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ORIGINAL ARTICLE

Inhibitors of Antigen-induced Degranulation of RBL-2H3 Cells Isolated from Wheat Bran

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Abstract Chromatographic separation of ethanol extract of wheat bran led to the isolation of five 5-alk(en)ylresorcinols, four aliphatic compounds, and one phenolic glycoside. These were, respectively: 5-*n*-heptadecylresorcinol (**1**), 5-*n*-14'-(*Z*)-heneicosylresorcinol (**2**), 5-*n*-nonadecylresorcinol (**3**), 5-*n*-heneicosylresorcinol (**4**), 5-*n*-tricosylresorcinol (**5**), 1-*O*-(9*Z*,12*Z*,15*Z*-octadecatrienoate) glycerol (**6**), 2-linoleoylglycerol (**7**), 1-*O*-(9*Z*,12*Z*-octadecatrienoate)glycerol (**8**), pinellic acid (**9**), and tachioside (**10**). Their structures were determined by 1D- & 2D-NMR and mass spectroscopy data analysis. The inhibitory effects of isolated constituents on the release of β-hexosaminidase from RBL-2H3 cells were examined. Inhibition was shown by 5-*n*-nonadecylresorcinol (**3**), 5-*n*-heneicosylresorcinol (**4**), pinellic acid (**9**), and tachioside (**10**).

Keywords 5-alk(en)ylresorcinol · β-hexosaminidase · RBL-2H3 cells · wheat bran

Introduction

Wheat is globally the most important cereal crop in terms of both areas cultivated and amount of grains produced. Wheat bran is a by-product of the milling of wheat into white flour and generally accounts for 14–19% of the grain weight (Maes and Delcour, 2002). Wheat bran is a good source of dietary fiber and is produced worldwide in enormous quantities as an important by-product of the cereal industry. Its extracts and chemicals have been reported to have antimutagenic and radical-scavenging (Brindzova et al., 2009), antioxidative (Yuan et al., 2005), protein glycation inhibitory (Wang et al., 2009), and antitumor activities (Reddy, 2000; Qu, 2005). In addition, previous studies on the phytochemical components of wheat bran have led to the isolation of many compounds, such as feruloylated oligosaccharides (Yuan et al., 2005), phenolic acids (Baublis et al., 2000), sterol ferulates, sterol, 5-alk(en)ylresorcinols (Iwatsuki et al., 2003), and lignans (Èukelj et al., 2011).

Type I (immediate hypersensitivity) allergic diseases are amongst the most common causes of chronic illness in the populations of industrialized countries. The clinical manifestations of type I allergic diseases are caused by the release of proinflammatory mediators, such as histamine, leukotrienes, and prostaglandins from IgE-sensitized effector cells (mast cells and basophils) when cell-bound IgE antibodies interact with the allergen (Choi et al., 2005). Mast cells play significant roles in allergic diseases. Upon activation by high-affinity IgE receptors (FcεRI), they release factors such as histamine, cytokines, and chemokines that ultimately cause allergic responses (Huang et al., 2008). Rat basophilic leukemia 2H3 (RBL-2H3) cells are tumor analogues of mucosal mast cells and express high levels of FcεRI on the cell membrane. These cells have been used extensively as a model for the study of mast cell degranulation through the antigen-induced aggregation of FcεRI (Marone, 2002; Wang, 2007). Among the various inflammatory mediators produced by mast cells, β-hexosaminidase is stored in the secretory granules of the cells and is released by exocytosis when mast cells are immunologically activated during the cross-linking of FcεRIs (Schwartz, 1981; Marquardt and Wasserman, 1983; Lee, 1999). Therefore, inhibition of the release of β-hexosaminidase has been used commonly as a reliable parameter to predict possible antiallergic activities of natural chemicals.

As part of our research program for the discovery of plant-derived inhibitors of the release of β-hexosaminidase, the ethanolic

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extract (IC_{50} = 46.5 μg/mL) of wheat bran was found to inhibited release of β-hexosaminidase in RBL-2H3. in the present study, the isolation of chemical constituents and structure elucidation of phenolic glycosides from wheat bran as well as the inhibitory effects of the isolated compounds on the release of β-hexosaminidase from RBL-2H3 cells were perfomed.

