JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

# Hordatine A β-D-Glucopyranoside from Ungerminated Barley Grains

Noriko Kohyama*,† and Hiroshi Ono‡

†NARO Institute of Crop Science, 2-1-18 Kannondai, Tsukuba, Ibaraki 305-8518, Japan

‡NARO Food Research Institute, 2-1-12 Kannondai, Tsukuba, Ibaraki 305-8642, Japan

*Supporting Information*

**ABSTRACT:** Hordatine A β-D-glucopyranoside was isolated from ungerminated barley grains for the first time and identified by MS spectrometry and one- and two-dimensional NMR spectroscopy. This compound was observed 20 days after flowering (DAF) and most abundant at 35 DAF when the dry weight of grains reached a maximum. The contents of the compound markedly decreased when grains were pearled from 85% to 70% yield, suggesting that the compound is localized in the aleurone layer. The compound was commonly observed in ungerminated mature grains among 10 cultivars, and its contents ranged from 103 to 254 nmol/g dry weight. Because hordatines have been reported to have antifungal activities, the compound may act as an antifungal component in the latter half of the maturing stages and in mature barley grains.

**KEYWORDS:** *barley, hordatine, mature grain, aleurone layer*

## INTRODUCTION

Hordatines are antifungal compounds abundant in barley (*Hordeum vulgare* L.) seedlings.[1] Hordatine A is a dimer of *p*-coumaroylagmatine, and hordatine B is an analogous dimer of *p*-coumaroylagmatine and feruloylagmatine.[2] In earlier reports, the mixture of D-glucopyranosides of hordatines A and B was called hordatine M since they were difficult to resolve by preparative TLC and ion-exchange chromatography.[3−5] Stoessl reported that NMR spectral data of the acetylated hordatine derivatives favored α-anomeric stereochemistry, though almost all naturally occurring phenolic glucosides have the β configuration.[2,3] Recently, Kageyama et al. isolated β-D-glucopyranosides of hordatines A and B as well as β-D-maltosyl hordatine A from malt acrospires and identified them by MS and NMR spectrometry.[6]

Hordatines are biosynthesized in two consecutive reactions. In the first, agmatine coumaroyltransferase (ACT) catalyzes the conjugation of agmatine and *p*-coumaroyl-CoA or feruloyl-CoA;[7] ACT activity in barley seedlings is greatest 3−4 days after germination.[5] Hordatine A is formed when *p*-coumaroylagmatine is incubated with a crude extract of barley seedling in the presence of hydrogen peroxide[8] as well as when *p*-coumaroylagmatine is oxidatively dimerized by horseradish peroxidase in vitro.[2] Thus, it is suggested that peroxidase catalyzes the oxidative coupling of agmatine conjugates to form hordatines in the second reaction. Though *p*-coumaroylagmatine and feruloylagmatine are also found in wheat,[9] hordatines are found only in barley and in a barley chromosome 2H addition line to wheat.[10] It has also been reported that hordatine contents increase in barley leaves after an infection of powdery mildew.[4,11] However, no hordatines have been detected in ungerminated seeds[4,5] or roots.[5,10]

Hordatines are also found in beer, made with barley malt, and exert effects on humans. Hordatine A is an agonist at the muscarinic $M_3$ receptor, which stimulates gastrointestinal motility by contracting the smooth muscle.[12] Hordatine A is also reported to bind to $\alpha_1$ adrenergic receptor as an antagonist.[13] β-D-Glucopyranosides of hordatines A and B as well as the β-D-maltoside of hordatine A stimulate the oral cavity and influence beer aftertaste as astringent substances.[6]

Barley grains (ungerminated seeds) contain many phenolic compounds in abundance, such as proanthocyanidins,[14−16] catechin,[14−17] *O*-glycosyl-*C*-glycosyl flavones,[18] and phenolic acids.[15,18,19] However, several constituents in crude phenolic extracts from mature barley grains are still unknown. To uncover such constituents, we purified one major compound by chromatography and identified it by MS and NMR spectrometry as hordatine A β-D-glucopyranoside. Its contents in grains were determined in maturing stages as well as after pearling and compared among 10 cultivars.

