oszds* mutants.[13] In proanthocyanidin-free barley mutants, proanthocyanidins do not accumulate.[14] However, in this study, we isolated and identified a very rare compound from a rice mutant, which indicates that various novel compounds may accumulate in plant mutants owing to changes in biosynthetic pathways.

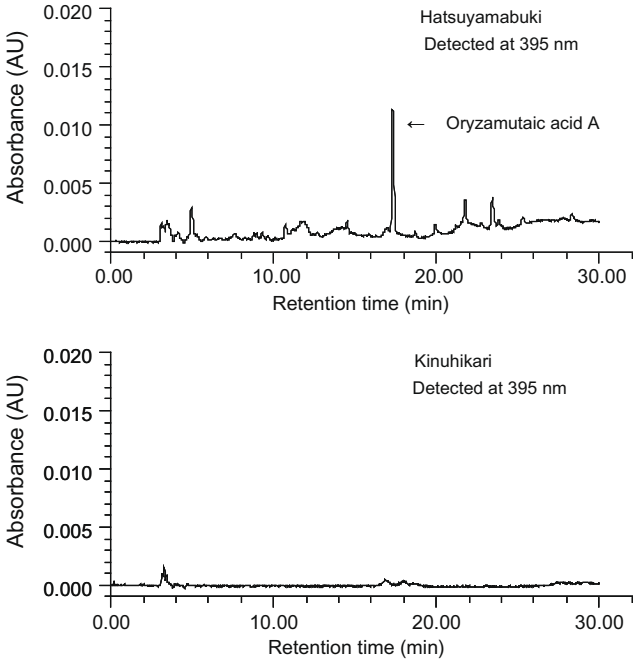

Figure 4. HPLC chromatogram of endosperm extracts of Hatsuyamabuki and Kinuhikari.

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Supplementary data

Experimental procedures IR, UV, ^{1}H NMR, ^{13}C NMR, DEPT 135, ^{1}H–^{1}H COSY, HMQC, and HMBC spectra of oryzamutaic acid A are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.02.082.

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5. A light yellow plate like crystal of $C_{23}H_{32}N_4O_6$ having approximate dimensions of $0.02 \times 0.02 \times 0.01$ mm was mounted in a loop. All measurements were made on a Rigaku RAXIS V imaging plate area detector at SPring8 beamline BL26B1 with wavelength adjusted to 0.71069. Indexing was performed from 20 oscillations that were exposed for 720 seconds. The crystal-to-detector distance was 200.04 mm. Cell constants and an orientation matrix for data collection corresponded to a primitive orthorhombic cell with dimensions: a = 9.793(17) Å, b = 12.401(15) Å, c = 24.555(16) Å, V = 2982(6) Å^3. For Z = 4 and F.W. = 460.53, the calculated density is 1.026 g/cm^3. The systematic absences of h00: h ± 2n, 0k0: k ± 2n, 00 l: l ± 2n, uniquely determine the space group to be: *P*212121 (#19). The data were collected at a temperature of −170 ± 1 °C to a maximum 2θ value of 45.5°. A total of 180 oscillation images were collected. A sweep of data was done using φ oscillations from –90.0 to 90.0° in 1.0° steps. The exposure rate was 720.0 (s/°). The detector swing angle was 0.07°. The crystal-to-detector distance was 200.04 mm. Readout was performed in the 0.100 mm pixel mode. Of the 13736 reflections that were collected, 3899 reflections were unique (R_{int} = 0.06); equivalent reflections were merged. The linear absorption coefficient, μ, for Mo-Kα radiation is 0.747 cm^{-1}. An empirical absorption correction was applied, which resulted in transmission factors ranging from 0.232 to 0.999. The data were corrected for Lorentz and polarization effects. The structure was solved by direct methods[15] and expanded using Fourier techniques.[16] The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined using the riding model. The final cycle of full-matrix least-squares refinement[17] on F^2 was based on 3899 observed reflections and 403 variable parameters and converged (large parameter shift was 0.00 times its esd) with unweighted and weighted agreement factors of: $R_1 = \sum ||F_o| - |F_c|| / \sum |F_o| = 0.0717$, $wR_2 = [\sum (w(F_o^2 - F_c^2)^2) / \sum w(F_o^2)^2]^{1/2} = 0.2046$. The standard deviation of an observation of unit weight[18] was 1.02. A Sheldrick weight scheme was used. The maximum and minimum peaks on the final deference Fourier map corresponded to 0.43 and −0.38 e$^-$/Å^3, respectively. The absolute structure was deduced based on Flack parameter, 0.0(19), using 1682 Friedel pairs.[19] Neutral atom scattering factors were taken from Cromer and Waber.[20] Anomalous dispersion effects were included in Fcale[21]; the values for Δf′ and Δf″ were those of Creagh and McAuley.[22] The values for the mass attenuation coefficients are those of Creagh and Hubbell.[23] All calculations were performed using the Crystal Structure[24] crystallographic software package except for refinement, which was performed using SHELXL-97.[25] These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road; Cambridge CB2 1EZ, UK; fax: +44 1223 336 033; email: deposit@ccdc.cam.ac.uk).
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