Antioxidative Phenolic Compounds from Japanese Barnyard Millet (*Echinochloa utilis*) Grains

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Three antioxidative phenolic compounds, one serotonin derivative and two flavonoids, were isolated from an ethanol extract of Japanese barnyard millet (cv. Kurohie) grains by Sephadex LH-20 chromatography and preparative high-performance liquid chromatography. Their structures were established to be *N*-(*p*-coumaroyl)serotonin, luteolin, and tricin on the basis of spectrometric data from ^{1}H and ^{13}C and two-dimensional nuclear magnetic resonance techniques, fast atom bombardment mass spectrometry, and Fourier transform infrared spectrophotometry. *N*-(*p*-Coumaroyl)-serotonin exhibited a strong antioxidant activity almost equivalent to that of butylated hydroxyanisole at the same concentration (w/v). Although the antioxidant activity of luteolin was lower than that of *N*-(*p*-coumaroyl)serotonin, it was nearly equal to that of quercetin, whereas the activity of tricin was lower than that of luteolin. All of them were newly isolated from Japanese barnyard millet grains.

Keywords: *Echinochloa utilis; N-(p-coumaroyl)serotonin; luteolin; tricin; antioxidant activity*

INTRODUCTION

The grains of Japanese barnyard millet are a traditional food in the cold districts of Japan, especially in the Tohoku district, where it is considered an important crop because of its ability to be stored for a long term as a food, as a seed with an extended germination ability. With respect to cultivation, the yield of grains is high (3 t ha^{-1} is expected), and its resistance to cold temperatures is extremely strong among cereals. From the nutritional aspect, its grains are highly nutritious, for example, rich in protein, lipid, vitamins B_1 and B_2, and nicotinic acid compared with other cereals, such as rice and wheat grains (Resources Council Science and Technology Agency, Japan, 1982). In more detail, the amino acid composition of its protein is good, and oleic and linoleic acids are abundant with respect to the fatty acid composition (Taira, 1983). Recently, Japanese barnyard millet grains have been used as food materials, in place of rice and wheat grains, for those patients with allergic disease including atopic dermatitis. Under these circumstances, its importance and the demand for it in Japan are now increasing.

Polyphenolic compounds such as flavonoids, phenolic acids, and proanthocyanidins, which are of great interest for their radical-scavenging activity, are expected to be effective in the prevention of many diseases and morbid states. Among the cereals, catechins and proanthocyanidins from barley (Porter, 1988) and buckwheat (Watanabe, 1998), isovitexin from rice (Ramarathnam et al., 1989), and phytic acid from various kinds of seeds are known as antioxidants. However, knowledge of antioxidant compounds in millets, including Japanese barnyard millet, is still superficial. In this paper are described the isolation and identification of antioxidant compounds from Japanese barnyard millet grains to obtain information on their contribution to health.

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MATERIALS AND METHODS

Materials. *(a) Varietal Differences in Antioxidant Activity in Eight Japanese Barnyard Millet Grains.* The seeds of eight main varieties (Kurohie, Waseshiro, Taiwan, Yoichiwase, Chousen, Suiraitan, Touya, and Futagomochi) of Japanese barnyard millet in the Tohoku district were used for the evaluation of antioxidant activity using their ethanolic extracts. They were cultivated and harvested at the Tohoku National Agricultural Experiment Station in 1994. After threshing, the seeds were stored at 4 °C until use. The glume was removed from the intact seeds by threshing and subsequent winnowing. Hulling was performed with gentle grinding using a grinder and then by winnowing. The grains were separated manually from the mixture of grains and caryopses.

(b) Isolation of Antioxidant Compounds from Japanese Barnyard Millet Grains. The seeds of Kurohie, used for the isolation of antioxidant compounds because its extract showed the strongest antioxidant activity among the varieties used, were harvested in 1998 at the Tohoku National Agricultural Experiment Station, and the preparation of the grains was performed in the same manner as described above.

Chemicals. Quercetin was obtained from Extrasynthèse S.A. (Genay, France). Butylated hydroxyanisole (BHA) was purchased from Nacalai Tesque Inc., Ltd. (Kyoto, Japan). Methyl linoleate, glycerol, *m*-nitrobenzyl alcohol, and serotonin hydrochloride were obtained from Tokyo Kasei Organic Chemicals Co., Ltd. (Tokyo, Japan), and methyl linoleate was purified as previously described (Terao and Matsushita, 1986). 2,2′-Azobis(2,4-dimethylvaleronitrile) (AMVN) and acetone-d_6 were obtained from Wako Pure Chemicals Industries (Osaka, Japan). DMSO-d_6 was purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI).

Extraction. Eight varieties of grains were ground by a vibrating sample mill, and a powdered sample (1 g) of each was extracted with 20 mL of ethanol under reflux in a water bath at 85 °C for 1 h. The extract was filtered with Advantec No. 5C (Toyo Inc.) filter paper and concentrated under reduced pressure at 40 °C. After filtration of the concentrated extract with the same filter paper, the filtrate was made up to 5 mL with ethanol. The powdered sample from Kurohie grains (500 g) were extracted with ethanol (1 L × 10), and the filtrate,

10.1021/jf990498s CCC: $18.00
Published on Web 10/08/1999

prepared in the same manner, was used for the isolation of antioxidant compounds.