## MATERIALS AND METHODS

**Plant Materials.** Barley cvs. Fiber-snow, Shunrai, Kashimamugi, Setsugenmochi, Nishinohoshi, Shirataenijo, Mannenboshi, Kirarimochi, Daishimochi, and Beau-fiber were grown in an experimental field at Tsukuba. When the first floret of the spikes was flowering, 170 spikes of cv. Fiber-snow were marked, and 10 were harvested every 5 days from the time of flowering to 40 DAF in 2012. Grain samples in the maturing stages were manually collected from the middle portion of the spikes in order to minimize variation of their maturities, stored at −80 °C until lyophilized, and ground in a TI-100 vibrating sample mill (CMT, Tokyo, Japan) for 90 s (mean volume diameter 48 μm). Mature grain samples were harvested in 2011 at 6 weeks after flowering time and dried under warm air at 40 °C until grain moisture content decreased to 10−12%. Whole grain flour was obtained by grinding mature grains in a Udy cyclone sample mill (Shizuoka seiki, Fukuroi, Japan) with a 1 mm screen or a TI-100 vibrating sample mill for 90 s (mean volume diameter 78 μm). A 180 g portion of mature grains of cv. Fiber-snow was pearled to 85%, 70%, and 55% yield using a TM-05 abrasive machine (Satake, Higashihiroshima, Japan) and ground using a TI-100 vibrating sample mill to obtain pearled grain

Received: September 24, 2012
Revised: January 9, 2013
Accepted: January 15, 2013
Published: January 15, 2013

 © 2013 American Chemical Society 

flour (mean volume diameter 45 $\mu$m). Water contents of samples were calculated by weight loss after heating at 135 °C for 60 min.

**Chemicals.** Prodelphinidin B3 (PDB3) and procyanidin B3 (PCB3) were purchased from Sigma-Aldrich (St. Lous, MO). Sephadex LH 20 resin was purchased from Amersham Biosciences (Uppsala, Sweden). Other chemicals were obtained from Wako Pure Chemical Industries (Osaka, Japan). 2-Methyl-2-propanol was distilled from calcium hydride before use for a quantitative reference material.

**Analytical Chromatography.** Phenolic constituents were extracted from 100 mg of whole grain flour of the maturing and mature grain samples or pearled grain flour with 1.0 mL of 75% acetone, shaking for 60 min at room temperature, in three or four replications. After centrifugation at 12 500 rpm for 10 min, the precipitate was re-extracted with 75% acetone twice. The 75% acetone extract evaporated under vacuum was dissolved in 3 mL of 2.5% acetic acid and applied to a solid-phase extraction cartridge Autoprep EDS-1 50-3 (Showa denko, Tokyo, Japan) preconditioned with 1 mL of MeOH, 3 mL of ultrapure water, and 1 mL of 2.5% acetic acid. After washing the cartridge with 3 mL of 2.5% acetic acid, a methanolic eluate (700 $\mu$L) was evaporated under vacuum, redissolved in 100 $\mu$L of 20% acetonitrile−20 mM ammonium phosphate buffer pH 3.0, and used as a crude phenolic fraction. A 20 $\mu$L aliquot of the crude phenolic fraction was applied on a 150 mm × 4.6 mm i.d. 5 $\mu$m TSKgel ODS-100 V column (Tosoh, Tokyo, Japan). The column was eluted with a gradient mobile phase consisting of 20 mM ammonium phosphate buffer (pH 3.0) (solvent A) and acetonitrile (solvent B) at 1.0 mL/min. The gradient was programmed as follows: 0−5 min, 5% B; 5−35 min, 5%−35% B; 35−40 min, 35%−50% B; 40−45 min, 50% B; 45−47 min, 50%−5% B; 47−57 min, 5% B. Elution was scanned between 240 and 400 nm with an SPD-M10AV diode array detector (Shimadzu, Kyoto, Japan). The amounts of compound **1** were calculated with the peak area at 300 nm using the isolated compound as a standard and expressed as nanomoles per gram of dry weight or nanomoles per grain. The concentration of a standard solution of compound **1** was calibrated by quantitative $^{1}$H NMR in the presence of a specific amount of 2-methyl-2-propanol as a quantitative reference material. The amounts of PDB3 and PCB3 were calculated with the peak area at 280 nm using calibration curves of authentic samples of PDB3 and PCB3, respectively, and expressed as nanomoles per grain. The coefficient of correlation and limit of detection were 0.9975 and 74.7 nM for compound **1**, 1.000 and 1.77 $\mu$M for PDB3, and 1.000 and 0.85 $\mu$M for PCB3, respectively.

