A Quinolone Alkaloid with Antioxidant Activity from the Aleurone Layer of Anthocyanin-Pigmented Rice

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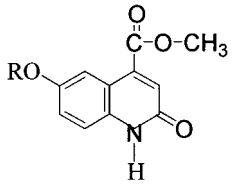
The aleurone layer of *Oryza sativa* cv. *Heugjinmi* yielded a new quinolone alkaloid, 4-carbomethoxy-6-hydroxy-2-quinolone (**1**), showing moderate antioxidative activity in a 1,1-diphenyl-2-picrylhydrazyl free-radical scavenging assay. Physical and spectroscopic evidence has determined the structure of the compound.

Free radicals have been implicated in a number of diseases, including brain disorders, platelet aggregation, atherosclerosis, and colon cancer.[1] Scavenging or trapping agents are compounds that typically react with the activated (electrophilic) forms of carcinogens and oxygen free radicals and can be detrimental by reacting with, and sometimes by destroying, critical cellular components including the polyunsaturated fatty acids that comprise lipoprotein particles and plasma membranes.[2] The term "antioxidant" has been used in a broad sense, referring to agents capable of interfering with processes involved in oxidative stress.

Rice (*Oryza sativa* L., Gramineae) is the staple food in many Asian countries. Varieties of rice include long-grain white, long-grain brown, glutinous brown, wild, basmati, brown basmati, jasmine, and rosotto. Brown rice has intact kernels that retain their bran layers and is somewhat more nutritious, but takes longer to cook than white rice. Cultivation of pigmented rice, through genetic engineering in the early 1970s, began a surge in the world production of various types of rice grains.[3] With growing concerns regarding national health and the expanding markets of health food, research in the industrial use of bioactive compounds from diverse crops evolved. Development of high-quality varieties containing increased levels of bioactive compounds may increase the nutritional value of the harvest grain. The anthocyanin-pigmented rice (*Oryza sativa* cv. *Heugjinmi*), having dark purple-colored grains, is broadly known as an enriched rice with improved organoleptic properties. It is also widely used as a food colorant in bread, ice creams, and liquor.[4,5] Cyanidin-3-O-β-D-glucoside, having oxygen radical absorbing capacity, is most abundant in anthocyanin-pigmented rice.[6]

As a part of our study on the antioxidant components of pigmented rice grain, a new quinolone alkaloid **1**, which has shown moderate antioxidant activity, was isolated from a 0.5% HCl in ethyl alcohol soluble fraction (IC_{50} = 28.9 μg/mL) using a 1,1-diphenyl-2-picrylhydrazyl free-radical scavenging assay.

Alkaloid **1**, obtained as needles, mp >320°, gave $[M]^+$ at *m/z* 219.0531 in the high-resolution mass spectrum, indicating molecular formula $C_{11}H_9NO_4$. Alkaloid **1** showed UV absorption bands at 242, 280, and 382 nm, which were unaffected by addition of acid, as well as the carbonyl absorption at 1658 cm^{-1}, and amide absorption at 1624 cm^{-1} in its IR spectrum, suggesting the presence of a 2-quinolone skeleton.[7] The presence of a carboxyl group, a phenolic hydroxyl group, and an amide group in the molecule was inferred by the IR bands (1708 and 3365 cm^{-1}) and D_2O exchangeable signals at δ 9.54 and 11.95 in the ^1H NMR spectrum, together with a positive $FeCl_3$ reaction. The ^1H NMR spectrum contained a one-proton singlet at δ 6.88 (H-3), a singlet at δ 3.92 (methoxyl), and ABX type signals of three aromatic protons. It was clear that the alkaloid had six nonprotonated carbons, four methine carbons, and one methyl carbon judging from the HMQC and DEPT spectra. The HMBC spectrum revealed the correlation between the methoxyl group protons and the carboxyl carbon at δ 165.9, which unambiguously assigned the presence of a carbomethoxyl moiety at C-4 of the 2-quinolone nucleus. The *meta*-coupled doublet at δ 7.49 (J = 2.6 Hz) in the deshielded position of the aromatic protons correlated with a quaternary carbon at δ 116.2 assigned to H-5. A NOESY cross-peak between the *ortho*-coupled doublet at δ 7.24 (J = 8.9 Hz) and the amidic proton at δ 11.95 was seen. These observations revealed that the hydroxyl group was attached at C-6. To confirm the location of the hydroxy group, the alkaloid was acetylated with pyridine and acetic anhydride to give **2**. In the ^1H NMR spectrum of **2**, the signals of H-5 and H-7 at δ 7.08 (J = 2.6 and 8.9 Hz) were shifted to δ 8.19 and 7.33, respectively.



1 R = H
2 R = Ac

Thus, the structure of the alkaloid **1** was elucidated as 4-carbomethoxy-6-hydroxy-2-quinolone. The occurrence of **1** in nature has not been previously reported.

Experimental Section

General Experimental Procedures. Melting points were measured on a Mitamura-Riken melting point apparatus and are uncorrected. The UV and IR spectra were recorded on Hitachi 3100 UV−vis and JASCO FT-IR-5300 spectrophotometers, respectively. A Bruker CXP-500 spectrometer was used to record NMR spectra (500 MHz for ^1H NMR and 125 MHz

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for ^{13}C NMR) with TMS as an internal standard in DMSO-d_6. EIMS and HRMS were obtained on a Hewelett Packard Model 5985B GC/MS and JMS700 spectrometer, respectively.

Chemicals. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals were purchased from commercial sources and were of the highest purity available.

Plant Material. A fully ground aleurone layer of anthocyanin-pigmented rice was supplied by the National Crop Experiment Station, Rural Development Administration (RDA), Suwon, Gyeonggi-do, Korea, in February 1999. A voucher specimen (OS-Suwon #415) has been deposited at the RDA.