Sephadex LH-20 Column Chromatography. The crude extract from Kurohie was subjected to Sephadex LH-20 (Pharmacia Co., Ltd.) column (46 mm i.d. × 940 mm) chromatography and eluted by ethanol with monitoring at 280 and 350 nm. Fractions of 10 mL were collected.

Preparative HPLC. Major peaks in the antioxidative fraction were collected by preparative HPLC under the following conditions.

(1) ODS Column. Conditions were as follows: column, Cosmosil 5C18 (Nacalai Tesque, Inc., Ltd., 20 mm i.d. × 250 mm); mobile phase, linear gradient of methanol−water (containing 2.5% acetic acid), 5−59% methanol over 48 min; flow rate, 7 mL/min; detection, UV detector (166 type, Beckman Co., Ltd.; 280 nm). Each collected peak fraction was concentrated at 40 °C under reduced pressure.

(2) Polymer-Based Column. Conditions were as follows: column, Shodex DE-2013 (Showa Denko K.K., 20 mm i.d. × 300 mm); mobile phase, methanol; flow rate, 7 mL/min; detection, UV detector (166 type, Beckman Co., Ltd.; 280 nm). Each collected peak fraction was concentrated at 40 °C under reduced pressure.

Analytical HPLC. To check the purity and obtain absorption spectra of the isolated compounds, they were analyzed by HPLC equipped with a photodiode array detector. The conditions of the HPLC were as follows: column, Cosmosil 5C18 (Nacalai Tesque Inc., Ltd., 4.6 mm i.d. × 250 mm); mobile phase, linear gradient of methanol−water (containing 2.5% acetic acid), 23−77% methanol over 120 min; flow rate, 1 mL/min; detection, photodiode array detector (168 type, Beckman Co., Ltd.; on-line acquisition of absorption spectra, 240−400 nm).

Detection of Indole Moiety. To characterize the isolated compound, its methanolic solution was mixed with Ehrlich's reagent (5% dimethylbenzaldehyde in 10% H_2SO_4) and heated. The color development of the mixture into a deep blue indicates the presence of an indole or pyrrole moiety with a nonsubstituted α-position.

Instrumental Analysis. *(a) Fast-Atom Bombardment Mass Spectrometry (FAB-MS).* The positive ion mode of the FAB-MS spectra of the isolated compounds was recorded on a JEOL JMS-AX505H mass spectrometer, which was operated at 10-kV accelerating potential. Compound **1** was dissolved in a mixture of glycerol and *m*-nitrobenzyl alcohol as the mounting matrix, and compounds **3** and **4** were mixed with glycerol. Ions were produced by bombardment with Ar atoms of 6-kV energy.

(b) ^1H and ^{13}C NMR. ^1H (399.65 MHz) and ^{13}C NMR (100.40 MHz) spectra of the isolated compounds were recorded on a JEOL JNM-EX 400 spectrometer with DMSO-d_6 at 40 °C or acetone-d_6/D_2O (1:1) solvent mixture at 30 °C. To determine the chemical shifts [given in δ values (ppm)], the peaks of δ 2.05 in ^1H NMR and δ 29.8 in ^{13}C NMR in acetone-d_6 and those of δ 2.50 in ^1H NMR and δ 39.5 in ^{13}C NMR in DMSO-d_6 were used as reference peaks. To determine the multiplicity of carbons, distortionless enhancement by a polarization transfer (DEPT) ^{13}C NMR experiment was performed.

(c) Two-Dimensional (2D) NMR Spectra. To confirm the assignment of the NMR spectrum of the isolated compounds, $^1H-^1H$ correlation spectroscopy (COSY), $^{13}C-^1H$ COSY with J_{CH} = 140 Hz, and correlation spectroscopy via long-range coupling (COLOC) with J_{CH} = 8 Hz were carried out. DMSO-d_6 was used as the solvent.

(d) Infrared Spectroscopy (IR). The IR spectrum was recorded by a Fourier transform infrared spectrophotometer, Perkin-Elmer Spectrum BX, with a microscope system fitted with an attenuated total reflectance (ATR) objective. The methanolic solution of the isolated compound was placed onto the gold mirror, and the solvent was evaporated. A sample spectrum was collected with an ATR crystal in contact with the sample. The background spectrum was collected in the same manner using the gold mirror.

Peroxyl Radical-Scavenging Activity. The peroxyl radical-scavenging activity of ethanol extracts of eight varieties and each sample of the purification step was determined as previously described (Watanabe et al., 1997) on the basis of the inhibition of the AMVN-initiated oxidation of methyl linoleate in solution. Briefly, 100 μL of ethanol extract from each variety or 50 μg of sample of each purification step was added to the solution of methyl linoleate (0.1 M, 1.0 mL; hexane/2-propanol, 8:3 v/v) and preincubated at 37 °C for 5 min. The reaction was started by adding 0.1 M AMVN in *n*-hexane/2-propanol solution (8:3 v/v, 0.1 mL). The reaction mixture was incubated with continuous shaking at 37 °C in the dark. The reaction products, methyl linoleate hydroperoxides (Me-LOOHs), were measured under the same conditions with HPLC in the literature (Watanabe et al., 1997). The antioxidant activity of extracts from eight varieties and fractions, obtained by Sephadex LH-20 chromatography, was expressed as lipid peroxidation. This value is calculated as the ratio of the rate of production of Me-LOOHs in the presence of each extract or fraction to the rate without a sample (the control) during the initial 90 min of the experiment. BHA and quercetin were used as reference standards for the investigation of antioxidant activity of the isolated compounds.

