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Food Chemistry 104 (2007) 1670–1677
Food Chemistry
www.elsevier.com/locate/foodchem

Characterization of antioxidant alkaloids and phenolic acids from anthocyanin-pigmented rice (*Oryza sativa* cv. *Heugjinjubyeo*)

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Received 4 December 2006; received in revised form 21 December 2006; accepted 7 March 2007

Abstract

As a part of our study on the analysis of bioactive secondary metabolites, we used bioactivity-guided fractionation and isolation methods to isolate five compounds from the ethylacetate-soluble fraction of the aleurone layer of *Oryza sativa* cv. *Heugjinjubyeo*, a highly developed rice cultivar; 4-carboethoxy-6-hydroxy-2-quinolone (**1**), ethyl-3,4-dihydroxybenzoic acid (**2**), 4-hydroxy-3-methoxyphenylacetic acid (**3**), 3,4-dihydroxybenzoic acid (**4**), and 4-hydroxy-3-methoxy cinnamic acid (**5**). These compounds showed significant antioxidant activity in a concentration-dependent manner through the scavenging of 1,1-diphenyl-2-picrylhydrazyl radicals. The structure of new compound **1** was elucidated on the basis of spectroscopic evidence, particularly the results obtained via hetero-nuclear multiple-bond connectivity and high-resolution MS spectroscopy.

Keywords: Anthocyanin-pigmented rice; Antioxidant; Alkaloid; Phenolic acids; 2D NMR; DPPH; Radical scavenging

1. Introduction

Select phytochemicals, originating from natural sources and exhibiting biological activity, are considered to be critical for human health. Several crops have proven to be important sources of a number of phytochemicals and secondary metabolites. Therefore, it appears reasonable to surmise that additional agents, thus far undiscovered, also exist. With the nationwide growth of interest in health and the expanding health food market, research into the industrial uses of secondary metabolites in crops has also increased. Development of high-quality varieties containing increased levels of bioactive compounds may increase the nutritional value of the harvest crops (Hyun & Chung, 2006). A great deal of recent research has been focussed on the development of new bioactive agents from cereals (Chung & Woo, 2001; Chung et al., 2006; Han, Ryu, & Kang, 2004; Iwatsuki et al., 2003; Suzuki, Okada, & Okuyama, 2003; Wenzig et al., 2005).

Rice (*Oryza sativa* Linn.) is the principle cereal consumed in Asia, and the primary staple for nearly half of the world's population. Anthocyanin-pigmented rice (*Oryza sativa* cvs. *Heugjinjubyeo*, *Heugnambyeo*, *Hongmi*, *Jakwangdo*, *Kilimheugmi*, *Sanghaehyeolla*, Suwon #405, Suwon #415, Suwon #420, and Suwon #425) was produced by genetic engineering techniques in Korea. This rice has a characteristic dark purple colour, which is attributable to a genomic modification affecting the aleurone layer of the rice grains.

Oryza sativa cv. *Heugjinjubyeo*, one of the anthocyanin-pigmented rice varieties, is well known for its taste and health-improving properties. Cyanidin-3-*O*-β-D-glucopyranoside is the most abundant pigment in purple rice (Ryu, Park, & Ho, 1998). It also has been known to have diverse physiological effects, protection against cytotoxicity (Chen et al., 2005), antineurodegenerative activity (Kim, Heo, Kim, Yang, & Lee, 2005), glycogen phosphorylase inhibition (Jakobs, Fridrich, Hofem, Pahlke, & Eisenbrand,

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doi:10.1016/j.foodchem.2007.03.020

2006), antioxidative activity (Kano, Takayanagi, Harada, Makino, & Ishikawa, 2005; Nam et al., 2006), and protective effects in carrageenan-induced pleurisy (Rossi et al., 2003).

In our ongoing efforts to characterize biological activities inherent in highly developed rice varieties (Chung & Woo, 2001; Chung, Han, Ko, & Shin, 2001; Chung & Han, 2002; Chung, 2002; Hyun & Chung, 2004), *Oryza sativa* cv. *Heugjinjubyeo* (Gramineae) was selected for study. This variety was chosen because the ethyl acetate (EtOAc)-soluble extract of the aleurone layer of rice was found to exhibit significant antioxidant activity, based on scavenging activity of stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals (IC_{50}: 18.5 μg/ml) (Chung et al., 1999). Bioassay-monitored fractionation of the active EtOAc extract using the DPPH antioxidant assay led to the isolation of one novel alkaloid (**1**) and four known phenolic acids (**2**–**5**), which were then evaluated for their individual biological activities. The structure of a new compound (**1**) was elucidated on the basis of spectroscopic evidence, particularly the results of hetero-nuclear multiple-bond connectivity and high-resolution mass spectroscopy.

