



Oryzamutaic acids B–G, new alkaloids from an *Oryza sativa* mutant with yellow endosperm

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ABSTRACT

Oryzamutaic acids B–G (**1–6**), structurally unique nitrogen-containing heterocyclic alkaloids, were isolated from the endosperm (polished rice) of an *Oryza sativa* mutant. The structures and relative stereochemistries of **1–6** were elucidated on the basis of spectroscopic and single-crystal X-ray diffraction analyses.

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Rice (*Oryza sativa*) is a major cereal crop in the world. The rare rice cultivar Hatsuyamabuki, which has yellow endosperm, was selected from the progeny obtained by treatment of the rice cultivar Kinuhikari with γ -rays. The agronomic traits of Hatsuyamabuki are almost the same as those of Kinuhikari except for the color of the endosperm. Recently, we isolated oryzamutaic acid A, a novel type of yellow pigment with a nitrogen-containing heterocyclic ring system, from the yellow endosperm (polished rice) of Hatsuyamabuki.¹ Oryzamutaic acid A has attracted great interest from biogenetic point of view. In our continuing study of yellow pigments in the endosperm of Hatsuyamabuki, we isolated oryzamutaic acids B–G (**1–6**). In this Letter, we describe the isolation and structure elucidation of **1–6**.

The endosperm (40 kg) of Hatsuyamabuki was extracted with 200 L of aq MeOH (MeOH/H₂O, 1:9) for 1 day at 25 °C. Then, 1.8 L of aq MeOH (MeOH/H₂O, 5:1) was added to the extract (648 g), and the solution was centrifuged at 3500 g for 10 min at 25 °C. The supernatant (368 g) was subjected to C₁₈ column chromatographies followed by C₁₈ HPLC to yield oryzamutaic acid B (**2**, 3.4 mg, 0.000009% yield) and C (**3**, 0.6 mg, 0.000002% yield) as yellow powder, and D (**4**, 46.0 mg, 0.000115% yield), E (**5**, 17.8 mg,

0.000045% yield), F (**6**, 1.8 mg, 0.000005% yield), and G (**7**, 5.2 mg, 0.000013% yield) as colorless powder.

Oryzamutaic acid B (**1**)² had the molecular formula, C₁₇H₂₃N₃O₄, established by HRESIMS [*m/z* 334.1759 (M+H)⁺, Δ –0.2 mmu] indicating eight degrees of unsaturation. The ¹³C NMR and DEPT 135 spectra resolved 17 carbon signals comprising four quaternary carbons, including two carbonyls, seven methine carbons, and six methylene carbons (Table 1). Oryzamutaic acid C (**2**)³ showed almost the same HRESIMS, ¹³C NMR, and DEPT 135 spectra as **1**. Oryzamutaic acid D (**3**)⁴ had the molecular formula, C₁₇H₂₅N₃O₄, established by HRESIMS [*m/z* 336.1916 (M+H)⁺, Δ –0.2 mmu] indicating seven degrees of unsaturation. The ¹³C NMR and DEPT 135 spectra resolved 17 carbon signals comprising four quaternary carbons, including two carbonyls, five methine carbons, and eight methylene carbons. Oryzamutaic acid E–G (**4–6**)^{5–7} showed almost the same HRESIMS, ¹³C NMR, and DEPT 135 spectra as **3**.

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

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Table 1
NMR spectropic data (800 MHz, D₂O) for oryzamutaic acid B–G (1–6)