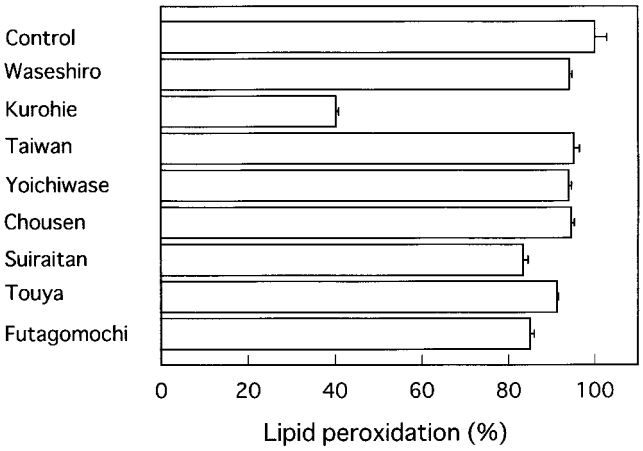


Figure 1. Rate of lipid peroxidation in the presence of each ethanolic extract from eight varieties of Japanese barnyard millet grains. Ethanol extract from 20 mg of each grain was used. Data are mean ± SD of three experiments.

RESULTS

Antioxidant Activity of Eight Varieties of Japanese Barnyard Millet Grains. Figure 1 shows the antioxidant activity of the ethanol extracts from Japanese barnyard millet grains. The extract from Kurohie showed the strongest antioxidant activity, and it was remarkably high compared with other varieties used, whereas Waseshiro, Taiwan, Yoichiwase, Chousen, and Touya showed slight activity and the activities of Suiraitan and Futagomochi varieties were somewhat higher than those of these varieties. On the basis of this result, Kurohie was used for the investigation of antioxidant compounds. The scheme for isolation of the antioxidant compounds from Kurohie is shown in Figure 2.

Antioxidant Activity of the Fractions Separated by Sephadex LH-20. The elution pattern of the crude extract by Sephadex LH-20 chromatography is shown in Figure 3. The eluate was divided into eight fractions (A−H), and they were concentrated under reduced pressure at 40 °C. Figure 4 shows the antioxidant activity of the fractions separated by Sephadex LH-20 chromatography. The activity order of the fractions was H, A, F, G ≈ B, E, C, D. In the analytical HPLC chromatogram of fractions H and A, only broad peaks were present. On the other hand, fraction F was the major fraction (Figure 3), and its analytical HPLC

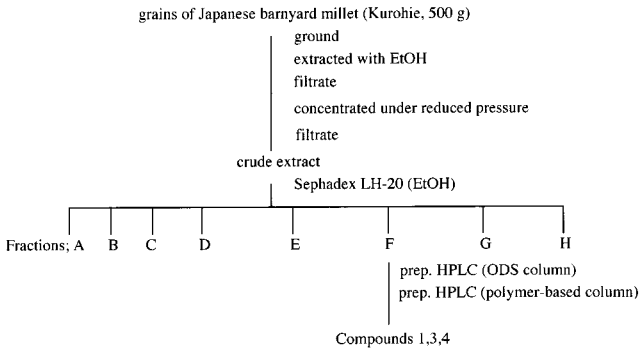


Figure 2. Scheme for preparation of antioxidant compounds from Japanese barnyard millet grains.

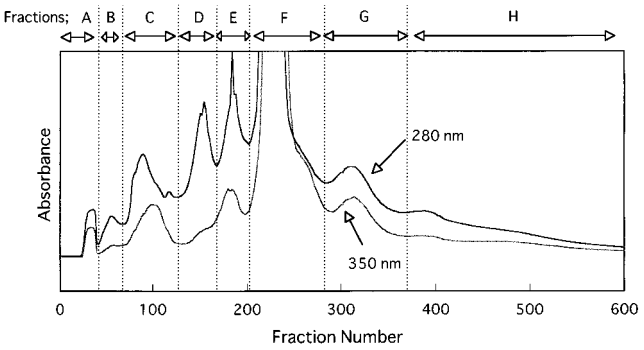


Figure 3. Chromatogram of the crude extract by Sephadex LH-20 chromatography eluted with ethanol. Eluate of initial 250 mL was discarded. Fractions of 10 mL were collected.