2. Materials and methods

2.1. Chemicals

DPPH was obtained from Sigma Chemical Co. (St. Louis, MO) The compounds were dissolved in dimethylsulfoxide (DMSO) and stored at −4 °C. Other chemicals were purchased from commercial sources and were of the highest purity available.

2.2. Sample material

The fully ground aleurone layer of *Oryza sativa* cv. *Heugjinjubyeo* 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 until used for experiments.

2.3. Instrumental analyses

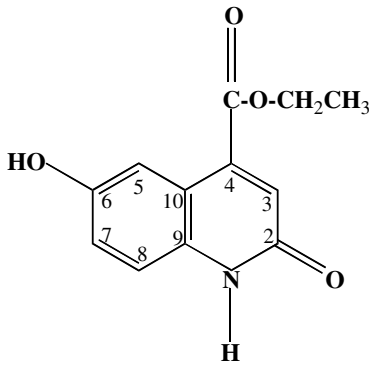
Melting points (mp) were determined using a Mitamura-Riken melting point apparatus and are uncorrected. A Hewelett Packard Model 5985B gas chromatography (GC)/MS system was used for electron impact mass spectrometry (EI-MS) and high resolution mass spectrometry (HR-MS) was performed using a JMS-700 spectrometer. The UV/Vis 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 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 ^1H–^1H correlation spectroscopy (COSY) and for experiments on 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). After samples developed, compounds were visualized by spraying plates with Dragendorff and $FeCl_3$ reagents for the identification of alkaloids and phenols. 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.

2.4. Assay for DPPH free radical-scavenging activity

This assay is based on the scavenging activity of stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals. Reaction mixtures containing 5 ml of test samples dissolved in DMSO and 95 ml of 300 mM DPPH in ethanol solution (final DPPH concentration) were incubated at 37 °C for 30 min in 96-well microfilter plates. Absorbance was then measured at 515 nm. Percent inhibition in sample treatments was determined by comparison with a DMSO-treated control group. IC_{50} values denote the concentration of sample required to scavenge 50% of DPPH free radicals. On the basis of reaction conditions, and in order to confirm the usefulness of the assay, commercial antioxidants, including ascorbic acid and 2(3)-*tert*-butyl-4-hydroxyanisole (BHA), were also evaluated for their free radical-scavenging activity.

2.5. Extraction and isolation of compounds

The dried and ground aleurone layer of *Oryza sativa* cv. *Heugjinjubyeo* (2 kg) was extracted 5 times with 80% ethyl alcohol (EtOH) for 24 h at room temperature. The combined dark-purple EtOH extracts (128.1 g) were partitioned between *n*-hexane and water, with the more polar layer then partitioned with chloroform ($CHCl_3$), EtOAc, and *n*-butanol (*n*-BuOH). The dried EtOAc-soluble fraction (20.8 g) was chromatographed over a silica gel vacuum column using a $CHCl_3$–methanol (MeOH) gradient to give 11 fractions. Fraction 4 (12.7 g) was further chromatographed on a silica gel open column using $CHCl_3$–MeOH (97:3 to 95:5, v/v), and subfractions 4–17 were rechromatographed on a Sephadex LH-20 column by elution with MeOH in order to give pale yellow solid materials. This yellow material was further purified by re-crystallization with highly purified MeOH to give the pure compound **1** (38.4 mg). Subfractions 87–123 were rechromatographed on a silica gel column by elution with aqueous EtOAc–MeOH (99:1 to 90:10, v/v) to isolate compounds **2**–**5**. Complete identification of isolated compounds made use of varieties of physical and chemical techniques: EI-MS spectrometry,



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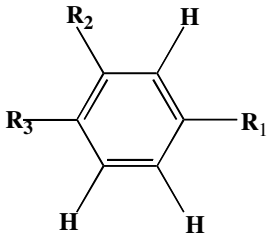

2 : R_1=CH_2COOCH_3, R_2=OH, R_3=OH
3 : R_1=CH_2COOH, R_2=OCH_3, R_3=OH
4 : R_1=COOH, R_2=OH, R_3=OH
5 : R_1=CHCHCOOH, R_2=OCH_3, R_3=OH

Fig. 1. Chemical structures of compounds **1**–**5**.

HREI-MS spectrometry, UV/Vis spectrophotometry, FT-IR spectrophotometry, ^{1}H NMR spectroscopy, and ^{13}C NMR spectroscopy (Fig. 1).

