

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

Eun Sil Kim, Jin Won Hyun,[†] Jin Chul Shin,[‡] and Ha Sook Chung^{*}

College of Natural Sciences, Duksung Women's University, Seoul 132-714, Korea

*E-mail: hasook@duksung.ac.kr

[†]College of Medicine and Applied Radiological Science Research Institute, Cheju National University, Jeju 690-756, Korea

[‡]National Institute of Crop Science, Rural Development Administration, Suwon 441-701, Korea

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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 cytotoxic⁵ and aldose inhibitory⁶ 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 ¹H NMR and ¹³C NMR (Table 1), indicated a molecular formula of C₂₃H₁₇O₁₀N. The ¹H NMR spectrum of compound (1) shows the presence of a carbomethoxy signal at δ 3.75, ABX-type aromatic protons on a 1,3,4-trisubstituted benzene moiety at δ 7.34, δ 7.32 and δ 6.80, *ortho*-coupled benzofuran moiety at δ 4.50 and δ 4.98, and 1,4-disubstituted benzene moiety at δ 5.83 and δ 5.88.³ The *ortho*- and *meta*-coupled protons at δ 7.32 were correlated with two singlet protons at δ 7.34 (*J* = 2.04) and δ 6.80 (*J* = 8.28) in ¹H-¹H COSY spectrum. The *ortho*-coupled two protons at δ 4.50 and δ 4.98 (*J* = 11.16) were correlated with two carbons at δ 71.50 and δ 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 δ 166.13 and δ 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 δ 6.73 were correlated with two carbon peaks at δ 145.05 and δ 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 D₂O exchangeable signals at δ 8.99 (one proton) and δ 11.90 (two protons) in the ¹H 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 δ 11.20 in its IR and ¹H NMR spectrum, thereby suggesting the presence of

a pyridone skeleton.⁸ In HMBC spectrum, the singlet proton at δ 6.86 and one carbonyl carbon at δ 163.28 indicated the presence of 6-pyridine molecules.⁴ The amide proton at δ 11.20 and two hydroxyl protons at δ 11.90 were correlated with carbons at δ 80.43 and δ 144.89, respectively. The peaks at δ 80.43 and δ 6.86 signals in pyridone moiety are also correlated with the peaks at δ 5.88 and δ 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 ¹H NMR and ¹³C NMR, indicated a molecular formula of C₆H₇O₂N. Compound (2) exhibited UV absorption bands at 241, 280 and 385 nm. The *o*- and *p*-coupled double doublet protons at δ 7.55 (*J* = 2.0 and 8.6 Hz) and *ortho*- and *para*-coupled doublet protons at δ 6.82 (*J* = 8.6 Hz) and δ 7.53 (*J* = 2.0 Hz) of the aromatic protons was correlated with a methane carbons at δ 152.40, δ 148.64 and δ 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 Hewlett 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 ¹H NMR

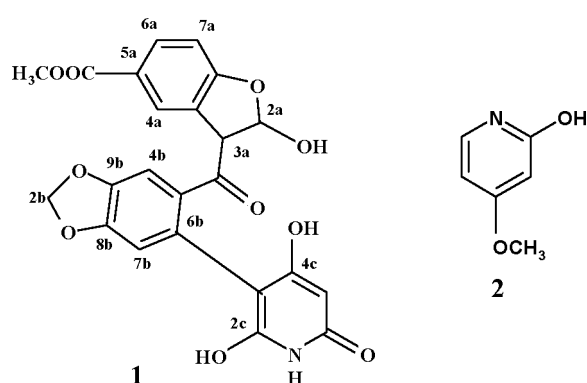


Figure 1. Chemical Structures of Compound 1 and 2.

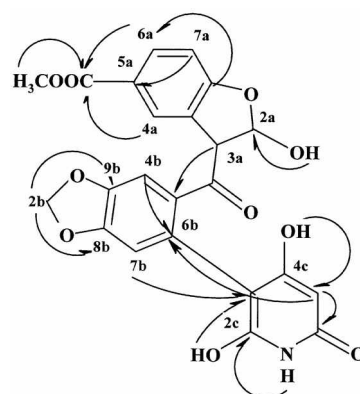


Figure 2. Selective HMBC Correlations of Compound 1.

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

Position	δ_{H} (J , Hz)	δ_{C}	^1H - ^1H 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 ₂ O)	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)			

^aTMS was used as the internal standard; chemical shifts are shown in the δ scale with J values in parenthesis. ^bs: 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 spectroscopic techniques were used for ^1H - ^1H 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 F₂₅₄ 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. *Heug-jinjubyeo* 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 CHCl₃-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 °C; UV λ_{max} (MeOH) (log ϵ): 240 (4.80), 278 (4.35), 382 (4.26) nm; IR ν_{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); ¹H NMR (500 MHz, DMSO-d₆) and ¹³C NMR (125 MHz, DMSO-d₆) data was described as Table 1.

2-Hydroxy-4-methoxypyridine (2). White amorphous powder from MeOH; mp 228 °C; UV λ_{max} (MeOH) (log ϵ): 240 (4.70), 280 (4.41), 385 (4.23) nm; IR ν_{max} (KBr) 3.450 (OH),

1.708, 1.660 (CO) cm⁻¹; HR EI-MS m/z : 125 ([M]⁻, calculated for 125.0466); ¹H NMR (500 MHz, DMSO-d₆): δ 3.31 (q, J = 2.0 Hz, OCH₃), 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); ¹³C NMR (125 MHz, DMSO-d₆): δ 56.42 (OCH₃), 113.88 (C-2), 125.19 (C-3), 148.64 (C-6), 152.40 (C-5), 170.15 (C-4).

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