Position	Oryzamutaic acid B (1)		Oryzamutaic acid C (2)		Oryzamutaic acid D (3)		Oryzamutaic acid E (4)		Oryzamutaic acid F (5)		Oryzamutaic acid G (6)	
	δ_C , mult.	δ_H (J in Hz)	δ_C , mult.	δ_H (J in Hz)	δ_C , mult.	δ_H (J in Hz)	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	176.2, qC		176.2, qC		176.0, qC		176.1, qC		176.1, qC		176.1, qC	
2	55.7, CH	3.72, t (6.2)	55.5, CH	3.71, t (6.3)	55.7, CH	3.73, t (6.1)	55.6, CH	3.70, t (6.2)	55.6, CH	3.70, t (6.2)	55.8, CH	3.71, t (6.2)
3a	31.3, CH ₂	2.01, m	31.5, CH ₂	2.04, m	31.3, CH ₂	1.99, m	31.5, CH ₂	2.02, m	31.4, CH ₂	2.03, m	31.1, CH ₂	1.96, m
3b		1.95, m		1.97, m		1.93, m		1.95, m		1.96, m		1.96, m
4a	24.3, CH ₂	2.37, t (8.1)	24.1, CH ₂	2.48, m	24.3, CH ₂	2.34, m	24.0, CH ₂	2.45, m	23.9, CH ₂	2.44, m	24.1, CH ₂	2.36, m
4b				2.36, m		2.31, m		2.29, m		2.28, m		2.28, m
5	101.0, qC		100.6, qC		99.8, qC		99.3, qC		98.6, qC		98.9, qC	
6	154.7, CH	7.40, s	154.9, CH	7.43, s	160.6, CH	7.36, s	160.6, CH	7.38, s	158.3, CH	7.44, s	158.0, CH	7.40, s
7a	130.5, CH	6.42, m	130.5, CH	6.43, m	55.2, CH ₂	3.64, m	55.2, CH ₂	3.66, m	58.2, CH ₂	3.68, m	58.1, CH ₂	3.66, m
7b						3.20, m		3.20, m		3.48, m		3.47, m
8a	113.9, CH	5.33, m	114.0, CH	5.34, m	25.8, CH ₂	1.78, m	25.8, CH ₂	1.78, m	30.3, CH ₂	1.78, m	29.7, CH ₂	1.77, m
8b						1.57, m		1.58, m		1.53, m		1.57, m
9a	22.0, CH ₂	2.20, m	22.1, CH ₂	2.22, m	23.7, CH ₂	1.89, m	23.7, CH ₂	1.90, m	24.5, CH ₂	1.61, m	24.2, CH ₂	1.60, m
9b				2.18, m		1.38, m		1.39, m				
10a	26.9, CH ₂	2.34, m	27.0, CH ₂	2.37, m	31.2, CH ₂	2.19, m	31.4, CH ₂	2.23, m	27.6, CH ₂	1.89, m	25.2, CH ₂	1.90, m
10b		1.66, m		1.71, m		1.33, m		1.38, m		1.60, m		1.58, m
11	58.5, CH	3.68, ddd (15.8, 11.7, 3.0)	58.4, CH	3.65, ddd (15.8, 11.3, 5.4)	60.0, CH	3.23, ddd (16.2, 11.0, 3.5)	59.9, CH	3.22, ddd (15.7, 11.6, 3.2)	62.8, CH	3.77, ddd (12.2, 8.6, 4.1)	62.9, CH	3.72, ddd (11.6, 8.3, 4.1)
12	40.8, CH	2.94, ddd (15.8, 11.5, 5.6)	39.9, CH	2.84, ddd (15.8, 11.3, 5.4)	41.7, CH	2.78, ddd (16.2, 11.6, 5.5)	41.0, CH	2.71, ddd (15.7, 11.5, 5.2)	38.0, CH	3.27, ddd (11.5, 7.8, 4.1)	39.1, CH	3.36, ddd (12.3, 7.6, 4.1)
13	167.2, qC		164.3, qC		166.4, qC		166.1, qC		164.4, qC		165.0, qC	
14	58.0, CH	4.22, d (6.3)	59.0, CH	4.11, dd (11.3, 5.4)	57.5, CH	4.20, d (6.1)	58.9, CH	4.06, dd (11.4, 5.4)	59.4, CH	4.08, dd (10.8, 5.2)	58.3, CH	4.16, dd (6.1, 1.4)
15a	26.4, CH ₂	2.32, m	27.1, CH ₂	2.46, m	26.1, CH ₂	2.30, m	27.1, CH ₂	2.41, m	27.6, CH ₂	2.37, m	26.9, CH ₂	2.28, m
15b		2.14, m		1.73, m		2.09, m		1.69, m		1.71, m		2.10, m
16a	20.2, CH ₂	2.05, m	22.9, CH ₂	2.23, m	20.6, CH ₂	1.98, m	23.4, CH ₂	2.14, m	21.5, CH ₂	1.86, m	19.0, CH ₂	1.70, m
16b		1.34, m		1.48, m		1.24, m		1.41, m		1.67, m		1.53, m
17	179.2, qC		179.2, qC		179.4, qC		179.6, qC		179.5, qC		179.8, qC	