Materials and Methods

Plant materials. Wheat bran was obtained from a local milling plant (Milex biotech Co., Gwangju, Korea). The bran was milled and passed through a 0.5-mm sieve. A voucher specimen (G36) was deposited at the Natural Product Chemistry Laboratory, Gyeonggi Institute of Science & Technology Promotion of the Natural Products Research Institute.

Instruments and reagents. Optical rotations were measured with a JASCO DIP-1000 polarimeter (Tokyo, Japan). UV and IR spectra were obtained on a JASCO UV-550 and a Perkin-Elmer model LE599 spectrometer (Manhattan, NY), respectively. 1D- and 2D- (^{1}H-^{1}H COSY, HMQC, and HMBC) NMR spectra were measured on a Varian 500 MHz (Agilent, Palo Alto, CA) and a Bruker Avance II 400 MHz NMR spectrometer (Rheinstetten, Germany) with tetramethylsilane as an internal standard, and chemical shifts are expressed as δ values. Electrospray ionization (ESI) mass spectra were obtained on a Q-Tof micro™ (Waters, Milford, MA) mass spectrometer. Preparative high performance liquid chromato-graphy (HPLC) was performed on a Shimadzu (LC-8A pump and SPD-20A UV/VIS detector, Kyoto, Japan) and a YMC-Pack ODS A column (250 mm × 20 mm I.D.; YMC, Allentown, PA) using a mixed solvent system of acetonitrile-water at a flow rate of 20 mL/min. Open column chromatography was performed using a silica gel (Kieselgel 60, 70–230 mesh, Merck, Darmstadt, Germany), Lichroprep RP-18 (40–63 mM, Merck), and thin layer chromatography (TLC) was performed using a pre-coated silica gel 60 F_{254} (0.25 mm, Merck). Compound 48/80, ketotifen, 2,4-dinitrophenol (DNP)-specific monoclonal IgE, 2,4-dinitrophenylated bovine serum albumin (DNP-BSA) were purchased from Sigma Chemical Co. (St. Louis, MO). All chemicals and solvents were of analytical grade and used without further purification.

Extraction and isolation. The dried wheat bran (1.7 kg) was extracted with EtOH (3×15 L) at room temperature (24 h). The extract was filtered and concentrated, *in vacuo*, and diluted with water. The aqueous solution was then sequentially partitioned with *n*-hexane (3×1.5 L), CH_2Cl_2 (3×1.5 L), and *n*-butyl alcohol (*n*-BuOH) (3×1.5 L). The *n*-hexane extract (24 g) was subjected to column chromatography on silica gel (70–230 mesh, 9×25 cm), eluted with CH_2Cl_2/CH_3OH (1:0) in increasing proportion of CH_3OH to yield ten fractions (G36H-3-1–10). Fraction G36H-3-10 (0.4 g) was subjected to silica gel column chromatography (70–230 mesh; Φ=7 cm, L=25 cm) and eluted with a stepwise gradient of *n*-hexane/acetone system (1:0, 90:1, 70:1, 50:1, 30:1, 10:1, 5:1, 3:1, 2:1, v/v) and yielded eight fractions (G36H-6-1–8). Fraction G36H-6-3 (550 mg) was further purified by means of semi-preparative HPLC eluting with acetonitrile/water (95→100% acetonitrile, gradient) at 20 mL/min to yield compounds **1** (12.2 mg), **2** (6.1 mg), **3** (107.5 mg), **4** (126.8 mg), and **5** (6.3 mg). Fractions G36H-6-8 (50 mg) was subjected to flash column chromatography on RP-18 (2×30 cm, 40–63 mm) eluting with acetonitrile-water (2:3, 3:2, 4:1, and 1:0) and semi-preparative HPLC eluting with an acetonitrile-water (70→100% acetonitrile, gradient, flow rate of 20 mL/min) to give **6** (4.5 mg), **7** (7.4 mg), and **8** (5.2 mg). The *n*-BuOH extract (10 g) was subjected to column chromatography on Diaion HP-20 (9×25 cm) eluting with H_2O/CH_3OH (1:0, 4:1, 3:2, 2:3, 1:4, 0:1) in increasing proportions of CH_3OH to yield six fractions (G36B-18-1–6). Fraction G36B-18-5 (420 mg) was further purified by semi-preparative HPLC eluting with acetonitrile - water (75→85% acetonitrile, gradient) at a flow rate of 20 mL/min to yield compound **9** (21.2 mg). In addition, compound **10** (10.5 mg) was obtained from fraction G36B-18-2 (110 mg) by silica gel column chromatography (70–230 mesh; Φ=3 cm, L=25 cm) using $CHCl_3$:CH_3OH:H_2O lower layer, 20:4:1, 10:3:1, 6:3:1, 6:4:1, v/v).