**Isolation and Identification of the Compound.** Whole grain flour of mature grain (1.0 kg) was extracted with 3 volumes (v/w) of 75% acetone stirring overnight at room temperature, and the filtered extract was concentrated to 10 mL under vacuum and defatted with 50 mL of *n*-hexane. The lyophilized lower phase was suspended in 15 mL of 0.1% TFA, applied onto a Sephadex LH-20 column (290 mL) equilibrated with 0.1% TFA, and successively eluted with 900 mL of 0.1% TFA, 900 mL of 20% EtOH−0.1% TFA, and 1600 mL of EtOH. Fractions 1−11 were collected every 250 mL after the absorbance at 280 nm began to increase. Fraction 2 (0.47 g) was dissolved in 0.1% TFA after lyophilization and fractionated on a 100 mm × 25 mm i.d. 10 $\mu$m $\mu$Bondapak C18 column (Waters, Milford, MA) using 15% EtOH−0.1% TFA at 5.0 mL/min as an eluent. The major constituent in fraction 2 was further purified on a 250 mm × 4.6 mm i.d. 5 $\mu$m Ascentis RP-Amide column (Supelco, Bellefonte, PA) eluted with 10%−25% EtOH−0.1% TFA at 1.0 mL/min by monitoring absorbance at 280 nm to yield compound **1**. The purity of the compound was determined from the ratio of peak area to the total area of peaks on the chromatogram at 300 nm by analytical chromatography.

High-resolution Fourier transform ion-cyclotron resonance (FT-ICR) mass spectra were recorded on an Apex II 70e (Bruker Daltonics Inc., Billerica, MA) by infusion of MeOH/$H_2O$ (50:50, v/v) solution in ESI positive mode. One- and two-dimensional NMR spectra were recorded at 298 K in $D_2O$ using an Avance 500 spectrometer (Bruker Biospin, Karlsruhe, Germany; with $^{1}$H at 500.13 MHz and $^{13}$C at 125.76 MHz) equipped with a DUL CryoProbe and an Avance 800 spectrometer (Bruker Biospin, Karlsruhe, Germany; with $^{1}$H at 800.23 MHz) equipped with a TCI CryoProbe and also in $CD_3OD$ using an Avance 800 spectrometer (with $^{1}$H at 800.23 MHz and $^{13}$C at 201.22 MHz). Chemical shifts were expressed downfield from tetramethylsilane (TMS). TMS (for $CD_3OD$) and 2-methyl-2-propanol (for $D_2O$, $\delta_H$ 1.23 ppm, $\delta_C$ 31.3 ppm) were used for internal standards. The two-dimensional homonuclear (DQF-COSY and NOESY) and two-dimensional heteronuclear ($^{1}$H−$^{13}$C HSQC and $^{1}$H−$^{13}$C HMBC) experiments were performed for structural assignments of the $^{1}$H and $^{13}$C signals using standard two-dimensional NMR pulse sequences of Bruker software.

**Acid Hydrolysis.** Compound **1** (0.1 mg) was dissolved in 0.6 mL of 6.0 M HCl and heated at 100 °C for 1 h under vacuum. After cooling, the solution was neutralized with 5.0 M NaOH, and aliquots were analyzed by HPLC as described above.

## RESULTS AND DISCUSSION

**Identification of Compound 1.** A typical HPLC chromatogram of the crude phenolic fraction of mature grains of barley cv. Fiber-snow is shown in Figure 1. Compound **1** was detected in the crude phenolic fraction at 280 and 300 nm as a major peak ($\lambda_{max}$ = 296 nm) with a retention time of 21.0 min. Its yield was 6.1 mg, and purity was 96.3% from HPLC analysis. High-resolution FT-ICR mass spectrometry gave an ion peak at $m/z$ 713.3619, which corresponds to a theoretical value for $C_{34}H_{49}N_8O_9$ = 713.3617. $^{1}$H NMR spectra showed that compound **1** consisted of hordatine A and a $\beta$-glucopyranosyl residue. The assignments of $^{1}$H and $^{13}$C NMR data recorded in $D_2O$ are shown in Table 1. The $\beta$ configuration at the glucosyl bond was confirmed by the large $J$ value (7.5 Hz) at the anomeric position. The HMBC correlation of the phenyl carbon at 158.77 ppm to the glucopyranosyl anomeric proton at 5.136 ppm and the NOESY correlation between the glucopyranosyl anomeric proton at 5.136 and phenyl proton at 7.155 ppm revealed that the position of the glycosyl linkage was the 4-*O*-position of the phenyl group. In the NOESY spectrum of the component, no cross peaks between H-2 (5.894 ppm) and H-3 (4.329 ppm) were observed. Yamaji et al. chemically synthesized stereoisomers with two adjacent chiral carbons in the five-membered ring of hordatine A and reported that hordatine A (trans isomer) showed no NOE signals between H-2 and H-3, though aperidine (cis isomer) showed clear NOE signals in these positions.[12] Therefore, compound **1**