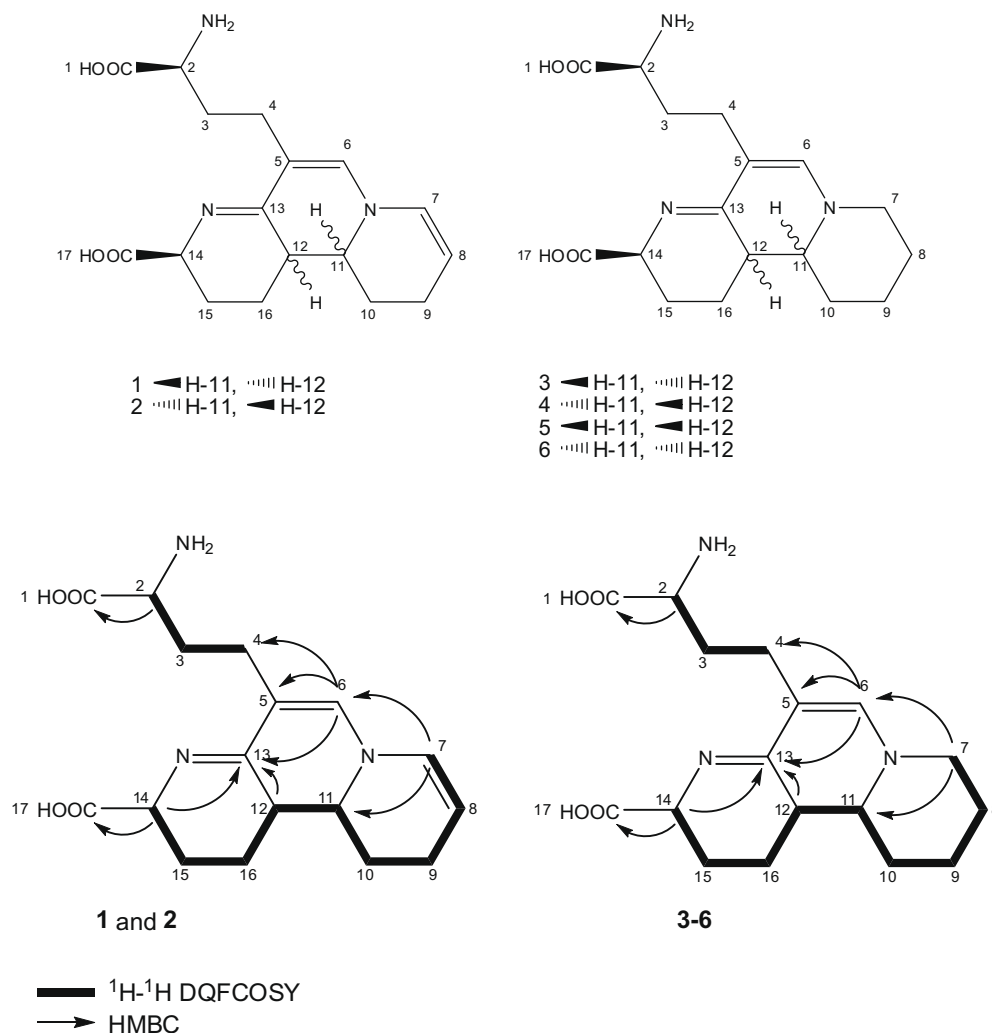


Figure 1. Selected 2D NMR correlations of oryzamutaic acids B–G (1–6).

C-12 and C-13. The HMBC 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.

Oryzamutaic acids B–G (1–6) are considered to be biogenetically derived from three amino acids because their C-2 and C-14

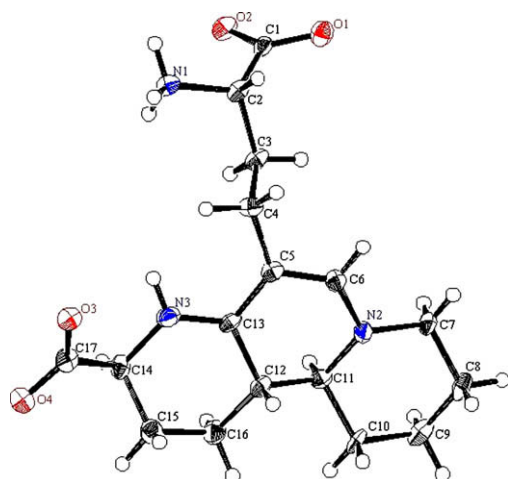


Figure 2. ORTEP drawing of oryzamutaic acid E (4).