5-*n*-Heptadecylresorcinol (1). Pale yellow powder; ^{1}H-NMR ($CDCl_3$/CD_3OD, 400 MHz) δ 6.16 (2H, d, *J*=2.5 Hz, H-4 and H-6), 6.12 (1H, t, *J*=2.5 Hz, H-2), 2.45 (2H, t, *J*=7.6 Hz, H-1'), 1.56 (2H, m, H-2'), 1.27 (approx. 28H, m, H-3'–16'), 0.88 (3H, t, *J*=7.0 Hz, H-17'); ^{13}C-NMR ($CDCl_3$/CD_3OD, 100 MHz) δ 156.2 (C-1 and C-3), 143.7 (C-5), 105.3 (C-6), 98.2 (C-2), 34.3 (C-1'), 30.3 (C-15'), 29.7 (C-2'), 28.03, 28.0, 27.9, 27.7, 27.68, 21.0 (C-16'), 12.0 (C-17'); ESIMS (negative) *m/z* 347 $[M - H]^-$.

5-*n*-14'-(*Z*)-Heneicosylresorcinol (2). Pale yellow oil; ^{1}H-NMR ($CDCl_3$/CD_3OD, 400 MHz) δ 6.16 (2H, d, *J*=2.4 Hz, H-4 and H-6), 6.12 (1H, t, *J*=2.4 Hz, H-2), 5.33 (2H, t, *J*=4.8 Hz, H-14' and H-15'), 2.45 (2H, t, *J*=7.5 Hz, H-1'), 2.01 (4H, m, H-13' and H-16'), 1.56 (2H, m, H-2'), 1.26 (approx. 30H, m, H-3'–20'), 0.89 (3H, t, *J*=7.0 Hz, H-21'); ^{13}C-NMR ($CDCl_3$/CD_3OD, 100 MHz) δ 156.4 (C-1 and C-3), 143.8 (C-5), 128.1 (C-14'), 128.07 (C-15'), 105.3 (C-6), 98.2 (C-2), 34.3 (C-1'), 30.4 (C-19'), 29.7 (C-2'), 28.1, 28.0, 27.9, 27.87, 27.7, 27.6, 25.4 (C-13), 25.2 (C-16), 20.6 (C-20'), 11.9 (C-21'); ESIMS (negative) *m/z* 401 $[M–H]^-$.

5-*n*-Nonadecylresorcinol (3). Pale yellow powder; ESIMS (negative) *m/z* 375 $[M–H]^-$, 751 $[2M–H]^-$; ^{1}H- and ^{13}C-NMR data are essentially the same as those of compound **1**.

5-*n*-Heneicosylresorcinol (4). Pale yellow powder; ESIMS (negative) *m/z* 403 $[M–H]^-$; ^{1}H- and ^{13}C-NMR data are essentially the same as those of compound **1**.

5-*n*-Tricosylresorcinol (5). Pale yellow powder; ESIMS (negative) *m/z* 431 $[M–H]^{-1}$; ^{1}H- and ^{13}C-NMR data are essentially the same as those of compound **1**.