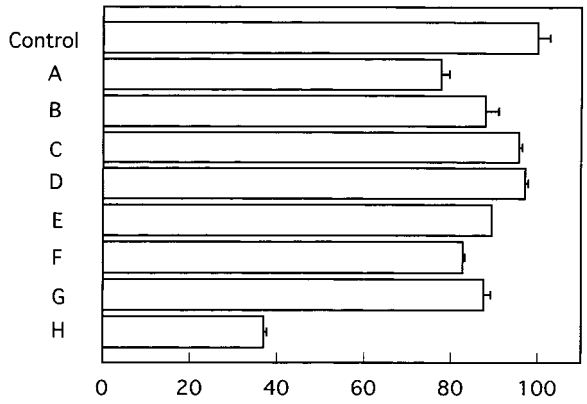


Figure 4. Rate of lipid peroxidation in the presence of fractions (4.2×10^{-3}% w/v) separated by Sephadex LH-20 chromatography. Data are mean ± SD of three experiments.

chromatogram with the absorption spectrum showed the presence of phenolic compounds including flavonoids. On the basis of these results, fraction F was used for the isolation of antioxidant compounds.

Antioxidant Activity of the Compounds Isolated by Preparative HPLC. Among the peaks in the chromatogram by HPLC analysis of fraction F (Figure 5), in addition to three major peaks 1, 2, and 4, peak 3, the absorption spectrum of which showed the typical shape of a flavone structure (λ_{max} = 232 sh, 246, 256, 286 sh, 336 nm) (Markham, 1982), was collected by preparative HPLC fitted with an ODS column. Compounds **1** and **3** were shown to be purified as one peak by HPLC analysis, but compounds **2** and **4** were contaminated. Consequently, compounds **2** and **4** were further purified with preparative HPLC fitted with a polymer-based column. Therefore, compound **4** was purified as one peak by HPLC analysis, but compound

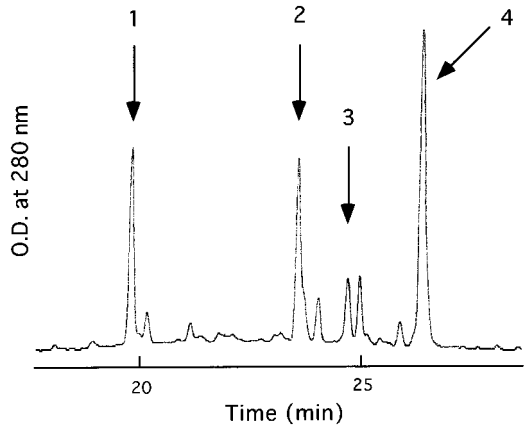


Figure 5. Chromatogram of fraction F by analytical HPLC. Four peaks were collected by preparative HPLC.

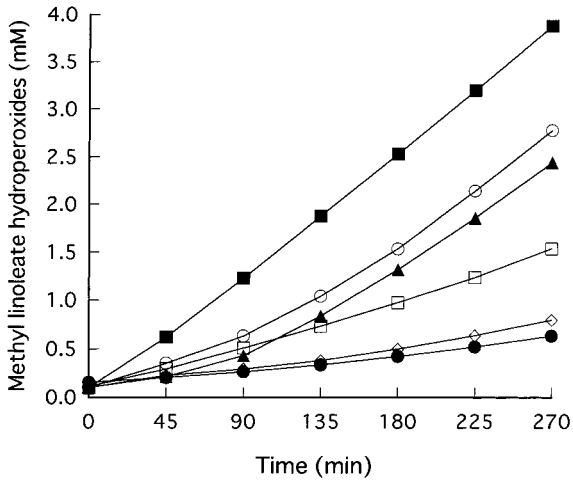


Figure 6. Effect of the isolated compounds (4.2×10^{-3}% w/v) from Japanese barnyard millet grains on AMVN-initiated oxidation of methyl linoleate in solution: (■) control; (●) BHA; (▲) quercetin; (◇) compound **1**; (□) compound **3**; (○) compound **4**. BHA and quercetin were used as reference standards.

2 was still contaminated. Accordingly, compounds **1**, **3**, and **4** were obtained, and their yields from 500 g of grains were 8.2, 1.9, and 18.0 mg, respectively. The antioxidant activity of the three isolated compounds is shown in Figure 6. Compound **1** showed the strongest antioxidant activity among the isolated compounds, and it was slightly lower than that of BHA at the same concentration (w/v). Although compound **3** showed slightly less activity than quercetin in the initial stage of the experiment, the amount of methyl linoleate hydroperoxides produced at and after 135 min of the experiment was lower than that of quercetin. The activity of compound **4** was lower than those of compound **3** and quercetin.

Identification of the Isolated Compounds. *(a) Compound **1**.* Table 1 shows the ^{13}C NMR spectra of the isolated compounds, showing the presence of a carbonyl group (δ 168.0) in compound **1**. On the other hand, the ^{1}H NMR spectra of the isolated compounds are shown in Table 2. AA′XX′ type aromatic proton signals at δ 6.79 (2H) and δ 7.38 (2H) indicated the presence of a *p*-hydroxyphenyl ring. The $^{13}C-^{1}H$ COSY experiment showed that the proton signal at δ 6.79 was correlated with the carbon signal at δ 116.2 and the proton signal at δ 7.38 was correlated with the carbon signal at δ 130.1. Two doublets at δ 6.41 (1H) and δ 7.33 (1H) in the ^{1}H NMR spectrum, the coupling constants of which