Assay for DPPH Free-Radical Scavenging Potential. This assay is based on the scavenging activity of stable 1,1-diphenyl-2-picrylhydrazyl free radicals.[8] Reaction mixtures containing test samples (dissolved in DMSO) and 300 μM DPPH ethanolic solution in 96-well microtiter plates were incubated at 37 °C for 30 min, since the optical density value was stable from 30 min up to 1 h, and absorbances were measured at 515 nm. Percent inhibition by sample treatment 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 evaluated for their free-radical scavenging activity. Alkaloid **1** showed moderate antioxidative activity, with an IC_{50} value of 36.4 μg/mL, and ascorbic acid and BHA were 21.8 and 20.6 μg/mL, respectively.

Extraction and Isolation. The dried and ground anthocyanin-pigmented rice powder (5 kg) was extracted with acetone, and the residue extracted with EtOH containing 0.5% HCl (× 3) at room temperature, overnight. The combined extracts were concentrated under reduced pressure to give a dark purple extract. The dried 0.5% HCl in the EtOH soluble fraction (52.1 g) was chromatographed over a silica gel column using a $CHCl_3$−MeOH gradient to give nine fractions. Fraction 7 was further chromatographed on a silica gel column using a $CHCl_3$−MeOH (92:8), and subfraction 16 was rechromatographed on a Sephadex LH-20 column by elution with MeOH in order to give alkaloid **1** (38.3 mg).

4-Carbomethoxy-6-hydroxy-2-quinolone (1): mp >320°, pale yellow needles (MeOH); UV λ_{max} (MeOH) (log ϵ) 242 (4.8), 280 (4.4), 382 (4.2) nm; λ_{max} (MeOH + HCl) (log ϵ) 242 (4.7), 280 (4.4), 380 (4.1) nm; λ_{max} (MeOH + NaOH) (log ϵ) 246 (4.9), 290 (4.6), 424 (4.1) nm; IR (KBr) ν_{max} 3365 (OH, NH), 1708, 1658 (CO), 1624 (CN) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 11.95 (1H, s, N*H*), 9.54 (1H, s, O*H*), 7.49 (1H, d, J = 2.6 Hz, H-5), 7.24 (1H, d, J = 8.9 Hz, H-8), 7.08 (1H, dd, J = 2.6, 8.9, H-7), 6.88 (1H, s, H-3), 3.92 (3H, s, OCH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 165.9 (s, *C*OO), 160.1 (s, C-2), 152.4 (s, C-6), 138.9 (s, C-9), 132.8 (s, C-10), 124.2 (d, C-3), 120.6 (d, C-7), 116.8 (d, C-8), 116.2 (s, C-4), 109.5 (d, C-5), 52.7 (q, OCH_3); HMBC, and NOESY data, see Figures 1 and 2; HREIMS *m*/*z* 219.0531 (calcd for $C_{11}H_9NO_4$, 219.0530); EIMS (70 eV) *m*/*z* 219 $[M]^+$ (100), 188 $[M - OCH_3]^+$ (19), 160 $[M - OCH_3 - CO]^+$ (40), 132 $[M - OCH_3 - CO - CO]^+$ (43).

Acetylation of Alkaloid 1. Alkaloid **1** (5.0 mg), dissolved in 0.2 mL of DMSO, was treated with 2.0 mL of acetic anhydride−pyridine (1:1) at room temperature overnight. The mixtures were added to distilled H_2O and then extracted with $CHCl_3$ three times. The $CHCl_3$ extract was concentrated under reduced pressure and chromatographed on a silica gel column, eluting with 1.0% $CHCl_3$ in MeOH to give the acetate (1.8 mg, **2**) as a pale yellow amorphous powder: mp, 178° (MeOH); UV λ_{max} (MeOH) (log ϵ) 242 (4.5), 280 (4.4), 382 (4.3) nm; λ_{max} (MeOH + HCl) (log ϵ) 242 (4.6), 280 (4.4), 381 (4.1) nm; λ_{max} (MeOH + NaOH) (log ϵ) 244 (4.4), 281 (4.3), 380 (4.1) nm; IR (KBr) ν_{max} 3370 (NH), 1708, 1658 (CO), 1624 (CN), 1236 (acetate) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 11.74 (1H, s, N*H*), 8.19 (1H, d, J = 2.6 Hz, H-5), 7.40 (1H, d, J = 8.9 Hz, H-8), 7.33 (1H, dd, J = 2.6, 8.9 Hz, H-7), 6.91 (1H, s, H-3), 4.07 (3H, s, OCH_3), 2.33 (3H, s, CH_3COO); HREIMS *m*/*z* 261.0638 (calcd for $C_{13}H_{11}NO_5$, 261.0639); EIMS (70 eV) *m*/*z* 261 $[M]^+$ (8), 230 $[M - OCH_3]^+$ (3), 219 $[M - HOAc]^+$ (100), 188 $[M - HOAc - OCH_3]^+$ (15), 160 $[M - HOAc - OCH_3 - CO]^+$ (64), 132 $[M - HOAc - OCH_3 - CO - CO]^+$ (89).

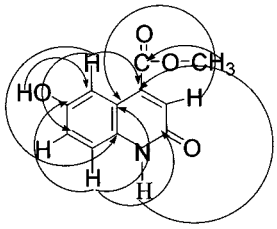

Figure 1. Selective HMBC correlations for alkaloid **1**.

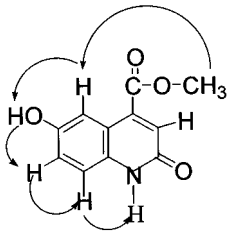

Figure 2. Observed NOESY correlations for alkaloid **1**.

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