bind a carboxyl group and a nitrogen atom. In addition, another nitrogen atom is contained in the molecules. The relative configuration of **4** was determined by single-crystal X-ray diffraction analysis⁸ (Fig. 2), and the absolute configuration was elucidated on the assumption that C-2 is in the configuration of L-amino acid. The relative stereochemistries of **1**–**3**, **5**, and **6** were deduced from analyses of ^1H – ^1H coupling constants and NOESY spectra (Fig. 3). The $J(\text{H-14}/\text{H-15b})$ and $J(\text{H-14}/\text{H-15a})$ values of **1** (6.3 and 0.0 Hz, respectively), **3** (6.1 and 0.0 Hz, respectively), and **6** (6.1 and 1.4 Hz, respectively) were similar to those of oryzamutaic acid A (5.9 and 0.0 Hz, respectively).¹ The NOESY correlations for H-15b/H-12 of **1**, **3**, and **6** indicated that both H-15b and H-12 were axial. Thus, the relative stereochemistries of C-14 to C-12 (through C-15 and C-16) of **1**, **3**, and **6** were similar to that of oryzamutaic acid A. The $J(\text{H-12}/\text{H-11})$ values of **1** (15.8 Hz) and **3** (16.2 Hz) and NOESY correlations for H-12/H-16a, H-12/H-10b, H-11/H-16b, H-11/H-10a, and H-16a/H-10a of **1** and **3** indicated that the relative stereochemistries of C-12 to C-11 of **1** and **3** were trans, like that of oryzamutaic acid A. The $J(\text{H-12}/\text{H-11})$ value of **6** (4.1 Hz) and NOESY correlations for H-16a/H-12, H-16a/H-11, H-12/H-11, H-12/H-10b, and H-11/H-10b of **6** indicated that the relative stereochemistry of C-12 to C-11 of **6** was cis. The $J(\text{H-14}/\text{H-15b})$ and $J(\text{H-14}/\text{H-15a})$ values of **2** (11.3 and 5.4 Hz, respectively) and **5** (10.8 and 5.2 Hz, respectively) were similar to those of **4** (11.5 and 5.2 Hz, respectively). The NOESY correlations for H-14/H-16b of **2** and **5** indicated that both H-14 and H-16b were axial, and the