Table 1. ^{13}C NMR Spectral Data for Serotonin and Compounds 1 and 4

	serotonin[a]	**1**[a]	**4**[b]
C1	124.9	124.0	
C2	108.9	111.6	163.5
C3	127.9	128.4	103.5
C4	102.7	103.0	181.6
C5	149.9	149.8	157.3
C6	112.0	111.7	98.8
C7	112.7	112.4	164.3
C8	131.9	131.9	94.1
C9	23.3	25.2	161.3
C10	40.2	40.3	103.6
C1′		126.9	120.4
C2′		130.1	104.5
C3′		116.2	148.1
C4′		158.8	139.9
C5′		116.2	148.1
C6′		130.1	104.5
C7′		140.7	
C8′		118.2	
C9′		168.0	
OCH_3			56.3

[a] Measured in acetone-d_6 + D_2O. [b] Measured in DMSO-d_6.

Table 2. ^{1}H NMR Spectral Data for Compounds 1, 3, and 4 (*J* in Hertz)[a]

	1	**3**	**4**
H1	7.05 d (2)		
H3		6.64 s	6.93 s
H4	6.86 d (2)		
H6	6.60 dd (2, 9)	6.19 d (2)	6.20 d (2)
H7	7.12 d (9)		
H8		6.44 d (2)	6.54 d (2)
H9	2.79 t (7)		
H10	3.43 q (7)		
H2′	7.38 d (9)	7.39 s (8)	7.31 s
H3′	6.79 d (9)		
H5′	6.79 d (9)	6.89 d (8)	
H6′	7.38 d (9)	7.40 dd (2,8)	7.31 s
H7′	7.33 d (14)		
H8′	6.41 d (14)		
2OCH_3			3.89 s

[a] Measured in DMSO-d_6.

were 14 Hz, were assigned to *trans*-olefinic protons. The former *trans*-olefinic proton was long-range correlated (^3J) with the quaternary carbon at δ 126.9 in the *p*-hydroxyphenyl ring by a COLOC experiment (Figure 7), whereas the latter *trans*-olefinic proton was correlated with the carbon signals at δ 130.1 for the *p*-hydroxyphenyl ring and at δ 168.0 for the carbonyl group. On the basis of this evidence, the presence of a *p*-coumaric acid moiety was clarified. On the other hand, the color development of the methanolic solution with Ehrlich's reagent into deep blue suggested the presence of an indole moiety. Furthermore, *meta*-related (2 Hz) protons at δ 6.86 and 6.60, *ortho*-related (9 Hz) protons at δ 6.60 and 7.12, and a carbon at δ 149.8 indicated that either aromatic carbon, C5 or C6, in this moiety was oxygenated. Two CH_2 groups at δ 25.2 and 40.3, determined by DEPT experiment, were elucidated to be adjacent to each other by ^{1}H−^{1}H COSY experiment. The large chemical shift of the proton signal of CH_2 at δ 3.43 showed that this group (δ 40.3) would be connected to an atom with large electronegativity such as oxygen, nitrogen, and halogens. From these data, it was suggested that this compound contained a serotonin (5-hydroxytryptamine) moiety. The fact that the ^{13}C NMR data for serotonin were essentially consistent with that of compound **1** except for the *p*-coumaric acid moiety supported the belief that this compound contained this

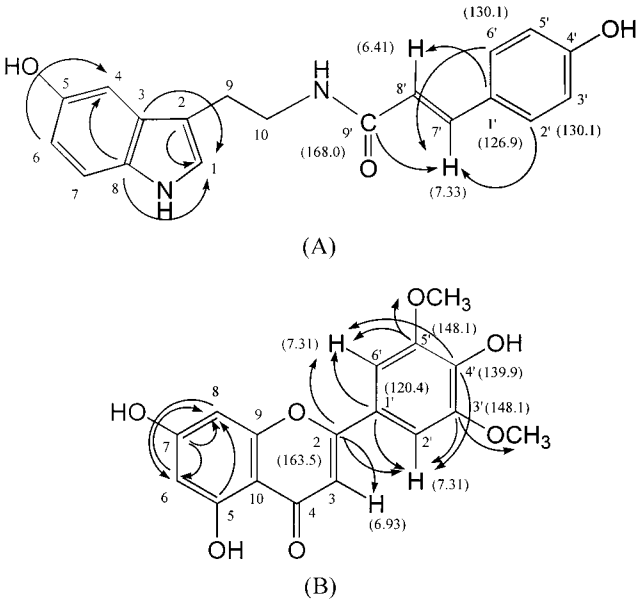
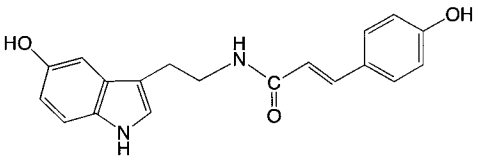
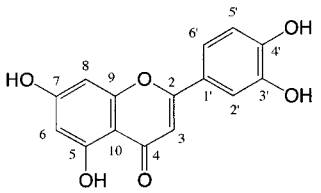


Figure 7. Heteronuclear long-range correlations observed by COLOC experiment: (A) compound **1**; (B) compound **4**. DMSO-d_6 was used as solvent.