1-*O*-(9*Z*,12*Z*,15*Z*-Octadecatrienoate)glycerol (6). Colorless oil; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.32–5.45 (6H, m, H-9', 10', 12', 13', 15', 16'), 4.23 (1H, dd, *J*=11.6, 4.4 Hz, H-1), 4.17 (1H, dd, *J*=11.6, 6.0 Hz, H-1), 3.96 (1H, m, H-2), 3.72 (1H, dd, *J*=11.6, 4.0 Hz, H-3), 3.62 (1H, dd, *J*=11.6, 6.0 Hz, H-3), 2.82 (4H, brt,

J=5.6 Hz, H-11', 14'), 2.38 (1H, t, *J*=7.6 Hz, H-2'), 2.08 (4H, m, H-8', 17'), 1.65 (2H, m, H-3'), 1.27-1.37 (8H, brs, H-4', 5', 6', 7'), 1.00 (3H, t, *J*=7.6 Hz, H-18'); ^{1}H-NMR (CD_3OD, 500 MHz) δ 5.29-5.41 (6H, m, H-9', 10', 12', 13', 15', 16'), 4.15 (1H, dd, *J*=11.5, 4.5 Hz, H-1), 4.06 (1H, dd, *J*=11.5, 6.5 Hz, H-1), 3.82 (1H, m, H-2), 3.56 (1H, dd, *J*=11.0, 5.5 Hz, H-3), 3.53 (1H, dd, *J*=11.5, 6.0 Hz, H-3), 2.81 (4H, brt, *J*=5.8 Hz, H-11', 14'), 2.35 (1H, t, *J*=7.5 Hz, H-2'), 2.09 (4H, m, H-8', 17'), 1.62 (2H, m, H-3'), 1.29-1.37 (8H, brs, H-4', 5', 6', 7'), 0.98 (3H, t, *J*=7.5 Hz, H-18'); ^{13}C-NMR (CD_3OD, 125 MHz) δ 174.1, 131.3, 129.7, 127.8, 127.79, 127.4, 126.8, 69.8, 65.7, 62.7, 33.5, 29.3, 29.27, 28.9, 28.8, 28.77, 26.7, 25.1, 25.0, 24.6, 20.7, 13.2; ESIMS (positive) *m/z* 375 $[M+Na]^+$.

2-Linoleoylglycerol (7). Colorless oil; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.31–5.38 (4H, m, H-9', 10', 12', 13'), 4.93 (1H, qui, *J*=5.0 Hz, H-2), 3.84 (4H, d, *J*=5.0 Hz, H-1, H-3), 2.77 (2H, brt, *J*=6.5 Hz, H-11'), 2.38 (1H, t, *J*=7.5 Hz, H-2'), 2.05 (4H, dd, *J*=13.5, 6.5 Hz, H-8', 14'), 1.64 (2H, m, H-3'), 1.27-1.37 (14H, brs, H-4', 5', 6', 7', 15', 16', 17'), 0.89 (3H, t, *J*=7.5 Hz, H-18'); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 174.1, 130.3, 130.0, 128.1, 127.9, 75.0, 62.6, 34.3, 31.5, 29.7, 29.6, 29.4, 29.2, 29.1, 29.08, 27.2, 27.19, 25.6, 24.9, 22.6, 14.1; ESIMS (positive) *m/z* 377 $[M+Na]^+$.

1-*O*-(9*Z*,12*Z*-Octadecatrienoate)glycerol (8). Colorless oil; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.31–5.41 (4H, m, H-9', 10', 12', 13'), 4.21 (1H, dd, *J*=11.6, 4.8 Hz, H-1), 4.16 (1H, dd, *J*=11.6, 7.0 Hz, H-1), 3.95 (1H, m, H-2), 3.71 (1H, dd, *J*=11.2, 4.0 Hz, H-3), 3.61 (1H, dd, *J*=11.5, 6.0 Hz, H-3), 2.78 (2H, brt, *J*=6.4 Hz, H-11'), 2.37 (1H, t, *J*=7.6 Hz, H-2'), 2.07 (4H, dd, *J*=13.6, 6.8 Hz, H-8', 14'), 1.63 (2H, m, H-3'), 1.27-1.41 (14H, brs, H-4', 5', 6', 7', 15', 16', 17'), 0.91 (3H, t, *J*=7.5 Hz, H-18'); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 174.4, 130.2, 130.0, 128.1, 127.9, 70.3, 65.1, 63.3, 34.1, 31.5, 29.7, 29.6, 29.3, 29.2, 29.1, 27.2, 27.18, 25.6, 24.9, 24.89, 22.6, 14.1; ESIMS (positive) *m/z* 377 $[M+Na]^+$.

