# New Alkaloids from *Oryza sativa* cv. *Heugjinjubyeo* Bran

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High-valued grains have been consistently used for taste and health improvements. The anthocyanin soluble fraction of *Oryza sativa* cv. *Heugjinjubyeo*, purple colored rice, showed the lowering effects on blood glucose and lipid levels in streptozotocin induced diabetic male rats.[1] It contains high values of phytochemicals with antioxidative,[2-4] cytotoxic[5] and aldose inhibitory[6] activities. Repeated chromatographic separation, involving open silica gel and Sephadex LH-20 columns, of the ethyl acetate soluble fraction of *Oryza sativa* cv. *Heugjinjubyeo* bran led to the isolation of new pyridine alkaloids (**1**) and (**2**) as white amorphous powder. Compound (**1**) was obtained as amorphous powder with a molecular weight of *m/z* 467, based on EI-MS data. It exhibited a molecular ion peak at *m/z* 468.0950 based on HR Positive FAB-MS data. These data, together with the data obtained by $^1$H NMR and $^{13}$C NMR (Table 1), indicated a molecular formula of $C_{23}H_{17}O_{10}N$. The $^1$H NMR spectrum of compound (**1**) shows the presence of a carbomethoxy signal at $\delta$ 3.75, ABX-type aromatic protons on a 1,3,4-trisubstituted benzene moiety at $\delta$ 7.34, $\delta$ 7.32 and $\delta$ 6.80, *ortho*-coupled benzofuran moiety at $\delta$ 4.50 and $\delta$ 4.98, and 1,4-disubstituted benzene moiety at $\delta$ 5.83 and $\delta$ 5.88.[3] The *ortho*- and *meta*-coupled protons at $\delta$ 7.32 were correlated with two singlet protons at $\delta$ 7.34 ($J$ = 2.04) and $\delta$ 6.80 ($J$ = 8.28) in $^1$H-$^1$H COSY spectrum. The *ortho*-coupled two protons at $\delta$ 4.50 and $\delta$ 4.98 ($J$ = 11.16) were correlated with two carbons at $\delta$ 71.50 and $\delta$ 82.99 in HMQC spectrum. In the selective HMBC spectrum of compound (**1**), correlations of the protons to carbons were observed as Fig. 2. The presence of the carbomethoxy group of compound (**1**) was also deduced from both the carbon signals at $\delta$ 166.13 and $\delta$ 51.55. The location of the carbomethoxy group was determined to be at the C-5 position in dihydrobenzofuran moiety by HMBC spectrum. The methylenedioxy protons at $\delta$ 6.73 were correlated with two carbon peaks at $\delta$ 145.05 and $\delta$ 145.72 on a benzodioxine moiety in selective HMBC long range spectrum.[7] The 2,4-dihydroxyl groups and an amide group in the molecule were inferred by the IR bands (1,710 and 3,410 cm$^{-1}$) and $D_2O$ exchangeable signals at $\delta$ 8.99 (one proton) and $\delta$ 11.90 (two protons) in the $^1$H NMR spectrum. Compound (**1**) exhibited UV absorption bands at 240, 278 and 382 nm. These bands remained unaffected by the application of acid, as did the carbonyl absorption band at 1,662 cm$^{-1}$ and the amide absorption band at 1,628 cm$^{-1}$ and at $\delta$ 11.20 in its IR and $^1$H NMR spectrum, thereby suggesting the presence of a pyridone skeleton.[8] In HMBC spectrum, the singlet proton at $\delta$ 6.86 and one carbonyl carbon at $\delta$ 163.28 indicated the presence of 6-pyridine molecules.[4] The amide proton at $\delta$ 11.20 and two hydroxyl protons at $\delta$ 11.90 were correlated with carbons at $\delta$ 80.43 and $\delta$ 144.89, respectively. The peaks at $\delta$ 80.43 and $\delta$ 6.86 signals in pyridone moiety are also correlated with the peaks at $\delta$ 5.88 and $\delta$ 128.01 in benzodioxine moiety by HMBC spectrum. Thus, the structure of compound (**1**) was identified as 3-[6-(2,4-dihydroxy-6-oxo-1,6-dihydro-pyridin-3-yl)-benzo[1,3]dioxole-5-carbonyl]-2-hydroxy-2,3-dihydrobenzofuran-5-carboxylic acid methyl ester.