3. Results and discussion

3.1. Isolation and structure determination of compound *1*

The dried and ground aleurone layers were extracted from *Oryza sativa* cvs. *Juanbyeo*, *Heugjinjubyeo*, *Ilpumbyeo*, *Jukjinjubyeo*, and *Obongbyeo* with 80% EtOH. All of these alcohol extracts of rice grains were evaluated initially for antioxidant activity, at a final concentration of 100 mg/ml, using a DPPH free radical-scavenging test system. The preliminary distribution pattern of antioxidant activity was categorized as active (>80% inhibition), moderately active (50–80% inhibition), or inactive (>50% inhibition). For the active rice grains showing over 80% inhibition in this primary screening, active compounds were isolated and their chemical structures were determined. Among the varieties studied, 80% ethanol extracts of *Oryza sativa* cv. *Heugjinjubyeo* showed strong antioxidant activity (Table 1).

The dried aleurone layer of *Oryza sativa* cv. *Heugjinjubyeo* (2 kg) was extracted and partitioned via successive extractions with *n*-hexane, $CHCl_3$, EtOAc, *n*-BuOH and H_2O. All solvent fractions were bio-assayed prior to the application of chromatographic separation for the isolation of bioactive compounds. The EtOAc fraction of *Oryza sativa* cv. *Heugjinjubyeo*, showed a strong antioxidant activity (Table 2) and was subjected to a series of activity-guided chromatographic separation steps to yield pure compounds. The dried EtOAc-soluble fraction was chromatographed over a silica gel vacuum column, using a $CHCl_3$–MeOH gradient to generate 11 fractions. Fraction 4 was then further chromatographed on a silica gel open column. The resulting subfraction with antioxidant activity was rechromatographed on a Sephadex LH-20 column in order to isolate the compounds. Each elution resulted in the formation of solid materials upon condensation. These were then additionally purified by re-crystallization with highly purified MeOH, to yield pure compound **1** (38.4 mg).

Table 1
Antioxidant activity of 80% ethyl alcohol extracts of aleurone layer of rice varieties in DPPH assay

Rice varieties	DPPH[a]
Oryza sativa cv. *Juanbyeo*	68.2
Oryza sativa cv. *Heugjinjubyeo*	85.6
Oryza sativa cv. *Ilpumbyeo*	58.8
Oryza sativa cv. *Jukjinjubyeo*	79.1
Oryza sativa cv. *Obongbyeo*	63.5

[a] DPPH free radical-scavenging activity (% Inhibition, 100 mg/ml).

Compound **1** was obtained as a pale yellow solid with a molecular weight of *m/z* 233, based on EI-MS data. It exhibited a protonated molecular ion peak at *m/z* 234 $[M+H]^+$ and a deprotonated molecular ion peak at *m/z* 232 $[M-H]^-$. These data, together with the data obtained by ^{1}H NMR and ^{13}C NMR (Table 3), indicated a molecular formula of $C_{12}H_{11}NO_4$. This conclusion was supported by the findings of HREI-MS in positive ion mode. Compound **1** exhibited UV absorption bands at 241, 276 and 385 nm. These bands remained unaffected by the application of acid, as did the carbonyl absorption band at 1660 cm^{-1} and the amide absorption band at 1625 cm^{-1} in its IR spectrum, thereby suggesting the presence of a 2-quinolone skeleton (Gray, 1993). The phenolic hydroxyl group, as well as an amide group in the molecule, was inferred by the IR bands (1708 and 3364 cm^{-1}) and D_2O exchangeable signals at δ 9.68 and δ 11.94 in the ^{1}H NMR spectrum, coupled with the positive $FeCl_3$ and Dragendorff reactions. The ^{1}H NMR spectrum contained one proton singlet at δ 6.87 (H-3), one triplet at δ 4.38, and one quartet at δ 1.36 for ethyl and methyl protons. The ABX type signals were detected at three aromatic protons

Table 2
Antioxidant activity of solvent fractions of aleurone layer of *Oryza sativa* cv. *Heugjinjubyeo* in DPPH assay

Solvent fractions	DPPH[a]
n-Hexane-soluble fraction	39.7
Chloroform-soluble fraction	51.6
Ethylacetate-soluble fraction	18.5
n-Butanol-soluble fraction	38.3
H_2O-soluble fraction	45.1

[a] DPPH free radical-scavenging activity (IC_{50}: μg/ml).