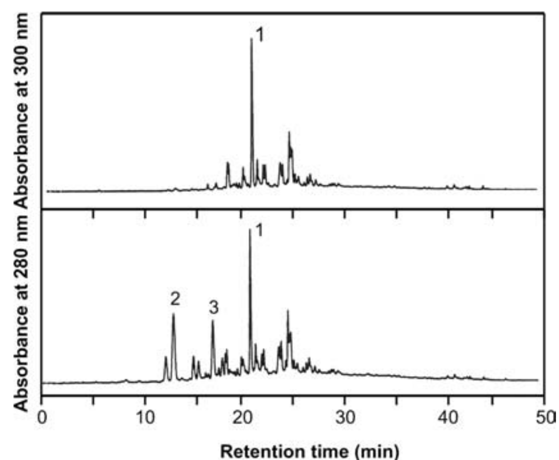


**Figure 1.** Chromatogram of the crude phenolic fraction from mature barley grains of cv. Fiber-snow. Peaks were detected by absorbance at 280 and 300 nm. Numbers indicate the peak of compound **1** (1), prodelphinidin B3 (2), and procyanidin B3 (3).

**Table 1. $^{1}H$ and $^{13}C$ NMR ($^{1}H$, 800.23 MHz; $^{13}C$ , 125.76 MHz, $D_2O$) Spectral Data for Compound 1**

| position | $^{1}H$ δ [ppm] | $^{1}H$ multiplicity | $^{1}H$ *J* [Hz] | $^{13}C$ δ [ppm] |
|---|---|---|---|---|
| 2,3-dihydro-3-benzofurancarboxamide | | | | |
| 2 | 5.894 | d | 7.0 | 89.61 |
| 3 | 4.329 | d | 7.0 | 58.41 |
| 3a | | | | 128.27 |
| 4 | 7.484 | br s | | 126.24 |
| 5 | | | | 130.25 |
| 6 | 7.593 | dd | 8.4, 1.7 | 132.10 |
| 7 | 6.993 | d | 8.4 | 112.07 |
| 7a | | | | 162.10 |
| CONH | | | | 175.01 |
| 2-(4-β-D-glucopyranosylphenyl) | | | | |
| 1 | | | | 135.38 |
| 2 | 7.406 | d[a] | 8.8 | 129.52 |
| 3 | 7.155 | d[a] | 8.8 | 118.55 |
| 4 | | | | 158.77 |
| 5 | 7.155 | d[a] | 8.8 | 118.55 |
| 6 | 7.406 | d[a] | 8.8 | 129.52 |
| Glc-1 | 5.136 | d | 7.5 | 101.63 |
| Glc-2 | 3.568 | dd | 9.3, 7.5 | 74.58 |
| Glc-3 | 3.597 | dd | 9.3, 8.9 | 77.24 |
| Glc-4 | 3.483 | dd | 9.8, 8.9 | 71.11 |
| Glc-5 | 3.624 | ddd | 9.8, 5.9, 2.2 | 77.87 |
| Glc-6 | 3.915 | dd | 12.5, 2.2 | 62.24 |
| | 3.735 | dd | 12.5, 5.9 | |
| 5-[3-{(4-guanidinobutyl)amino}-3-oxo-1-propenyl] | | | | |
| 1 | 7.467 | d | 15.8 | 142.31 |
| 2 | 6.486 | d | 15.8 | 119.71 |
| 3 | | | | 170.66 |
| butyl-1 | 3.317 (2H) | br t | 6.5 | 40.67 |
| butyl-2 | 1.614 (2H) | m | | 27.41 |
| butyl-3 | 1.614 (2H) | m | | 27.01 |
| butyl-4 | 3.201 (2H) | br t | 6.5 | 42.45 |
| guanidino | | | | 158.45 |
| *N*-(4-guanidinobutyl) | | | | |
| butyl-1 | 3.267 | dt | 13.7, 6.5 | 40.64 |
| | 3.326 | dt | 13.7, 6.6 | |
| butyl-2 | 1.555 (2H) | m | | 27.34 |
| butyl-3 | 1.509 (2H) | m | | 26.96 |
| butyl-4 | 3.126 (2H) | br t | 7.1 | 42.41 |
| guanidino | | | | 158.30 |