Pinellic acid (9). Gummy, white solid; $[\alpha]_D^{25}$ –18.3° (c 0.1, CH_3OH); IR (KBr) ν_{max} 3541, 3352, 2930, 2847, 1691, 1456, 1311, 1069 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3/CD_3OD$) δ 5.73 (1H, dd, *J*=15.6, 6.0 Hz, H-10), 5.65 (1H, dd, *J*=15.6, 6.4 Hz, H-11), 4.06 (1H, dd, *J*=6.4 Hz, H-9), 3.89 (1H, t, *J*=6.4 Hz, H-12), 3.41 (1H, m, H-13), 2.28 (2H, t, *J*=7.5 Hz, H-2), 1.61 (3H, m, H-3, H-14b), 1.52 (1H, m, H-8b), 1.32 (16H, brs, H-4, 5, 6, 7, 8b, 14a, 15, 16, 17), 0.89 (3H, t, *J*=7.0 Hz, H-18); ^{1}H-NMR (400 MHz, pyridine-d_5) δ 6.42 (1H, dd, *J*=15.6, 5.6 Hz, H-10), 6.35 (1H, dd, *J*=15.6, 5.2 Hz, H-11), 4.53 (2H, m, H-9 and H-12), 3.96 (1H, m, H-13), 2.51 (2H, t, *J*=7.4 Hz, H-2), 1.29 (aliphatic methylene), 0.83 (3H, t, *J*=6.8 Hz, H-18); ^{13}C-NMR (100 MHz, pyridine-d_5) δ 176.4 (C-1), 137.0 (C-11), 131.3 (C-10), 76.6 (C-12), 75.6 (C-13), 72.2 (C-9), 38.9 (C-8), 35.2 (C-2), 34.0 (C-14), 32.7 (C-16), 30.3 (C-5 or C-6), 30.1 (C-5 or C-6), 29.9 (C-4), 26.6 (C-7), 26.4 (C-3 or C-15), 26.0 (C-3 or C-15), 23.4 (C-17), 14.6 (C-18); ESIMS (positive) *m/z* 353.85 $[M+Na]^+$, 683.42 $[2M+H]^+$; ESIMS (negative) *m/z* 329.72 $[M–H]^-$, 659.83 $[2M–H]^-$.

Tachioside (10). Colorless needles; $[\alpha]_D^{25}$ –35.5° (c 0.15, CH_3OH); UV (MeOH) λ_{max} (log ε) 206 (4.25), 227 (sh, 3.84), 285 (3.58) nm; IR (KBr) ν_{max} 3429, 1610, 1514, 1370, 1278, 1070, 1041, 993 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 6.69 (1H, d, *J*=2.8 Hz, H-2), 6.66 (1H, d, *J*=8.8 Hz, H-5), 6.46 (1H, dd, *J*=8.8, 2.8, H-6), 4.67 (1H, d, *J*=7.6 Hz, H-1'), 3.73 (3H, s, OCH_3), 3.71 (1H, dd, *J*=11.6, 4.8 Hz, H-6'), 3.44 (1H, d, *J*=11.6, 6.0 Hz, H-6'), 3.10 – 3.31 (4H, m, H-2', 3', 4', 5'); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 151.2 (C-4), 148.2 (C-2), 141.7 (C-1), 115.6 (C-6), 102.8 (C-3), 102.1 (Glc-1), 77.5 (Glc-3), 77.2 (Glc-5), 73.7 (Glc-2), 70.4 (Glc-4), 61.3 (Glc-6), 55.9 (OCH_3); ESIMS (positive) *m/z* 325 $[M+Na]^+$, 627 $[2M+H]^+$; ESIMS (negative) *m/z* 301 $[M–H]^-$, 603 $[2M–H]^-$.