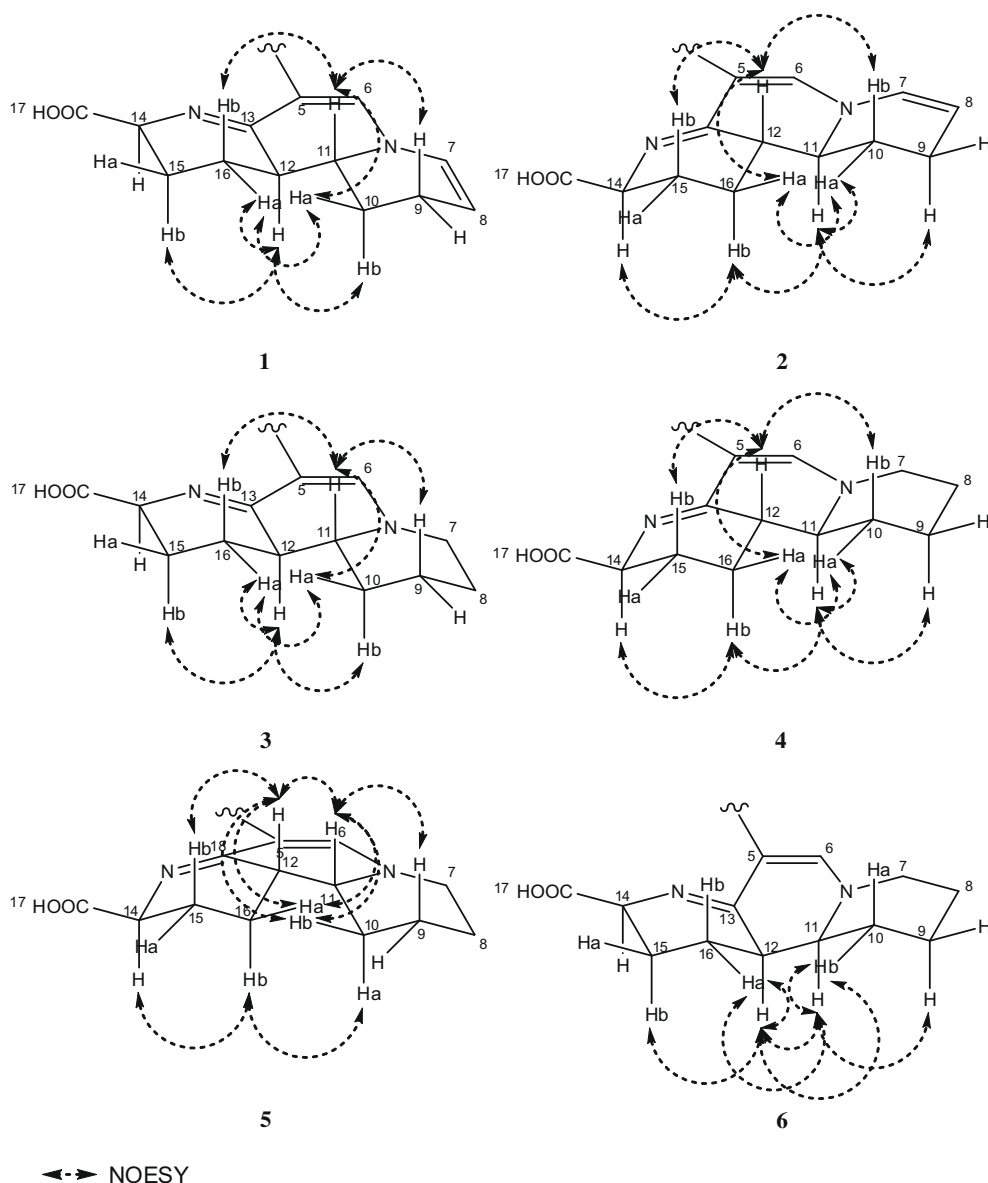


Figure 3. Selected NOESY correlations of oryzamutaic acids B–G (1–6).

correlations for H-15b/H-12 of **2** and **5** indicated that both H-15b and H-12 were axial. Thus, the relative stereochemistry of C-14 to C-12 (through C-15 and C-16) of **2** and **5** was similar to that of **4**. The $J(\text{H-12}/\text{H-11})$ value of **2** (15.8 Hz) and NOESY correlations for H-16a/H-12, H-12/H-10b, H-16b/H-11, H-11/H-10a, and H-16a/H-10a of **2** indicated that the relative stereochemistry of C-12 to C-11 of **2** was trans, like that of **4**. The $J(\text{H-12}/\text{H-11})$ value of **5** (4.1 Hz) and NOESY correlations for H-16a/H-12, H-16a/H-11, H-12/H-11, H-12/H-10b, and H-11/H-10b of **5** indicated that the relative stereochemistry of C-12 to C-11 of **5** was cis.

Oryzamutaic acids A and B–G (1–6) are structurally unique nitrogen-containing heterocyclic alkaloids. Oryzamutaic acids A, B (**1**), and C (**2**), 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. In contrast, oryzamutaic acids D–G (**3–6**) lack the double bonds between C-7 and C-8 and are colorless. Single-crystal X-ray diffraction indicated that N-2 of **4** was sp^2 hybridized with some sp^3 character. Thus, the resonance structure expanding 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 acid A, **1**, and **2**.

Oryzamutaic acid D (**3**) inhibited the root and shoot growth of lettuce (*Lactuca sativa*) seedling (I_{25} , i.e., 25% inhibitory concentration, 1.5 and 2.0 mM, respectively).