[a] AA′XX′ type.

was suggested to have a trans configuration in the five-membered ring and identified as hordatine A β-D-glucopyranoside (Figure 2).

Kageyama et al. isolated hordatine A β-D-glucopyranoside from barley malt and reported $^{1}H$ and $^{13}C$ NMR data in $CD_3OD$.[6] Our results obtained in $CD_3OD$ were in reasonable agreement with their results. Kageyama et al. also reported hordatine derivatives with *cis*-cinnamoyl moieties as minor components from barley malt.[20] The compound isolated our study only has the signal of the *trans*-cinnamoyl moiety.

Stoessl reported that the D-glucopyranosides of hordatines A and B from barley seedlings were of the α configuration;[3] however, these data seem to be insufficient to exclude a β configuration for hordatine D-glucopyranosides. Therefore, hordatine A β-D-glucopyranoside, isolated from ungerminated grain in the present study, could be one of the members of

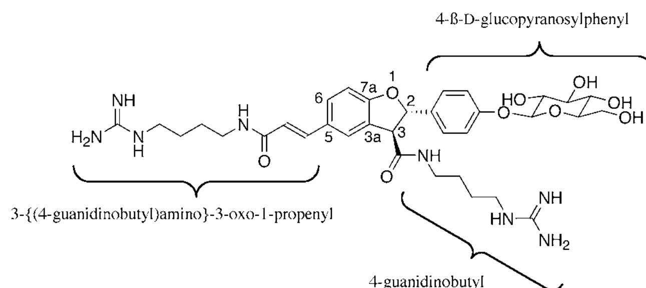


**Figure 2.** Chemical structure of compound **1** isolated from mature barley grains.

hordatine M described in previous reports.[3−5] The reason is as follows: Stoessl obtained two diacetylated products from hordatine M and recorded their $^{1}H$ NMR spectra at a Larmor frequency of 60 MHz in $D_2O$. The anomeric signals in each product appeared at 5.45 ppm as a noisy broad singlet with a half width of 15 Hz and partially overlapped with the HDO signal. The low-field shift of anomeric signals was interpreted as a consequence of a diamagnetic aromatic ring current. Stoessl described the signal as "poorly resolved doublets" and roughly estimated their coupling constants to be less than 5 Hz. Since the coupling constants of the α and β configurations of the glucopyranosyl structure were 3.0−3.5 and 7.0−7.7 Hz, respectively,[21] the reported value was too large to conclude α configuration. In addition, broad doublets appeared at 6.34 (H-2) and 4.67 ppm (H-3), whose half widths were 15−16 Hz, similar to that of the anomeric signal, had far larger coupling constants of 7 Hz than the estimated *J* value of the anomeric proton.

Because D-glucosyl hordatine B[1,3−6] and D-maltosyl hordatine A[6] were reported to coexist with D-glucosyl hordatine A in barley seedling and malt, it is possible that these compounds may be present in the ungerminated grain. However, this is not clear in the present study. When hordatine A β-D-glucopyranoside was hydrolyzed under acidic conditions, a major peak ($\lambda_{max}$ = 300 nm) was observed at 23.8 min. This peak most likely corresponds to hordatine A. In earlier studies of hordatines from barley seedlings, hordatine M was found in lesser amounts than the aglycones, hordatines A and B.[4,10] In contrast, Bird and Smith reported that hordaine M was detected at much higher levels than hordatines A and B and reached a maximum level at 6 or 7 days after germination in the dark or light-grown conditions, respectively.[5] In our conditions, no peak suggestive of hordatine A was detected in the crude phenolic fraction from mature grains. Hordatine A may be stored as the glycosylated form in mature grains and partially produced by hydrolysis of the glycosylated form after germination.