Culture of RBL-2H3 cells. RBL-2H3 is a basophilic leukemia cell line. Cells were grown in minimum essential medium eagle (MEM) containing 15% fetal bovine serum (FBS), penicillin, streptomycin (Welgene, Daegu, Korea) and 2 mM glutamine at 37°C in 5% CO_2.

MTT assay for cell viability. Cell viability was assessed by 3-(4,5-dimethyl-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) colorimetric assay. RBL-2H3 cells were treated with MTT (5 mg/mL) in serum-free DMEM. After 3 h at 37°C and 5% CO_2 incubation, 100 μL of 100% DMSO was added to all wells to dissolve the insoluble purple formazan product into a colored solution. Absorbance was measured at 540 nm using an ELISA reader.

Inhibition of the release of β-hexosaminidase from RBL-2H3 cells. Before the assay, cells were dispensed into 24-well plates at 5×10^5 cells per well and cultured overnight. The media was changed, and cells were treated with 50 ng/mL of DNP-specific IgE. The cells were sensitized by incubation for 4 h at 37°C in 5% CO_2. The cells were washed twice with 500 μL piperazine-N,N-bis-(2-ethanesulfonic acid) (PIPES) buffer (25 mM PIPES, 119 mM NaCl, 5 mM KCl, 40 mM NaOH) and incubated in PIPES buffer containing 5.6 mM glucose, 1 mM $CaCl_2$ and 0.1% BSA for an additional 10 min at 37°C. The cells were then exposed to test materials for 20 min at 37°C and treated with 25 ng/mL of the antigen DNP-BSA for 30 min at 37°C to activate the cells. The supernatant was transferred to a 96-well plate and incubated with substrate (1 mM *p*-nitrophenyl-N-acetyl-β-D-glucosaminide) for 1 h at 37°C. The reaction was stopped by adding 0.1 M $Na_2CO_3/NaHCO_2$ or 2 M glycine. Absorbance was measured using an ELISA reader (SPECTRA MAX 340PC, Molecular Devices, Sunnyvale, CA) at 405 nm.

Results and Discussion

The structures of **1-9** were identified as 5-*n*-heptadecylresorcinol (**1**) (Iwatsuki et al., 2003), 5-*n*-14'-(*Z*)-heneicosylresorcinol (**2**) (Suzuki et al., 1997), 5-*n*-nonadecylresorcinol (**3**) (Ahn et al., 1996), 5-*n*-heneicosylresorcinol (**4**), 5-*n*-tricosylresorcinol (**5**), and 1-*O*-(9*Z*,12*Z*,15*Z*-octadecatrienoate) glycerol (**6**) (Jung et al., 2000), 2-linoleoylglycerol (**7**) (Swaroop et al., 2005), 1-*O*-(9*Z*,12*Z*-octadecatrienoate) glycerol (**8**) (Choi et al., 2004), and