Table 3
^{1}H NMR, ^{13}C NMR, HMBC and NOESY spectral data of Compound **1**

Position	δ^1H[a] (m, J in Hz)	^{13}C[b] correlations	HMBC	NOESY
2		160.1 (s)[c]		
3	6.87	124.0 (d)	C-4, COO	
4		116.2 (s)		
5	7.48 (d[d], J=2.6)	109.4 (d)	C-4, 6, 7, 10	
6		152.4 (s)		
7	7.08 (dd, J = 2.6, 8.9)	120.6 (d)	C-6, 10	H-8
8	7.25 (d, J=8.9)	116.9 (d)	C-4, 5, 6, 7	NH, H-7
9		139.3 (s)		
10		132.8 (s)		
COO		165.1 (s)	C-4	
CH_2	4.38 (t, J = 5.0)	61.7 (t)	COO	H-5
CH_3	1.36 (q, J = 5.0)	13.8 (q)	C-5, COO	
NH	11.94 (br s)		C-4, 7	H-8
OH	9.68 (br s)		C-5, 6, 7	H-5, 7

[a] TMS was used as the internal standard; chemical shifts are shown in the δ scale with J values in parentheses; measured at 500 MHz in DMSO-d_6.
[b] Measured at 125 MHz in DMSO-d_6.
[c] Multiplicity from DEPT experiments (Θ_y = 45°, 90° and 135°).
[d] s: singlet, br s: broad singlet, d:doublet, dd: double doublet, t: triplet, q: quartet.

(Fig. 2). According to ^{13}C NMR and HMQC (Figs. 3 and 4) and distortionless enhancement by polarization transfer (DEPT; Θ_y = 45°, 90° and 135°) spectra, it was clear that compound **1** had six non-protonated carbons, four methane carbons, one ethyl carbon, and one methyl carbon. The HMBC spectrum, for long-range correlation with proton and carbon, revealed a correlation between the ethyl and methyl group protons and the carboxyl carbon at δ 165.1, which unambiguously indicated the presence of a carboethoxyl moiety at C-4 of the 2-quinolone nucleus. The *meta*-coupled doublet at δ 7.48 (J = 2.6 Hz) in the deshielded position of the aromatic protons was correlated with a quaternary carbon at δ 116.2, assigned to H-4 (Fig. 5). The cross peak between the *ortho*-coupled doublet at δ 7.25 (J = 8.9 Hz) and the amide proton at δ 11.94 was also observed from Nuclear Overhauser Enhancements Spectroscopy (NOESY) (Fig. 6). These observations showed that the hydroxyl group was attached at the C-6 location. Thus, the structure of compound **1** was identified as 4-carboethoxy-6-hydroxy-2-quinolone. The occurrence of **1** in nature has not been previously reported.

3.2. Spectral data of compound *1*

4-Carboethoxy-6-hydroxy-2-quinolone (**1**): Pale yellow plates from MeOH; positive in $FeCl_3$ and Dragendorff reaction; mp >320°; UV λ_{max} (MeOH) (log ε): 241 (4.76), 281 (4.40), 385 (4.20) nm; λ_{max} (MeOH + HCl) (log ε): 241 (4.75), 276 (4.43), 385 (4.18) nm; λ_{max} (MeOH + NaOH) (log ε): 248 (4.90), 291 (4.62), 424 (4.15) nm; IR (KBr) ν_{max} 3,364 (OH, NH), 1708, 1660 (CO), 1625 (CN) cm^{-1}; HREI-MS *m/z* 233.0587 (calculated for $C_{12}H_{11}NO_4$ 233.0584); EI-MS (70 eV) *m/z* (relative intensity, %): 233 $[M]^+$ (34.2), 219 $[M-CH_2]^+$ (100), 188