Compound (**2**) was obtained as white amorphous powder with a molecular weight of *m/z* 125 (observed 125.0466, estimated 125.0477), based on EI-MS and HR-MS data. These data, together with the data obtained by $^1$H NMR and $^{13}$C NMR, indicated a molecular formula of $C_6H_7O_2N$. Compound (**2**) exhibited UV absorption bands at 241, 280 and 385 nm. The *o*- and *p*-coupled double doublet protons at $\delta$ 7.55 ($J$ = 2.0 and 8.6 Hz) and *ortho*- and *para*-coupled doublet protons at $\delta$ 6.82 ($J$ = 8.6 Hz) and $\delta$ 7.53 ($J$ = 2.0 Hz) of the aromatic protons was correlated with a methane carbons at $\delta$ 152.40, $\delta$ 148.64 and $\delta$ 125.19, respectively. These observations showed that the hydroxyl and methoxyl group were attached at the C-2 and C-4 locations, respectively. Thus, the structure of compound (**2**) was identified as 2-hydroxy-4-methoxy pyridine. Several researches were reported that mono-substitutions on the pyridine ring at the 2-,4- and 5-positions, as well as several di-substituted variants, also were examined.[9] Up to date, compounds (**1**) and (**2**) were isolated as natural products for the first time.

## Experimental Section

**General**. Melting points (mp) were determined using a Mitamura-Riken melting point apparatus and are uncorrected. A Hewelett Packard Model 5985B Gas chromatography (GC)/MS system was used for electron impact mass spectrometry (EI-MS) and high resolution mass spectrometry (HR-MS) was performed using a JMS-700 spectrometer. The Ultraviolet/Visible spectra were recorded on a Hitachi 3100 UV/Vis spectrophotometer and Infrared (IR) spectra were detected on a JASCO Fourier transform (FT)-IR-5300 spectrophotometer. A Bruker AMX500 spectrometer was used to record nuclear magnetic resonance (NMR) spectra (500 MHz for $^1$H NMR

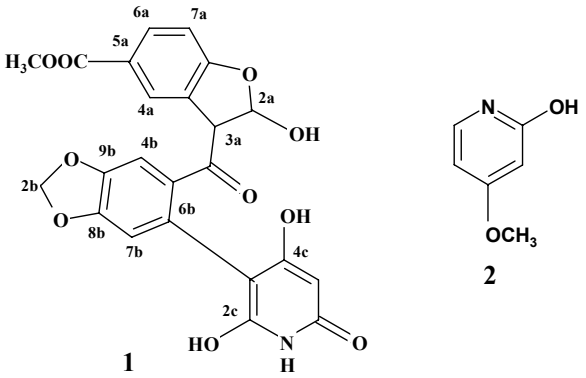


**Figure 1.** Chemical Structures of Compound **1** and **2**.

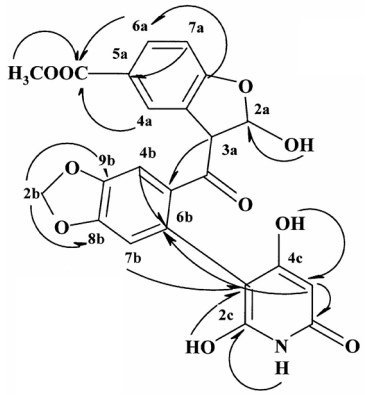


**Figure 2.** Selective HMBC Correlations of Compound **1**.

**Table 1.** $^{1}$H NMR and $^{13}$C NMR Spectral Data of Compound (**1**)[a]

| Position | $\delta_H$ (*J*, Hz) | $\delta_C$ | $^{1}$H-$^{1}$H COSY | HMBC (H to C) |
|---|---|---|---|---|
| 2a | 4.50 (d,[b] 11.16) | 71.50 | H-3a | C-6a |
| 3a | 4.98 (d, 11.16) | 82.99 | H-2a | $COOCH_3$, C-5b |
| 4a | 7.34 (d, 2.04) | 116.22 | H-6a | |
| 5a | | 150.42 | | |
| 6a | 7.32 (dd, 2.04, 8.28) | 121.72 | H-4a, 7a | $COOCH_3$ |
| 7a | 6.80 (d, 8.28) | 115.28 | H-6a | C-5a |
| 8a | | 162.50 | | |
| 9a | | 115.06 | | |
| 2b ($OCH_2O$) | 6.73 (s) | 101.50 | | C-8b, 9b |
| 4b | 5.83 (s) | 95.01 | | |
| 5b | | 119.33 | | |
| 6b | | 128.01 | | |
| 7b | 5.88 (s) | 96.01 | | C-2b, 3c |
| 8b | | 145.05 | | |
| 9b | | 145.72 | | |
| 2c | | 144.89 | | |
| 3c | | 80.43 | | |
| 4c | | 150.42 | | |
| 5c | 6.86 (s) | 115.31 | | C-6b, 6c, 4c-OH |
| 6c | | 163.28 | | |
| CO (a) | | 166.13 | | |
| CO (b) | | 197.60 | | |
| $OCH_3$ | 3.75 (q) | 51.55 | | C-5a, 2c |
| NH | 11.20 (br s) | | | C-2c |
| OH | 8.99 (s) | | | |
| OH | 11.90 (br s) | | | |