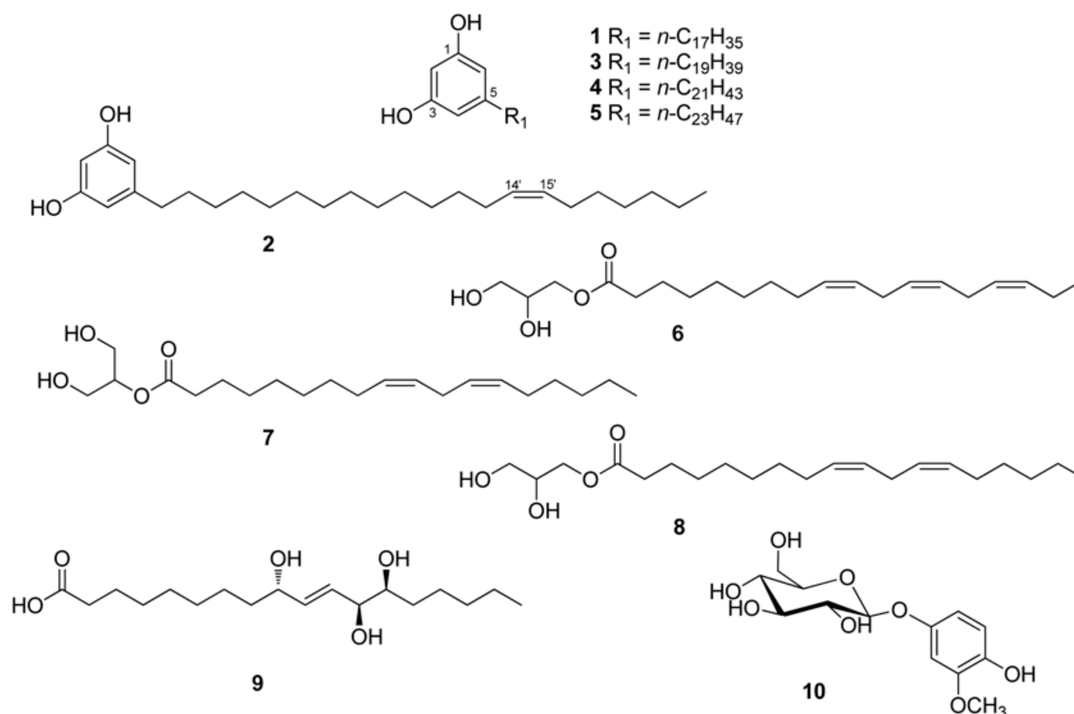

Fig. 1 Structures of compounds **1**–**10** from wheat bran.

pinellic acid (**9**) (Kim et al., 2009) by comparing of ^{1}H-, ^{13}C-NMR and MS data with the literature (Fig. 1).

Compound **10** was obtained as a whitish amorphous powder. The positive and negative ESI-MS showed quasimolecular ion peaks at *m/z* 325 $[M+Na]^+$ and 301 $[M–H]^-$, respectively, indicating a molecular weight of 302. According to the ESI-MS, ^{1}H- and ^{13}C-NMR spectral data, the molecular formula of **10** was determined to be $C_{13}H_{18}O_8$. The IR absorption at 3429 cm^{-1} for hydroxyl groups and 1597 cm^{-1} for an aromatic ring suggested that compound **10** was a phenolic compound, and the UV absorption also indicated the presence of an aromatic moiety (206 and 285 nm) (Chang and Inui, 2005). In addition, the IR spectral data of **10** suggested the presence of a glycosidic C–O (1070 and 1041 cm^{-1}) (Kim et al., 2004). The ^{1}H-NMR spectrum exhibited three aromatic proton resonances at δ_H 6.69 (1H, d, *J*=2.8 Hz), 6.66 (1H, d, *J*=8.8 Hz), and 7.46 (1H, dd, *J*=8.8, 2.8 Hz), which were indicative of a 1,3,4-trisubstituted phenyl ring. In addition, the ^{1}H-NMR spectrum showed signals assignable to a methyl ester group at δ_H 3.73. The β-glucosyl protons were at δ_H 4.67 (1H, d, *J*=7.6 Hz) for the anomeric proton, δ_H 3.71 (dd, *J*=11.6, 4.8 Hz) and 3.44 (1H, dd, *J*=11.6, 6.0 Hz) for the C-6' protons, appearing as a doublet of doublets, and between δ_H 3.1 to 3.4 as multiplets for the other sugar protons. Moreover, the presence of a glucopyranosyl moiety in compound **10** was confirmed by the ^{13}C-NMR spectrum, which showed six signals at δ_C 104.7 (C-1), 75.3 (C-2), 78.2 (C-3), 72.1 (C-4), 75.8 (C-5), and 65.4 (C-6). The glucose unit was determined to be attached to C-1 by the cross-peak in the HMBC spectrum between H-1' at δ 4.67 of the glucose unit with C-1 at δ_C 141.7 of the benzene ring. Thus, compound **10** was supposed to be a tachioside (4-hydroxy-3-methoxyphenyl-*O*-β-D-glucopyranoside) by comparing the spectral data with those reported in the literature (Inoshiri et al., 1987). To the best of our knowledge, a tachioside (**10**) was isolated from this plant for the first time in the present study. To identify compounds with inhibitory effects on mast cell degranulation, bioassay-guided separation was performed. The inhibitory effects of compounds **1**–**10** on the release of β-hexosaminidase from RBL-2H3 cells were evaluated. RBL-2H3 cells were incubated overnight in 24-well cluster plates with DNP-specific IgE. The medium was replaced with a PIPES buffer that contained the indicated concentration of isolated compounds, before stimulation with DNP-BSA, to measure the release of β-hexosaminidase. Antiallergic compounds, including ketotifen fumarate, caused inhibition, with IC_{50} values of 61.3 μM. Table 1 shows the inhibitory effects of 5-alk(en)ylresorcinol, aliphatic, and phenolic glycoside compounds isolated from wheat bran on antigen-induced β-hexosaminidase release from RBL-2H3 cells. All compounds, except 5-*n*-heptadecylresorcinol (**1**) and 5-*n*-tricosylresorcinol (**5**), inhibited the release of β-hexosaminidase, with IC_{50} values ranging between 87.2 and 173.6 μM. The inhibitory activity of pinellic acid (**9**) was the most potent among the constituents of wheat bran. Cell viability measured by the MTT assay showed that none of the tested compounds had any significant cytotoxicity to RBL-2H3 cells at concentrations that inhibited the release of β-hexosaminidase (data not shown).In conclusion, we found anti type I allergic activities of isolates from wheat bran that inhibited