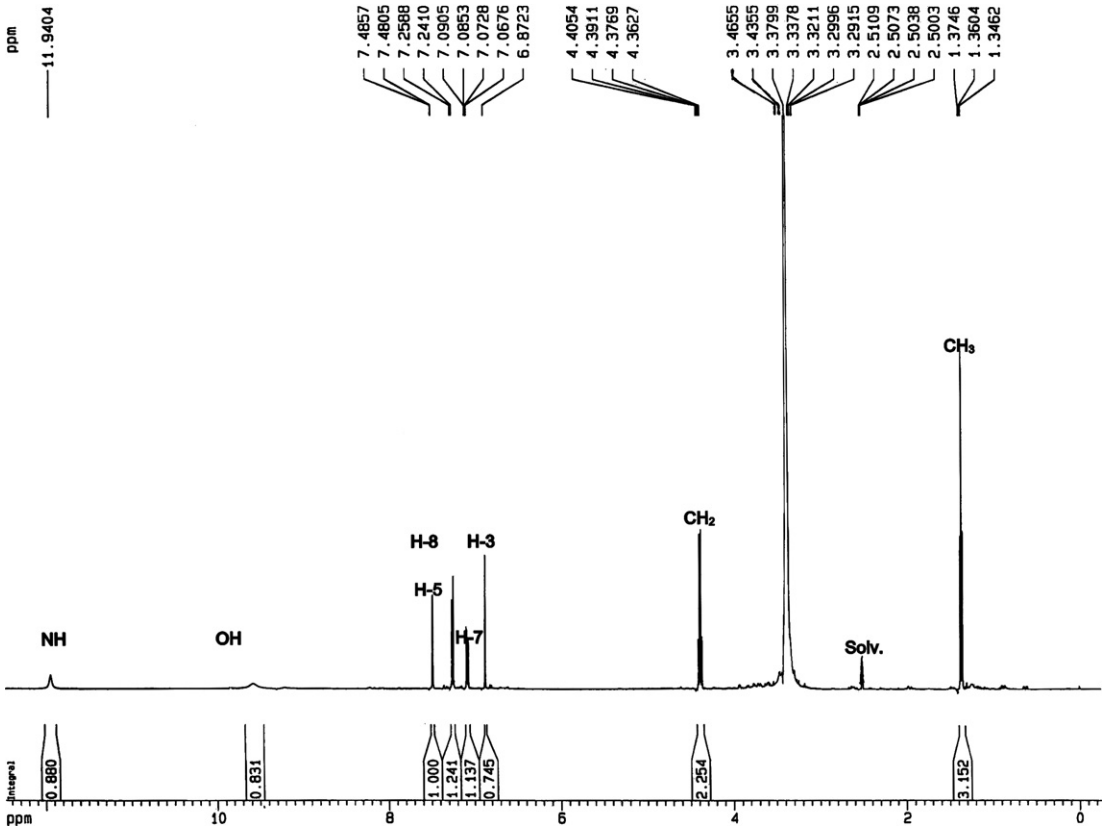

Fig. 2. ^{1}H NMR spectrum of compound **1** (500 MHz, DMSO-d_6).

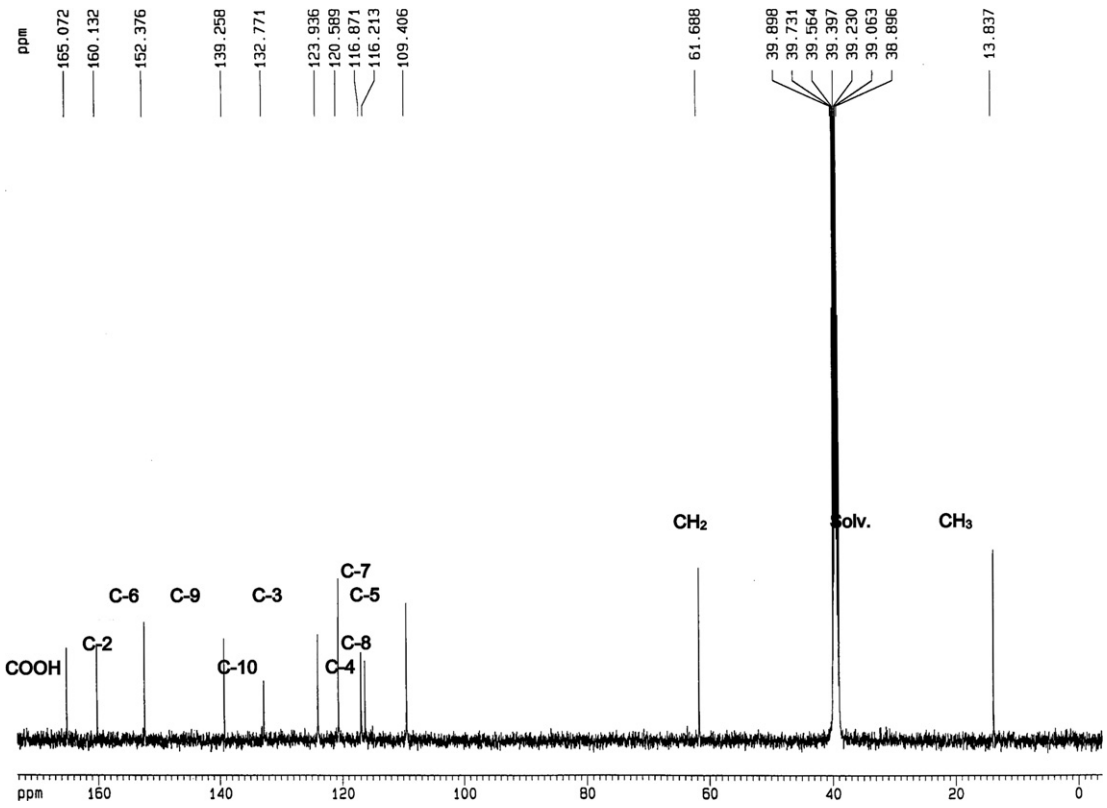

Fig. 3. ^{13}C NMR spectrum of compound **1** (125 MHz, DMSO-d_6).