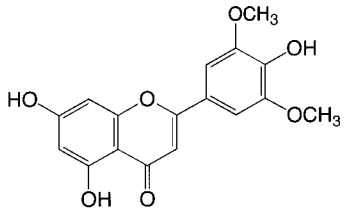
N-(*p*-coumaroyl)serotonin

Compound 1



Luteolin

Compound 3



Tricin

Compound 4

Figure 8. Structures of the isolated antioxidant compounds from Japanese barnyard millet grains.

structure. Moreover, the FT-IR spectrum indicated the presence of a −CO−NH− group (1651 cm^{-1}). In addition, a molecular ion at *m*/*z* 323 $[M + H]^+$ was detected by FAB-MS analysis. From these results, this compound was identified as *N*-(*p*-coumaroyl)serotonin (Figure 8).

*(b) Compound **3**.* As already described in the paragraph on preparative HPLC, the spectral data from the

HPLC chromatogram with a photodiode array detector suggested that this compound contained a flavone structure. The ^{1}H NMR spectrum showed that three aromatic proton signals at δ 7.39, 6.89, and 7.40 were assigned to H2′, H5′, and H6′ in ring B, respectively, on the basis of their coupling constants and the data from the ^{1}H−^{1}H COSY spectrum. Two *meta*-related (2 Hz) protons, δ 6.19 and 6.44, were assigned to H6 and H8 in ring A, respectively. In addition, a molecular ion at *m*/*z* 287 $[M + H]^+$ was detected by FAB-MS analysis. On the basis of these results, this compound was identified as luteolin (Figure 8).

(c) Compound ***4***. The spectral data from the HPLC chromatogram with a photodiode array detector (λ_{max} = 236, 260, 290 sh, 336 nm) suggested that this compound also contained a flavone structure. In the ^{1}H NMR spectrum, a *meta*-related (2 Hz) doublet at δ 6.20 (1H) and δ 6.54 (1H) was assigned to H6 and H8 in ring A, respectively. A singlet proton signal at δ 7.31 (2H) was deduced from aromatic protons. The ^{13}C−^{1}H COSY experiment showed that these protons were correlated with the carbon signal at δ 104.5. In the COLOC spectrum, two aromatic protons at δ 7.31 were correlated with carbon signals at δ 120.4, 139.9, 148.1, and 163.5 (Figure 7). Among them, except for the carbon at δ 163.5, which was assigned to C2 from the correlation with H3 at δ 6.93 in the COLOC experiment, the rest of the carbon signals were deduced to be derived from ring B. Consequently, the carbon signals at δ 104.5, 120.4, 139.9, and 148.1 were assigned to C2′ and C6′, C1′, C4′, and C3′ and C5′, respectively. The data from ^{1}H and ^{13}C NMR indicated the presence of two methoxyl groups in this compound. Their positions were determined by the COLOC experiment as 3′ and 5′ (Figure 7). In addition, a molecular ion at *m*/*z* 331 $[M + H]^+$ was detected by FAB-MS analysis. On the basis of these results, this compound was identified as tricin (Figure 8).

DISCUSSION

Three antioxidant phenolic compounds, one serotonin derivative and two flavonoids, have been isolated and identified. *N*-(*p*-Coumaroyl)serotonin and its derivatives had already been isolated from safflower seeds (Sakamura et al., 1980) and oil cake as strong 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavengers (Zhang et al., 1996, 1997). Also in this experiment, *N*-(*p*-coumaroyl)serotonin exhibited a strong peroxyl radical-scavenging activity almost equivalent to that of BHA. Serotonin, which is known as a messenger in the brain and also acts as a hormone in the intestine, is a metabolite from tryptophan, and its strong antioxidant activity using the active oxygen method (AOM) test and the DPPH method was reported (Kajimoto et al., 1979). On the other hand, Terao et al. (1993) reported that the radical scavenging activity of *p*-coumaric acid using the AMVN-initiated lipid peroxidation system was low. Consequently, the antioxidant activity of *N*-(*p*-coumaroyl)serotonin would be derived from the serotonin moiety. Although aromatic amino acids such as tryptophan, tyrosine, and histidine are also strong antioxidants, their antioxidant kinetics are still unclear (Yamaguchi, 1976). Further study is required to understand the antioxidant kinetics of serotonin. In addition to antioxidant activity, it was recently reported that this compound had antiinflammatory activity by inhibition of proinflammatory cytokine synthesis from human monocytes in vitro (Kawashima et al., 1998). This evidence supports the utility of Japanese barnyard millet grains, which contain no allergic components, as foodstuffs with biological activity. Various activities of *N*-(*p*-coumaroyl)serotonin will be found in the future.