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2. Oryzamutic acid B (**1**) yellow powder; $[\alpha]_D^{20} +610$ (c 0.010, H₂O); UV (H₂O) λ_{\max} 393 nm (ϵ 8800); ATR-FTIR ν 3234, 2926, 1560, 1400, 1375, and 1291 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; HRESIMS m/z 334.1759 (M+H)⁺ (calcd for C₁₇H₂₄N₃O₄, 334.1761).
3. Oryzamutic acid C (**2**) yellow powder; $[\alpha]_D^{20} -300$ (c 0.002, H₂O); UV (H₂O) λ_{\max} 392 nm (ϵ 12,000); ATR-FTIR ν 3261, 2958, 1570, 1400, and 1286 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; HRESIMS m/z 334.1751 (M+H)⁺ (calcd for C₁₇H₂₄N₃O₄, 334.1761).
4. Oryzamutic acid D (**3**) colorless powder; $[\alpha]_D^{20} +430$ (c 0.048, H₂O); UV (H₂O) λ_{\max} 365 nm (ϵ 17,000); ATR-FTIR ν 3211, 2926, 2859, 1560, 1528, 1363, 1304, 1254, and 1201 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; HRESIMS m/z 336.1916 (M+H)⁺ (calcd for C₁₇H₂₆N₃O₄, 336.1918).
5. Oryzamutic acid E (**4**) colorless powder; $[\alpha]_D^{20} +4.2$ (c 0.033, H₂O); UV (H₂O) λ_{\max} 360 nm (ϵ 4700); ATR-FTIR ν 3234, 2956, 2941, 1575, 1392, 1294, and 1252 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; HRESIMS m/z 336.1915 (M+H)⁺ (calcd for C₁₇H₂₆N₃O₄, 336.1918).
6. Oryzamutic acid F (**5**) colorless powder; $[\alpha]_D^{20} +300$ (c 0.014, H₂O); UV (H₂O) λ_{\max} 367 nm (ϵ 13,000); ATR-FTIR ν 3212, 2932, 2859, 1574, 1526, 1387, 1362, 1306, 1258, and 1201 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; HRESIMS m/z 336.1912 (M+H)⁺ (calcd for C₁₇H₂₆N₃O₄, 336.1918).
7. Oryzamutic acid G (**6**) colorless powder; $[\alpha]_D^{20} +22$ (c 0.017, H₂O); UV (H₂O) λ_{\max} 368 nm (ϵ 10,000); ATR-FTIR ν 3211, 2941, 2864, 1560, 1389, 1363, 1311, 1254, and 1196 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; HRESIMS m/z 336.1914 (M+H)⁺ (calcd for C₁₇H₂₆N₃O₄, 336.1918).
8. A colorless needle-crystal of C₁₇H₂₅N₃O₄·6H₂O having approximate dimensions of 0.10 × 0.05 × 0.05 mm was mounted on a MicroMount (MiTeGen). All measurements were made on a Rigaku RAXIS V imaging plate area detector at beamline BL26B1 of the SPring-8 synchrotron facility; the wavelength was adjusted to 0.71069 Å. The crystal-to-detector distance was 179.75 mm. Cell constants and an orientation matrix for data collection corresponded to a primitive monoclinic cell with dimensions: $a = 12.879(3)$ Å, $b = 4.7657(11)$ Å, $c = 18.440(5)$ Å, $\beta = 97.566(3)^\circ$, $V = 1121.9(5)$ Å³. For $Z = 2$ and $FW = 442.49$, the calculated density is 1.310 g/cm³. Based on the systematic absence of $0k0: k \neq 2n$, packing considerations, a statistical analysis of intensity distribution, and the successful solution and refinement of the structure, the space group was determined to be $P2_1$ (#4). The data were collected at a temperature of -180 ± 1 °C to a maximum 2θ value of 48.3°. Of the 11,082 reflections that were collected, 3469 were unique ($R_{\text{int}} = 0.081$); equivalent reflections were merged. The linear absorption coefficient, μ , for Mo K α radiation is 1.071 cm⁻¹. An empirical absorption correction was applied which resulted in transmission factors ranging from 0.178 to 0.995. The data were corrected for Lorentz and polarization effects. The structure was solved by direct methods⁹ and expanded using Fourier techniques.¹⁰ The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined using the riding model. The final cycle of full-matrix least-squares refinement¹¹ on F^2 was based on 3419 observed reflections and 272 variable parameters and converged (large parameter shift was 0.00 times its esd) with unweighted and weighted agreement factors of: $R_1 = \sum ||F_o| - |F_c|| / \sum |F_o| = 0.0805$, $wR_2 = \{ \sum [w(F_o^2 - F_c^2)] / \sum w(F_o^2) \}^{1/2} = 0.2372$. The standard deviation of an observation of unit weight¹² was 1.08. Unit weights were used. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.42 and -0.64 e⁻/Å³, respectively. All calculations were performed using the CRYSTALSTRUCTURE¹³ and SHELXL-97.¹⁴ The X-ray crystallographic data has been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 746590. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road; Cambridge CB2 1EZ, UK (fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk).
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11. Least squares function minimized: (SHELXL97) $\sum w(F_o^2 - F_c^2)^2$, where w = least squares weights.
12. Standard deviation of an observation of unit weight: $[\sum w(F_o^2 - F_c^2)^2 / (N_o - N_v)]^{1/2}$, where: N = number of observation, N_v = number of variables.
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