$[M-CH_2-OCH_3]^+$ (45.7), 160 $[M-CH_2-OCH_3-CO]^+$ (53.8), 132 $[M-CH_2-OCH_3-CO-CO]^+$ (34.6); ^{1}H NMR and ^{13}C NMR data are described in Table 3.

3.3. Isolation and structure determination of compounds 2–5

Subfractions (320.2 mg) of EtOAc-soluble fraction of *Oryza sativa* cv. *Heugjinjubyeo* with antioxidant activity (IC_{50}: 20.1 μg/ml) were re-chromatographed on a silica gel column by elution with aqueous EtOAc–MeOH (99:1, v/v) to isolate bioactive compounds. Isolated compounds were further purified by re-crystallization with pure MeOH to give ethyl-3,4-dihydroxybenzoic acid (**2**), 4-hydroxy-3-methoxyphenylacetic acid (**3**), 3,4-dihydroxybenzoic acid (**4**), and 4-hydroxy-3-methoxycinnamic acid (**5**). Compounds **2**–**5** were positive in $FeCl_3$ and showed a characteristic phenolic skeleton from ^{1}H NMR and ^{13}C NMR spectral data.

3.4. Spectral data of compounds 2–5

Ethyl-3,4-dihydroxybenzoic acid (**2**): mp 172–173 °C, white plates from MeOH; positive in $FeCl_3$ test; IR ν_{max} (KBr, cm^{-1}): 3490 (OH), 1602 (C=C), 1610 (C=C), 1026 (CO); EI-MS (70 eV) *m/z* (relative intensity, %): 182 $[M]^+$ (21.0), 164 $[M-H_2O]^+$ (70.9), 154 $[M-CO]^+$ (20.1), 146 $[M-2H_2O]^+$ (19.1), 137 $[M-OCH_2CH_3]^+$ (100.0); ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.27 (3H, q, CH_3), 4.23 (2H, t, CH_2), 6.81 (1H, d, $J = 8.0$, H-5), 7.32 (1H, dd, $J = 2.0$, 8.0, H-6), 7.39 (1H, d, $J = 2.0$, H-2), 9.38 (1H, br s, OH), 9.65 (1H, br s, OH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 14.2 (CH_3), 59.9 (CH_2), 115.2 (C-5), 116.2 (C-2), 120.7 (C-1), 121.6 (C-6), 144.9 (C-3), 150.2 (C-4), 165.6 (COO).

4-Hydroxy-3-methoxyphenylacetic acid (**3**): mp 143 °C; white needles from MeOH; positive in $FeCl_3$ test; IR ν_{max} (KBr, cm^{-1}): 3430 (OH), 1692 (C=C), 1621 (C=C), 1036 (CO); EI-MS (70 eV) *m/z* (relative intensity, %): 182 $[M]^+$ (25.8), 164 $[M-H_2O]^+$ (11.0), 154 $[M-CO]^+$ (20.1), 137 $[M-OCH_2CH_3]^+$ (100.0) 122 $[M-OCH_3CH_2CH_3]^+$ (12.1); ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.87 (3 H, q, OCH_3), 4.25 (2H, t, CH_2), 6.75 (1H, d, $J = 8.0$, H-5), 6.80 (1H, d, $J = 2.0$, H-2), 6.87 (1H, dd, $J = 2.0$, 8.0, H-6), 9.50 (1H, br s, OH), 11.8 (1H, br s, COOH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 55.0 (OCH_3), 60.1 (CH_2), 111.2 (C-5), 114.5 (C-1), 125.0 (C-6), 126.0 (C-1), 145.0 (C-4), 146.6 (C-3), 176.9 (COOH).

3,4-Dihydroxybenzoic acid (**4**): mp 194–195 °C; white needles from MeOH; positive in $FeCl_3$ test; IR ν_{max} (KBr, cm^{-1}): 3222 (OH), 1679 (C=C), 1614 (C=C), 1095 (CO); EI-MS (70 eV) *m/z* (relative intensity, %): 154 $[M]^+$ (100.0), 136 $[M-H_2O]^+$ (15.9), 118 $[M-2H_2O]^+$ (19.1); ^{1}H NMR (500 MHz, DMSO-d_6): δ 6.80 (1H, d, $J = 8.0$, H-5), 7.32 (1H, dd, $J = 2.0$, 8.0, H-6), 7.33 (1H, d, $J = 2.0$, H-1), 9.27 (1H, br s, OH), 9.66 (1H, br s, OH), 12.80 (1H, br s, COOH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 115.3 (C-5), 116.7 (C-2), 121.8 (C-1), 122.3 (C-6), 144.9 (C-3), 150.1 (C-4), 167.6 (COOH).