[a]TMS was used as the internal standard; chemical shifts are shown in the $\delta$ scale with *J* values in parenthesis. [b]s: singlet; br s: broad singlet; d: doublet; dd: double doublet; q: quartet.

and 125 MHz for $^{13}$C NMR) with tetramethylsilane (TMS) as an internal standard and DMSO-$d_6$ as a NMR solvent. Two-dimensional NMR spectroscophic techniques were used for $^{1}$H-$^{1}$H correlation (COSY), for heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC). Thin-layer chromatographic (TLC) analysis was performed on 0.25 mm silica gel Kiesel gel 60 $F_{254}$ plates (Merck, Darmstadt, Germany). Silica gel (Merck 60 A, 230-400 mesh, ASTM) and Sephadex LH-20 (25-100 μm; Pharmacia Fine Chemicals, Piscataway, NJ) were used for open column and flash column chromatographic separation.

**Plant material**. The fully ground *Oryza sativa* cv. *Heugjinjubyeo* bran was supplied by the National Crop Experiment Station, Rural Development Administration (RDA), Suwon, Korea. A voucher specimen has been deposited at the RDA. The samples were kept in a refrigerator before use for experiments.

**Extraction and isolation**. The dried and ground *Oryza sativa*

cv. *Heugjinjubyeo* bran (1.0 kg) was extracted 5 times with 80% ethyl alcohol (EtOH) for 24 hours at room temperature. The combined dark-purple EtOH extracts (54.7 g) were partitioned with *n*-hexane and water. After then, water layer partitioned with ethyl acetate (EtOAc). The dried EtOAc soluble fraction (4.6 g) was chromatographed over a Sephadex LH-20 column by elution with MeOH to give 13 fractions according to TLC profiles using Dragendorff's reagent.[10] Fractions 8-9 (180.9 mg) were chromatographed over a dried silica gel vacuum column using a $CHCl_3$-MeOH (94:5 to 92:10, *v/v*) gradient to give 21 subfractions. Subfractions 7-12 (75.1 mg) were rechromatographed on a Sephadex LH-20 column by elution with MeOH in order to give white solid materials. This material was further purified by re-crystallization with highly purified MeOH to yield the pure compound **(1)** (11.6 mg). Subfractions 3-4 (51.2 mg) was rechromatographed on a silica gel column using *n*-hexane-EtOAc (95:5 to 82:8, *v/v*) gradient to give single spot materials in TLC profiles. This material was further crystallized with highly purified MeOH to yield the pure compound **(2)** (9.7 mg).

**3-[6-(2,4-Dihydroxy-6-oxo-1,6-dihydro-pyridin-3-yl)-benzo[1,3]dioxole-5-carbonyl]-2-hydroxy-2,3-dihydro-benzofuran-5-carboxylic acid methyl ester (1).** White amorphous powder from MeOH; mp 202 $^{o}$C; UV $\lambda_{max}$ (MeOH) (log ε): 240 (4.80), 278 (4.35), 382 (4.26) nm; IR $\nu_{max}$ (KBr) 3,410 (OH, NH), 1,710, 1,660 (CO), 1,628 (CN) $cm^{-1}$; HR positive FAB-MS m/z: 468 ($[M+H]^+$, calculated for 468.0950); $^1$H NMR (500 MHz, DMSO-$d_6$) and $^{13}$C NMR (125 MHz, DMSO-$d_6$) data was described as Table 1.

**2-Hydroxy-4-methoxypyridine (2).** White amorphous powder from MeOH; mp 228 $^{o}$C; UV $\lambda_{max}$ (MeOH) (log ε): 240 (4.70), 280 (4.41), 385 (4.23) nm; IR $\nu_{max}$ (KBr) 3,450 (OH), 1,708, 1,660 (CO) $cm^{-1}$; HR EI-MS m/z: 125 ($[M]^+$, calculated for 125.0466); $^1$H NMR (500 MHz, DMSO-$d_6$): δ 3.31 (q, $J$ = 2.0 Hz, $OCH_3$), 6.82 (d, $J$ = 8.6 Hz, H-6), 7.53 (d, $J$ = 2.0 Hz, H-3), 7.55 (dd, $J$ = 2.0, 8.6 Hz, H-5); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ 56.42 ($OCH_3$), 113.88 (C-2), 125.19 (C-3), 148.64 (C-6), 152.40 (C-5), 170.15 (C-4).

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