Both of the isolated flavonoids belong to flavone. Luteolin and its glycosides are widely distributed in many plants, and they have various activities, such as antioxidant, antiinflammatory, cancer-preventive, and antiarrhythmic activities (Duke, 1992). On the other hand, tricin is distributed in wild and cultivated wheats (*Triticum*, *Aegilops*, *Hordeum*) (Cooper et al., 1994), Cyperaceae (Harborne et al., 1985), *Artemisia* (Liu and Mabry, 1981, 1982), *Phoenix canariensis* (Garcia et al., 1981), *Poa hoecu* (Rofi and Pomilio, 1985), etc. It was isolated as one of the antitumor constituents from Thymelaeaceae and also exhibited noteworthy antileukemic activity (Lee et al., 1981). The presence of tricin in the grains of Japanese barnyard millet is of interest because it is reported that tricin is a characteristic grass flavone (Harborne and Hall, 1964; Estiarte et al., 1997). Flavone and flavonol glycosides may be important deterrents in the leaves of most angiosperms (Harborne, 1988); tricin was also reported as an inhibitor of the feeding activity of the aphid *Schizaphis graminum* in wheat (*Triticum aestivum*) (Dreyer and Jones, 1981) and of the boll weevil *Anthonomus grandis* in *Arundo donax* (Miles et al., 1993) and *Eleocharis dulcis* Trin (Miles et al., 1994). For these reasons, tricin in Japanese barnyard millet grains would also act as a defense system against insects, and its content in plants may be much higher than that in grains.

Although the initial peroxyl radical-scavenging activity of luteolin was slightly lower than that of quercetin, the amount of increase in methyl linoleate hydroperoxides at and after 135 min in the experiment was smaller than that for quercetin. This result indicates that the working period of luteolin as an antioxidant is longer than that of quercetin, which is known as a strong antioxidant. On the other hand, the peroxyl radical-scavenging activity of tricin was lower than those of luteolin and quercetin, and it would be attributable to the methylation of the hydroxy groups (3′ and 5′) in ring B. In flavonoids, the 3′,4′-dihydroxy structure (catechol structure) is important for expression of radical-scavenging activity. Moreover, the trihydroxy structure (pyrogallol structure) contributes to a 3-fold increase in radical-scavenging activity compared with that of the catechol structure in catechins (Matsuzaki and Hara, 1985). From this evidence, the antioxidant activity of tricetin (5,7,3′,4′,5′-pentahydroxyflavone), which has a pyrogallol structure in ring B, would be higher than that of luteolin and tricin.

It was reported that the contents of phenolics and tannins in Japanese barnyard millet were low (Suman et al., 1992). Although the yields of isolated compounds suggest that these compounds are not so abundant in Japanese barnyard millet grains, they are expected to be effective in the body by oral administration. The strong antioxidant activity of the extract from Kurohie would be partly attributed to the isolated compounds. The investigation of the content of these compounds in other varieties would clarify their contribution to the activity. Thre are <100 varieties of Japanese barnyard millet, and investigation of the content of the antiallergic substance, *N*-(*p*-coumaroyl)serotonin, among the varieties will show their utility as foodstuffs for allergic

patients. Formerly, millet including Japanese barnyard millet was noted to be an important foodstuff in Japanese eating habits, and it would be considered to be very valuable due to its content of useful antioxidant compounds and the fact that it is highly nutritious.

ACKNOWLEDGMENT

I thank Mr. T. Saito, Tohoku National Agricultural Experiment Station, for his help in cultivation of Japanese barnyard millet and preparing the powdered sample of its grains.

LITERATURE CITED

Cooper, R.; Lavie, D.; Gutterman, Y.; Evenari, M. The distribution of rare phenolic type compounds in wild and cultivated wheats. *J. Arid Environ.* **1994**, *27*, 331−336.

Dreyer, D. L.; Jones, K. C. Feeding deterrency of flavonoids and related phenolics towards *Schizaphis graminum* and *Myzus persicae*: Aphid feeding deterrents in wheat. *Phytochemistry* **1981**, *20*, 2489−2493.

Duke, J. A. *Handbook of Biologically Active Phytochemicals and Their Activities*; CRC Press: Boca Raton, FL, 1992.

Estiarte, M.; Peñuelas, J.; Cañigueral, S.; Casals, I. A reverse-phase HPLC method for tricin separation from wheat leaves. *Cereal Chem.* **1997**, *74*, 495−496.

Garcia, B.; Marco, J. A.; Seoane, E.; Tortajada, A. Triterpenes, waxes, and tricin in *Phoenix canariensis*. *J. Nat. Prod.* **1981**, *44*, 111−113.

Harborne, J. B. *Introduction to Ecological Biochemistry*; Academic Press: London, U.K., 1988.

Harborne, J. B.; Hall, E. The occurrence of tricin and of glycoflavones in grasses. *Phytochemistry* **1964**, *3*, 421−428.

Harborne, J. B.; Williams, C. A.; Wilson, K. L. Flavonoids in leaves and inflorescences of Australian Cyperaceae. *Phytochemistry* **1985**, *24*, 751−766.

Kajimoto, G.; Ikebe, Y.; Yoshida, H.; Yamashoji, S.; Kotake, Y. Studies on the antioxidative activities of metabolites of several amino acids. *J. Jpn. Soc. Food Nutr.* **1979**, *32*, 41−46.

Kawashima, S.; Hayashi, M.; Takii, T.; Kimura, H.; Zhang, H. L.; Nagatsu, A.; Sakakibara, J.; Murata, K.; Oomoto, Y.; Onozaki, K. Serotonin derivative, *N*-(*p*-coumaroyl)serotonin, inhibits the production of TNF-α, IL-1α, IL-1*β*, and IL-6 by endotoxin-stimulated human blood monocytes. *J. Interferon Cytokine Res.* **1998**, *18*, 423−428.