**Accumulation of Hordatine in Grains during Maturation.** Contents of hordatine A β-D-glucopyranoside in grains of cv. Fiber-snow in maturity stages are shown in Table 2. The compound was undetectable in grains at 5, 10, and 15 DAF, but it accumulated after 20 DAF, increased up to 35 DAF, and decreased slightly at 40 DAF. Table 2 also shows the contents of proanthocyanidins, the predominant phenolic constitutions in barley grains, during maturation. PDB3 and PCB3 increased after 10 DAF, and their contents per grain decreased after 30 and 20 DAF, respectively. Thus, the onset and peak time of accumulation of hordatine A β-D-glucopyranoside were later than those of PDB3 and PCB3. The dry weights of grains increased up to 30 DAF and remained almost constant from 30 to 40 DAF (Table 2), such that accumulation of hordatine A β-

**Table 2. Grain Weight and Contents of Hordatine A β-D-Glucopyranoside and Proanthocyanidins in the Grains cv. Fiber-Snow during Maturation[a]**

| DAF | grain fresh weight (mg/grain) | grain dry weight (mg/grain) | hordatine A β-glucopyranoside (nmol/grain) | PDB3 (nmol/grain) | PCB3 (nmol/grain) |
|---|---|---|---|---|---|
| 5 | 17.0 ± 1.4 | 4.5 ± 0.4 | ND | ND | ND |
| 10 | 34.3 ± 1.3 | 9.6 ± 0.4 | ND | 1.0 ± 0.0 | 6.1 ± 0.2 |
| 15 | 46.9 ± 2.3 | 16.6 ± 0.8 | ND | 9.7 ± 0.2 | 13.6 ± 0.4 |
| 20 | 56.5 ± 3.8 | 24.9 ± 1.7 | 0.95 ± 0.12 | 18.8 ± 0.4 | 17.2 ± 0.5 |
| 25 | 62.1 ± 4.4 | 32.3 ± 2.3 | 2.49 ± 0.14 | 24.0 ± 1.6 | 12.8 ± 1.1 |
| 30 | 64.6 ± 3.1 | 37.6 ± 1.8 | 3.61 ± 0.19 | 25.5 ± 0.8 | 11.3 ± 0.4 |
| 35 | 55.1 ± 5.4 | 38.7 ± 3.8 | 3.88 ± 0.39 | 20.6 ± 1.3 | 8.8 ± 0.6 |
| 40 | 45.8 ± 2.9 | 36.5 ± 2.3 | 3.30 ± 0.25 | 17.8 ± 0.4 | 7.9 ± 0.1 |

[a]Contents are expressed as mean ± standard deviation ($n$ = 3). ND is not detected. PDB3 and PCB3 are prodelphinidin B3 and procyanidin B3, respectively.

D-glucopyranoside in maturing grains was apparently completed before grain dry weight reached its maximum. The contents of hordatine A β-D-glucopyranoside were 3.88 nmol per grain (100 nmol/g dry weight) at maximum level in cv. Fiber-snow, far lower than the contents of hordatine M (130 nmol per shoot in dark-grown barley) reported in barley seedlings.[5] This may explain why hordatines have not previously been found in grains before germination.

**Distribution of Hordatine As Determined by Pearling.** The effect of pearling on contents of hordatine A β-D-glucopyranoside was studied in the grains of cv. Fiber-snow (Figure 3). When grains were pearled to 85% yield, 97% of the compound in the whole grains remained in the pearled grains. In contrast, only 19% and 3% of the compound were retained in grains pearled to 70% and 55% yield, respectively. Therefore, it is suggested that very little hordatine A β-D-glucopyranoside is contained in either the outermost layer or the endosperm of barley grains and that the compound is localized in the aleurone layer. When barley grains are processed into rolled barley or rice-shaped barley, which are popular as staple foods in Japan, barley grains are usually pearled to 50%−60% yield. Therefore, the compound is very scarce in foods derived by standard pearling.

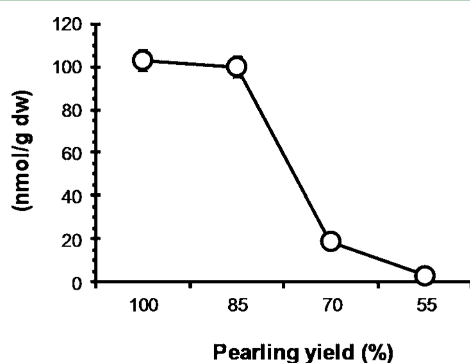


**Figure 3.** Contents of hordatine A β-D-glucopyranoside in pearled grains of cv. Fiber-snow. Contents are expressed as mean ± standard deviation ($n$ = 4).

