A New 2-Arylbenzofuran with Antioxidant Activity from the Black Colored Rice (*Oryza sativa* L.) Bran

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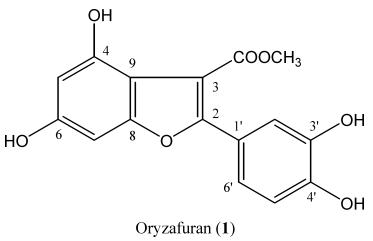
From the black colored rice bran of *Oryza sativa* cv. Heugjinjubyeo, a new 2-arylbenzofuran, 2-(3,4-dihydroxyphenyl)-4,6-dihydroxybenzofuran-3-carboxylic acid methyl ester, oryzafuran (1), was isolated. Its structure has been elucidated on the basis of spectral data. This compound showed strong antioxidative activity in a 1,1-diphenyl-2-picrylhydrazyl free radical scavenging assay.

Key words *Oryza sativa*; Poaceae; black colored rice; 2-arylbenzofuran; oryzafuran; antioxidative activity

Rice (*Oryza sativa* L., Poaceae) is the principle cereal food in Asia and the staple food of nearly half of the world's population.[1)] Black colored rice has long been consumed in Korea, Japan and China and is considered to be a healthy food. We were especially interested in the antioxidative and radical scavenging properties of rice because of the potential of such properties to provide protection against reactive oxygen species and free radicals, which have been implicated in more than 100 diseases.[2)] There have already been some reports concerning the antioxidative compounds found in black colored rice. γ-Oryzanol and tocopherol are well known as an antioxidant in rice bran.[1)] The major pigment, cyanidin 3-*O*-β-D-glucoside (C3G), has been reported to be one of the major antioxidant compounds.[3,4)] However, little information exists on the antioxidative effects of black colored rice, although the presence of antioxidants such as polymeric procyanidins[2)] and 4-carbomethoxy-6-hydroxy-2-quinolone[5)] has been reported. In the course of our investigations of bioactive substances from diverse crops, we found that black colored rice, especially Heugjinjubyeo, exhibited significant antioxidant activity.[3,6)] The purpose of the present study therefore was to isolate and identify other types of antioxidants present black colored rice bran, specially the BuOH soluble extract of Heugjinjubyeo bran.

The defatted BuOH soluble fraction of the MeOH extract from the Heugjinjubyeo bran was repeatedly subjected to silica gel column and Sephadex LH-20 column chromatography, followed by reverse-phase semipreparative HPLC to yield 2-(3,4-dihydroxyphenyl)-4,6-dihydroxybenzofuran 3-carboxylic acid methyl ester, oryzafuran (**1**), together with four known compounds, quercetin (**2**),[7)] protocatechuic acid (**3**),[8)] vanillic acid (**4**)[8)] and palmitic acid (**5**).[9)] Of these compounds, **2**—**5** were identified by direct comparisons of their spectroscopic data with those reported.



Oryzafuran (**1**)

Oryzafuran (**1**) was obatained as pale brown needle crystals. The molecular formula of **1** was determined as $C_{16}H_{12}O_7$ by high-resolution (HR)-EI-MS. The UV spectrum of **1** showed absorption maxima at 250 and 340 nm that shifted in alkali to 260 and 385 nm, respectively, closely resembling a 2-aryl-3-carbonylbenzofuran derivative.[10,11)] The ^{1}H-NMR spectrum of **1** shows the presence of a carbomethoxy signal at δ 3.82, ABX-type aromatic protons on a 1,3,4-trisubstituted benzene moiety (δ 7.16, 6.83, 7.08) and *meta*-coupled aromatic protons on a 1,2,3,5-tetrasubstituted benzene moiety (δ 6.46, 6.21). The presence of the carbomethoxy group was also deduced from both the carbon signals (δ 167.1, 52.6) in the ^{13}C-NMR spectrum of **1** and the absorption at 1637 cm^{-1} in its IR spectrum. The location of the carbomethoxy group was determined to be at the C-3 position from the heteronuclear multiple bond connectivity (HMBC) spectrum (Table 1). The placement of the carbomethoxy group at C-3 was confirmed the nuclear Overhauser effect spectroscopy (NOESY) spectrum of **1**, which showed NOE interactions between the methyl protons and the H-2′,5′,6′ protons. Thus, the structure of oryzafuran (**1**) was characterized as 2-(3,4-dihydroxyphenyl)-4,6-dihydroxybenzofuran 3-carboxylic acid methyl ester. Oryzafuran (**1**) is the only 2-arylbenzofuran carrying a carbomethoxy group in plant kingdom.

Table 1. NMR Spectral Data of Oryzafuran (**1**) in DMSO-d_6

Position	δ_H	J (Hz)	δ_C (DEPT)	HMBC (H→C)
2			157.4 (C)	
3			106.3 (C)	
4			151.0 (C)	
5	6.46	d, 2.0	98.7 (CH)	C-7, C-9
6			155.2 (C)	
7	6.21	d, 2.0	89.2 (CH)	C-5, C-9
8			156.7 (C)	
9			106.9 (C)	
1′			120.1 (C)	
2′	7.16	d, 2.5	115.7 (CH)	C-4′, C-6′, C-2
3′			147.4 (C)	
4′			144.9 (C)	
5′	6.83	d, 8.0	115.4 (C)	C-1′, C-3′
6′	7.08	dd, 8.0, 2.5	120.5 (C)	C-2′, C-4′, C-2
$\underline{C}OOCH_3$			167.1 (C)	
$COO\underline{CH}_3$	3.82	s	52.6 (CH_3)	$\underline{C}OOCH_3$, C-3