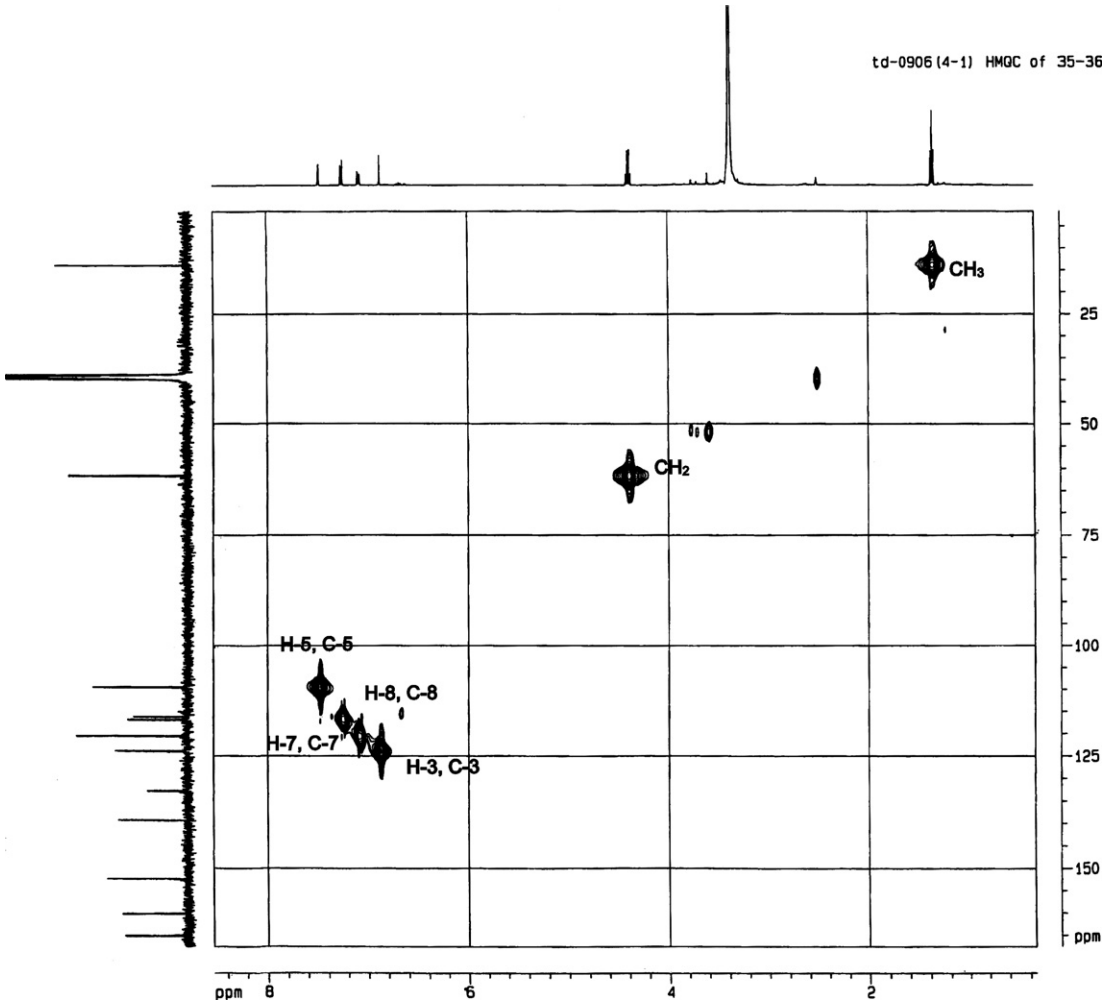

Fig. 4. HMQC spectrum of compound **1** (125 MHz, DMSO-d_6).

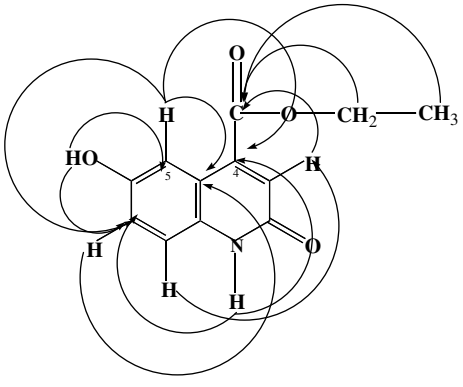

Fig. 5. Selective HMBC correlations of compound **1** (125 MHz, DMSO-d_6).

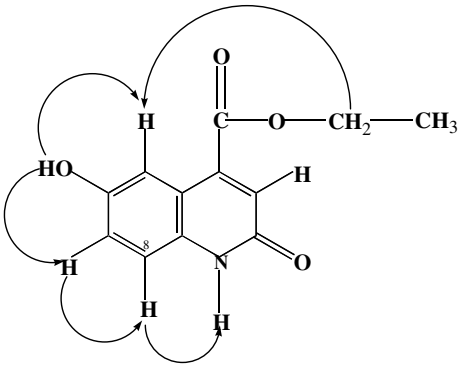

Fig. 6. Observed NOESY correlations of compound **1**.