**Hordatine Contents in Several Barley Cultivars.** The contents of hordatine A β-D-glucopyranoside in grains were determined in 10 barley cultivars (Table 3). All 10 cultivars examined contained the compound in mature grains in amounts ranging from 103 to 254 nmol/g dry weight. There was no significant difference between normal amylose and waxy barley in the contents of the compound. The contents in hulled barley (167 ± 66 nmol/g dry weight) were insignificantly lower than those in hull-less barley (210 ± 41 nmol/g dry weight), whereas the contents in two-rowed barley (241 ± 9 nmol/g dry weight) were far higher than those in six-rowed barley (147 ± 44 nmol/g dry weight). Because water contents of these mature grains were 11.1%−12.1%, the average concentrations of the compound in mature grains were estimated to be 0.79−1.9 mM among 10 cultivars. According to Stoessl and Unwin, the hordatines inhibit spore germination of *Helminthosporium sativum* and several other fungi in vitro at concentrations as low as 10 μM.[1] The concentration of hordatine A β-D-glucopyranoside in mature grains was well above that at which hordatines showed these antifungal activities in vitro.

**Table 3. Contents of Hordatine A β-D-Glucopyranoside (nmol/g dry weight) in Mature Barley Grains[a]**

| cultivar | genotype | hordatine A β-glucopyranoside |
|---|---|---|
| hulled barley | | |
| Fiber-snow | 6-rowed | 103 ± 5 |
| Shunrai | 6-rowed | 110 ± 11 |
| Setsugenmochi | 6-rowed, waxy | 124 ± 7 |
| Kashimamugi | 6-rowed | 177 ± 9 |
| Nishinohoshi | 2-rowed | 236 ± 15 |
| Shirataenijo | 2-rowed | 254 ± 12 |
| hull-less barley | | |
| Mannenboshi | 6-rowed | 150 ± 15 |
| Daishimochi | 6-rowed, waxy | 218 ± 18 |
| Kirarimochi | 2-rowed, waxy | 235 ± 12 |
| Beau-fiber | 2-rowed | 239 ± 16 |

[a]Contents are expressed as mean ± standard deviations ($n$ = 4).

This is the first report stating that hordatine A β-D-glucopyranoside, a compound in the hordatine family, is found in ungerminated barley seeds. The compound is commonly observed in mature barley grains. Further studies are necessary to confirm the effects of the compound on spore germination of fungi and resistance against barley diseases caused by fungi, such as *Fusarium* head blight. However, the presence of hordatine A β-D-glucopyranoside in the latter half of the maturing process and in mature barley grains suggests its possible in vivo antifungal activity.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

This material is available free of charge via the Internet at http://pubs.acs.org.

## AUTHOR INFORMATION


### Corresponding Author

*Phone/fax: +81-29-838-8868. E-mail: norikok@affrc.go.jp.


### Notes

The authors declare no competing financial interest.

## ABBREVIATIONS USED

ACT, agmatin coumaroyltransferase; DAF, days after flowering; ESI-TOF, electrospray ionization time of flight; FT-ICR, Fourier transform ion cyclotron resonance; PDB3, prodelphinidin B3; PCB3, procyanidin B3; TMS, tetramethylsilane

## REFERENCES


(1) Stoessl, A.; Unwin, C. H. The antifungal factors in barley. V. Antifungal activity of the hordatines. *Can. J. Bot.* **1970**, *48*, 465−470.

(2) Stoessl, A. The antifungal factors in barley − The constitutions of hordatines A and B. *Tetrahedron Lett.* **1966**, *21*, 2287−2292.

(3) Stoessl, A. The antifungal factors in barley. IV. Isolation, structure, and synthesis of the hordatines. *Can. J. Chem.* **1967**, *45*, 1745−1760.

(4) Smith, T. A.; Best, G. R. Distribution of the hordatines in barley. *Phytochemistry* **1978**, *17*, 1093−1098.

(5) Bird, C. R.; Smith, T. A. Agmatine metabolism and hordatine formation in barley seedlings. *Ann. Bot.* **1984**, *53*, 483−488.