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Table 2. DPPH Free Radical Scavenging Activity of **1**—**4**

Compounds	EC_{50} (μg/ml)
Oryzafuran (**1**)	1.58±0.001
Quercetin (**2**)	2.73±0.004
Protocatechuic acid (**3**)	2.33±0.007
Vanillic acid (**4**)	>20.00
Ascorbic acid	3.35±0.006

Four isolated compounds (**1**—**4**) were assessed for antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, the results of which are presented in Table 2. The measured EC_{50} value of **1** (1.58±0.001 μg/ml) showed that **1** has a significant activity against the DPPH radical, which was more potent than other phenolic compounds (**2**—**4**). It has been reported that the radical scavenging activity of one of the 2-arylbenzofuran derivative, bolusanthin IV from *Bolusanthus speciosus* was lower than that of quercetin or ascorbic acid.[12] The strong antioxidant activities of quercetin (**2**)[13,14] and other phenolic acids such as protocatechuic and vanillic acids (**3**, **4**) have been previously reported.[15,16] In these results, it was postulated that these isolated phenolic compounds contribute to the antioxidant activity of black colored rice, Heugjinjubyeo, bran. The stronger scavenging activity of the MeOH extract of black colored rice bran than the corresponding extracts from other varieties may be due to the presence of high content of C3G and of other phenolic compounds isolated from this study.[2] These data suggest that black colored rice, Heugjinjubyeo, may have some health benefits associated with the relief of oxidative stress.

Experimental

General Procedures Melting points were measured on a Büchi B-540 apparatus and are uncorrected. The UV spectra were recorded on a HP 8453 UV–Vis spectrophotometer in MeOH solution. The IR spectra were measured on a JASCO FT/IR-5300 spectrophotometer as KBr disks. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded on a Bruker CXP-500 spectrometer. Chemical shifts are shown in δ values with tetramethylsilane (TMS) as an internal reference. EI-MS were obtained on a Hewelett Packard Model 5989B GC/MS spectrometer and HR-EI-MS were measured on a JMS700 spectrometer.

Plant Material Black colored rice, Heugjinjubyeo, was grown at the National Crop Experiment Station, Rural Development Administration (RDA), Suwon, Gyeonggi-do, Korea, in 2002. A voucher specimen (IT191964) has been deposited at the RDA.

Extraction and Separation Rice bran was obtained by milling brown rice with an Automatic rice tester (SY 94+RAT22400, SsangYong Machinery Co., Ltd., Korea). The rice bran (10 kg) was extracted six times with MeOH under reflux. The combined MeOH extract was concentrated under reduced pressure and the residue was partitioned between *n*-hexane and water. The aqueous layer was further partitioned with BuOH. The BuOH layer was concentrated under reduced pressure to give a dark purple residue. The BuOH fraction (58 g) was chromatographed over a silica gel column (10×100 cm) using $CHCl_3$–MeOH (98 : 2→85 : 15, gradient) system to give 24 fractions. Fraction 16 (326 mg) was rechromatographed on a Sephadex LH-20 column (5×100 cm) by elution with water–MeOH (98 : 2→85 : 15, gradient) system to give five fractions (Fr. 16-1—Fr. 16-5). Fraction 16-3 (128 mg) was further separated by preparative HPLC (Nomura Chemical ODS, i.d. 10×250 mm, 25% MeCN in H_2O, 1 ml/min) to give compound **1** (24 mg) and **2** (14 mg). Fractions 20 (124 mg) and 24 (47 mg) were separately fractionated by repeated column chromatography on Sephadex LH-20 and ODS by elution with water–0.1% trifluoracetic acid in MeOH (1 : 0→0 : 1, gradient) system. Compound **3** (73 mg) and **4** (26 mg) were obtained from fraction 20, and compound **5** (19 mg) was obtained from fraction 24, respectively.

Oryzafuran (**1**): Pale brown needles (MeOH), mp 251—252 °C. UV λ_{max} (MeOH) nm (log ε): 250 (4.31), 340 (4.17). UV λ_{max} (MeOH+NaOH) nm (log ε): 260 (4.40), 385 (4.22). IR (KBr) cm^{-1}: 3582, 3493, 3321, 1637, 1500. HR-EI-MS *m/z*: 316.0594 (Calcd for $C_{16}H_{12}O_7$: 316.0583). EI-MS (70 eV) *m/z*: 316 $[M]^+$ (100), 285 $[M-CH_3O]^+$ (8.8), 284 $[M-CH_3OH]^+$ (39.7), 256 $[M-CH_3OH-CO]^+$ (47.9), 228 $[M-CH_3OH-2\times CO]^+$ (23.1), 200 $[M-CH_3OH-3\times CO]^+$ (6.6), 171 (8.3), 149 (5.8). ^{1}H-NMR (DMSO-d_6, 500 MHz) and ^{13}C-NMR (DMSO-d_6, 125 MHz): Table 1.

Scavenging Effect on DPPH Radical The DPPH radical scavenging activity of the samples were estimated according to the method of Hatano *et al.*[17] Samples in MeOH (0.5 ml) added to a solution of DPPH radical in EtOH (60 μM, 0.5 ml), and the reaction mixture was left to stand for 30 min at room temperature in the dark. The scavenging activity of samples at 1.25, 2.5, 5, 7.5 and 10 μg/ml was estimated by measuring the absorption of the mixture at 515 nm, which reflects the amount of DPPH radical remaining in the solution. The scavenging activity was expressed as the EC_{50}, the concentration of samples required for scavenging 50% of DPPH radical in the solution. Ascorbic acid (Junsei Chemical Co., Ltd., Japan) was used as a standard agent.

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