4-Hydroxy-3-methoxycinnamic acid (**5**): mp 181–184 °C; white needles from MeOH; positive in $FeCl_3$ test; IR ν_{max} (KBr, cm^{-1}): 3430 (OH), 1692 (C=C), 1621 (C=C), 1036 (CO); EI-MS (70 eV) *m/z* (relative intensity, %): 194 $[M]^+$ (100.0), 179 $[M-CH_3]^+$ (19.1) 176 $[M-H_2O]^+$ (8.0), 163 $[M-OCH_3]^+$ (3.0); ^1H NMR (500 MHz, DMSO-d_6): δ 3.84 (3H, q, OCH_3), 6.39 (1H, d, $J = 12.0$, CH), 6.82 (1H, d, $J = 8.2$, H-5), 7.10 (1H, d, $J = 8.2$, H-6), 7.29 (1H, d, $J = 2.0$, H-2), 7.52 (1H, d, $J = 12.0$, CH), 9.57 (1H, br s, OH), 12.0 (1H, br s, COOH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 55.6 (OCH_3), 111.0 (C-6), 115.4 (C-3), 115.5 (CH), 122.6 (C-2), 126.0 (C-1), 144.1 (CH), 147.8 (C-5), 148.9 (C-4), 168.0 (COOH).

Table 4
Antioxidant activities of compounds **1**–**5** in DPPH assay

Compounds	DPPH (IC_{50})	
	μg/ml	μM
1	19.7	87.1
2	17.4	70.2
3	24.5	143
4	13.7	56.5
5	28.9	127
Ascorbic acid[a]	22.0	124
BHA[a]	21.0	114

[a] Control compounds.

3.5. Antioxidant activity of compounds *1*–*5*

New alkaloid **1** and known phenolic acids **2**–**5** were characterized by spectroscopic methods. These compounds showed significant antioxidant activity in a concentration-dependent manner through their capacity for scavenging DPPH free radicals. The IC_{50} of compound **1** was 19.7 μg/ml and 87.1 μM, that of compound **2** was 17.4 μg/ml and 70.2 μM, compound **3** had an IC_{50} of 24.5 μg/ml and 143 μM, compound **4** showed a value of 13.7 μg/ml and 56.5 μM, and the IC_{50} of compound **5** was 28.9 μg/ml and 127 μM. The alkaloids in crops comprise the largest single class of secondary plant substances. They are nitrogenous bases with one or more nitrogen atoms, which are usually incorporated into a cyclic structure. Alkaloids often have dramatic physiological activities, hence their wide use in medicine (Harborne, 1984). Compounds **2**–**5** showed phenolic characteristics in a $FeCl_3$ reaction and in ^1H NMR and ^{13}C NMR spectral data. Compounds **2** and **4** were significantly active as free radical-scavengers in the DPPH assay, and their activities compared favourably with the activity observed with a number of standards in this same assay (Table 4). Compounds **3** and **5** were only moderately active in this assay, possibly because there was a lower degree of hydroxylation in their phenol rings than in those of compounds **2** and **4**. It is well known that phenolic compounds have potent antioxidant properties and free radical-scavenging capabilities (Shashidi & Wanasundara, 1992). Fruits, vegetables, and cereal are known to be major dietary sources of phenolic compounds that can prevent oxidative damage, platelet aggregation, heart disease, and cancer in humans (Daniel, Meier, Schlatter, & Frischknecht, 1999; Morton, Caccetta, Puddey, & Croft, 2000; Newmark, 1996).

Overall results showed that purple-coloured rice, *Oryza sativa* cv. *Heugjinjubyeo*, is rich in secondary metabolites, especially quinolone alkaloid and phenolic acids. This variety of rice contains materials with a strong antioxidant activity and can serve as a good source of dietary bio-functional phytochemicals.

4. Conclusions

There is growing interest in natural extracts and pure components as food additives in the health food market. Development of high-quality varieties containing increased levels of bioactive compounds may increase the nutritional value of harvest grains. The aleurone layer of *Oryza sativa* cv. *Heugjinjubyeo* was found to have potent antioxidant activity. This shows that phytochemicals, alkaloids, and phenolic acids are responsible for antioxidant activity. The present study concludes that anthocyanin-pigmented rice, which possesses potent antioxidant components, may contribute, not only to the reduction of oxidative stress diseases but also to improvements in the colour and taste of foods made with rice.

Acknowledgements

This study was supported by the Specific Research-Promoting Joint Projects, National Institute of Crop Science, Rural Development Administration, Korea.

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