Lee, K.-H.; Tagahara, K.; Suzuki, H.; Wu, R.-Y.; Haruna, M.; Hall, I. H. Antitumor agents. 49. Tricin, kaempferol-3-O-*β*-D-glucopyranoside and (+)-nortrachelogenin, antileukemic principles from *Wikstroemia indica*. *J. Nat. Prod.* **1981**, *44*, 530−535.

Liu, Y.-L.; Mabry, T. J. Flavonoids from *Artemisia frigida*. *Phytochemistry* **1981**, *20*, 1389−1395.

Liu, Y.-L.; Mabry, T. J. Flavonoids from *Artemisia ludoviciana var. ludoviciana*. *Phytochemistry* **1982**, *21*, 209−214.

Markham, K. R. Ulltraviolet-visible absorption spectroscopy. In *Techniques of Flavonoid Identification*; Academic Press: London, U.K., 1982; pp 36−51.

Matsuzaki, T.; Hara, Y. Antioxidative activity of tea leaf catechins. *Agric. Chem. Soc. Jpn.* **1985**, *59*, 129−134.

Miles, D. H.; Tunsuwan, K.; Chittawong, V.; Kokpol, U.; Choudhary, M. I.; Clardy, J. Boll weevil antifeedants from *Arundo donax*. *Phytochemistry* **1993**, *34*, 1277−1279.

Miles, D. H.; Tunsuwan, K.; Chittawong, V.; Hedin, P. A.; Kokpol, U. Boll weevil antifeedants from *Eleocharis dulcis* Trin. *J. Agric. Food Chem.* **1994**, *42*, 1561−1562.

Porter, L. J. Flavans and proanthocyanidins. In *The Flavonoids. Advances in Research since 1980*; Harborne, J. B., Ed.; Chapman and Hall: London, U.K., 1988; pp 21−62.

Ramarathnam, N.; Osawa, T.; Namiki, M.; Kawakishi, S. Chemical studies on novel rice hull antioxidants. 2. Identification of isovitexin, a C-glycosyl flavonoid. *J. Agric. Food Chem.* **1989**, *37*, 316−319.

Resources Council, Science and Technology Agency, Japan. *Standard Tables of Food Composition in Japan*, 4th revised ed.; Tokyo, 1982.

Rofi, R. D.; Pomilio, A. B. 5,7,3′-trihydroxy-4′,5′-dimethoxyflavone and other phenolics from *Poa huecu*. *Phytochemistry* **1985**, *24*, 2131−2132.

Sakamura, S.; Terayama, Y.; Kawakatsu, S.; Ichihara, A.; Saito, H. Conjugated serotonins and phenolic constituents in safflower seed (*Carthamus tinctorius* L.). *Agric. Biol. Chem.* **1980**, *44*, 2951−2954.

Suman, C. N.; Monteiro, P. V.; Ramachandra, G.; Sudharshana, L. *In-vitro* enzymic hydrolysis of the storage proteins of Japanese barnyard millet (*Echinochloa frumentacea*). *J. Sci. Food Agric.* **1992**, *58*, 505−509.

Taira, H. Influence of flooded culture on lipid content and fatty acid composition of Japanese barnyard millet. *Rep. Natl. Food Res. Inst.* **1983**, *43*, 54−57.

Terao, J.; Matsushita, S. The peroxidizing effect of α-tocopherol on autoxidation of methyl linoleate in bulk phase. *Lipids* **1986**, *21*, 255−260.

Terao, J.; Karasawa, H.; Arai, H.; Nagao, A.; Suzuki, T.; Takama, K. Peroxyl radical scavenging activity of caffeic acid and its related phenolic compounds in solution. *Biosci., Biotechnol., Biochem.* **1993**, *57*, 1204−1205.

Watanabe, M. Catechins as antioxidants from buckwheat (*Fagopyrum esculentum* Moench) groats. *J. Agric. Food Chem.* **1998**, *46*, 839−845.

Watanabe, M.; Ohshita, Y.; Tsushida, T. Antioxidant compounds from buckwheat (*Fagopyrum esculentum* Moench) hulls. *J. Agric. Food Chem.* **1997**, *45*, 1039−1044.

Yamaguchi, N. Effect of the food components on oxidative stabilities of fat and oil. *J. Jpn. Oil Chem. Soc.* **1976**, *25*, 249−256.

Zhang, H. L.; Nagatsu, A.; Sakakibara, J. Novel antioxidants from safflower (*Carthamus tinctorius* L.) oil cake. *Chem. Pharm. Bull.* **1996**, *44*, 874−876.

Zhang, H. L.; Nagatsu, A.; Watanabe, T.; Sakakibara, J.; Okuyama, H. Antioxidative compounds isolated from Safflower (*Carthamus tinctorius* L.) oil cake. *Chem. Pharm. Bull.* **1997**, *45*, 1910−1914.

Received for review May 12, 1999. Revised manuscript received August 9, 1999. Accepted August 23, 1999.

JF990498S