(6) Kageyama, N.; Inui, T.; Fukami, H.; Komura, H. Elucidation of chemical structures of components responsible for beer aftertaste. *J. Am. Soc. Brew. Chem.* **2011**, *69*, 255−259.

(7) Burhenne, K.; Kristensen, B. K.; Rasmussen, S. K. A new class of *N*-hydroxycinnamoyltransferases. Purification, cloning, and expression of a barley agmatine coumaroyltransferase (EC 2.3.1.64). *J. Biol. Chem.* **2003**, *278*, 13919−13927.

(8) Negrel, J.; Smith, T. A. Oxidation of *p*-coumaroylagmatine in barley seedling extracts in the presence of hydrogen peroxide or thiols. *Phytochemistry* **1984**, *23*, 739−741.

(9) Jin, S.; Yoshida, M. Antifungal compound, feruloylagmatine, induced in winter wheat exposed to a low temperatue. *Biosci. Biotechnol. Biochem.* **2000**, *64*, 1614−1617.

(10) Nomura, T.; Sue, M.; Horikoshi, R.; Tebayashi, S.; Ishihara, A.; Endo, T. R.; Iwamura, H. Occurrence of hordatines, the barley antifungal compounds, in a wheat-barley chromosome addition line. *Genes Genet. Syst.* **1999**, *74*, 99−103.

(11) von Röpenack, E.; Parr, A.; Schulze-Lefert, P. Structural analyses and dynamics of soluble and cell wall-bound phenolics in a broad spectrum resistance to the powdery mildew fungus in barley. *J. Biol. Chem.* **1998**, *273*, 9013−9022.

(12) Yamaji, N.; Yokoo, Y.; Iwashita, T.; Nemoto, A.; Koike, M.; Suwa, Y.; Wakimoto, T.; Tsuji, K.; Nukaya, H. Structural determination of two active compounds that bind to the muscarinic $M_3$ receptor in beer. *Alcohol: Clin. Exp. Res.* **2007**, *31*, 9S−14S.

(13) Wakimoto, T.; Nitta, M.; Kasahara, K.; Chiba, T.; Yiping, Y.; Tsuji, K.; Kan, T.; Nukaya, H.; Ishiguro, M.; Koike, M.; Yokoo, Y.; Suwa, Y. Structure−activity relationship study on $\alpha_1$ adrenergic receptor antagonists from beer. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5905−5908.

(14) McMurrough, I.; Madigan, D.; Smyth, M. R. Semipreparative chromatographic procedure for the isolation of dimeric and trimeric proanthyocyanidins from barley. *J. Agric. Food Chem.* **1996**, *44*, 1731−1735.

(15) Holtekjølen, A. K.; Kinitz, C.; Knutsen, S. H. Flavanol and bound phenolic acid contents in different barley varieties. *J. Agric. Food Chem.* **2006**, *54*, 2253−2260.

(16) Dvorakova, M.; Moreira, M. M.; Dostalek, P.; Skulilova, Z.; Guido, L. F.; Barros, A. A. Characterization of monomeric and oligomeric flavan-3-ols from barley and malt by liquid chromatography−ultraviolet detection−electrospray ionization mass spectrometry. *J. Chromatogr. A* **2008**, *1189*, 398−405.

(17) Friedrich, W.; Galensa, R. Identification of a new flavanol glucoside from barley (*Hordeum vulgare* L.) and malt. *Eur. Food Res. Technol.* **2002**, *214*, 388−393.

(18) Ferreres, F.; Kršková, Z.; Gonçalves, R. F.; Valentão, P.; Pereira, J. A.; Dušek, J.; Martin, J.; Andrade, P. B. Free water-soluble phenolics profiling in barley (*Hordeum vulgare* L.). *J. Agric. Food Chem.* **2009**, *57*, 2405−2409.

(19) Yu, J.; Vasanthan, T.; Temelli, F. Analysis of phenolic acids in barley by high-performance liquid chromatography. *J. Agric. Food Chem.* **2001**, *49*, 4352−4358.

(20) Kageyama, N.; Inui, T.; Fukami, H.; Komura, H. Structures in the hordatine family with *cis*-cinnamoyl moieties. *J. Am. Soc. Brew. Chem.* **2012**, *70*, 133−136.

(21) Van der Veen, J. M. An n.m.r. study of the glycoside link in glycosides of glucose and galactose. *J. Org. Chem.* **1963**, *28*, 564−566.