Table 1 Inhibitory effects of constituents from wheat bran on the release of β-hexosaminidase from RBL-2H3 cells

	Inhibition (%)			
	25 μg/mL	50 μg/mL	100 μg/mL	IC_{50} μg/mL (μM)
5-*n*-Heptadecylresorcinol (**1**)	-16.8±2.7[a]	20.4±1.4	23.9±2.0	171.6 (493.2)
5-*n*-14'-(*Z*)-Heneicosylresorcinol (**2**)	45.3±7.0	66.5±2.5	87.3±2.2	43.0 (107.0)
5-*n*-Nonadecylresorcinol (**3**)	39.7±0.8	67.3±0.9	76.9±2.8	38.4 (102.0)
5-*n*-Heneicosylresorcinol (**4**)	20.9±1.5	67.4±2.3	88.9±2.4	40.6 (100.5)
5-*n*-Tricosylresorcinol (**5**)	-1.7±3.8	13.1±3.6	30.1±4.4	159.9 (370.3)
1-*O*-(9*Z*,12*Z*,15*Z*-octadecatrienoate) glycerol (**6**)	43.7±0.9	74.5±1.1	78.5±0.5	32.7 (92.9)
2-Linoleoylglycerol (**7**)	40.5±0.9	53.2±2.2	55.2±1.8	59.8 (168.9)
1-*O*-(9*Z*,12*Z*-octadecatrienoate) glycerol (**8**)	32.8±2.0	46.8±1.3	66.2±1.5	61.4 (173.6)
Pinellic acid (**9**)	46.2±2.3	71.1±1.7	93.5±3.4	28.7 (87.2)
Tachioside (**10**)	41.2±1.1	69.4±0.7	75.8±0.7	32.7 (108.5)
KF[b]	49.2±2.4	69.3±1.6	83.4±1.5	26.1 (61.3)

[a]Data are presented as a means ± SD of three separate experiments. [b]Ketotifen fumarate was used as the positive control (Morikawa et al., 2010).

the degranulation of mast cells. According to these results 5-alk(en)ylresorcinol, aliphatic derivatives, and phenolic glycoside isolated from wheat bran may prevent type I allergy associated with the release of β-hexosaminidase. However, further studies are needed to elucidate how these active compounds inhibit the release of β-hexosaminidase.

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