Bioorganic & Medicinal Chemistry 18 (2010) 7052–7057

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Chalcone glycosides isolated from aerial parts of *Brassica rapa* L. 'hidabeni' suppress antigen-stimulated degranulation in rat basophilic leukemia RBL-2H3 cells

Tomohiro Itoh [a], Masayuki Ninomiya [b], Yoshinori Nozawa [a,c], Mamoru Koketsu [b,*]

[a] Gifu International Institute of Biotechnology, 1-1 Naka-fudogaoka, Kakamigahara, Gifu 504-0838, Japan
[b] Department of Materials Science and Technology, Faculty of Engineering, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan
[c] Tokai Gakuin University, 1-1 Naka-Kirino, Kakamigahara, Gifu 504-8511, Japan

ARTICLE INFO

Article history:
Received 25 May 2010
Revised 3 August 2010
Accepted 4 August 2010
Available online 9 August 2010

Keywords:
Brassica rapa L. 'hidabeni'
Chalcone glycoside
Type I allergy
RBL-2H3 cells
NADPH oxidase

ABSTRACT

We isolated three chalcone glycosides along with other glycoside constituents from the aerial parts of *Brassica rapa* L. 'hidabeni' and examined the effects of these compounds on the antigen-stimulated degranulation in rat basophilic leukemia RBL-2H3 cells. Treatments with both 4′-*O*-β-D-glucopyranosyl-4-hydroxy-3′-methoxychalcone (**C1**) and 4′-*O*-β-D-glucopyranosyl-3′,4-dimethoxychalcone (**C2**) markedly inhibited antigen (Ag)-stimulated degranulation. To gain further insight into the inhibitory mechanisms by **C1** and **C2**, we examined early intracellular signaling events, Ca^{2+} mobilization and intracellular reactive oxygen species (ROS) production. Both **C1** and **C2** did not affect early intracellular signaling events but exhibited the suppression of intracellular ROS production through NADPH oxidase (NOX) inactivation. From these results, we proposed that the inhibitory effects of **C1** and **C2** on Ag-stimulated degranulation were mainly due to suppression of intracellular Ca^{2+} elevation by suppression of intracellular ROS production through NOX inactivation. Our findings suggest that **C1** and **C2** would be beneficial to alleviate symptoms of type I allergy.

1. Introduction

Brassica vegetables belong to the Cruciferous family, which are cultivated in many countries. Turnip (*Brassica rapa* L. var. *glabra* or *B. rapa* L. var. *rapa*), cabbage (*Brassica oleracea* L. var. *capitata*), brussels sprout (*B. oleracea* var. *gemmifera*), and broccoli (*B. oleracea* var. *italica*) are vested on this family. The chemical constituents of turnip are flavonoids, phenylpropanoids, norisoprenoids, and sulfur-containing compounds.[1–4] To date, it has been reported that turnip has anti-oxidant[5,6] and hepatoprotective effects.[7] *B. rapa* L. 'hidabeni', one of the popular Japanese turnip, is a kind of red turnip. And it is mainly raised and consumed as traditional vegetable in Gifu. Recently, our phytochemical investigations of the aerial parts of this plant resulted in the isolation of three chalcone glycosides, along with other glycoside constituents (Fig. 1).[8]

Type I allergy or immediate-type hypersensitivity is implicated in a various allergic symptoms including rhinitis, conjunctivitis, bronchial asthma, and urticaria. Mast cells are key effector cells that cause these allergic symptoms. High affinity IgE receptor (FcεRI) expresses the surface of mast cells and basophils, and immunoglobulin E (IgE) binds to FcεRIα chain. Cross-linking of IgE-mediated by the binding of multivalent antigen on the surfaces of mast cells induces the release of intragranular mediators such as histamine, arachidonic acid metabolites, proteases, serotonin, and heparin.[9–11] These mediators trigger immediate allergic responces.[12]

To date, several natural products inhibited Ag-stimulated degranulation in cellular system.[13–16] In this study, we also found that chalcone glycosides isolated from the aerial parts of *B. rapa* L. 'hidabeni' significantly suppressed Ag-stimulated degranulation. Although both treatments of **C1** and **C2** suppressed calcium (Ca^{2+}) mobilization and intracellular ROS production, these compounds did not affected early intracellular signaling events following Ag

Abbreviations: Ag, antigen; BSA, bovine serum albumin; C1, 4′-*O*-β-D-glucopyranosyl-4-hydroxy-3′-methoxychalcone; **C2**, 4′-*O*-β-D-glucopyranosyl-3′,4-dimethoxychalcone; **C3**, 4,4′-di-*O*-β-D-glucopyranosyl-3′-methoxychalcone; **CC**, corchoionoside C; $[Ca^{2+}]i$, intracellular free calcium concentration; CM-H_2DCF-DA, 5-(and-6-)-carboxy-2′,7′-dichlorodihidrofluorescein diacetate; DCF, 2′,7′-dichlorofluorescein; DCFH, 2′,7′-dichlorohydrofluorescein; DNP, dinitrophenol; DPPH, 1,1-diphenyl-2-picrylhydrazyl; DPI, diphenyleneiodonium chloride; Dscg, disodium cromoglycate; ERK, extracellular signal-regulated kinase; EGTA, *O,O*′-bis(2-aminoethyl)ethyleneglycol-*N,N,N*′,*N*′-tetraacetic acid; FcεRI, high affinity IgE receptor; GS, glucoacetosyringone; HRP, horseradish peroxidase; IgE, immunoglobulin E; SAPK/JNK, stress-activated protein kinase/c-jun-N-terminal kinase; Lyn, Src family protein kinase; MAPK, mitogen-activated protein kinase; NOX, NADPH oxidase; cPLA_2, cytosolic phospholipase A_2; PLC, phospholipase C; PN, picein; PVDF, polyvinylidene fluoride; Syk, spleen tyrosine kinase; ROS, reactive oxygen species.

* Corresponding author. Tel./fax: +81 58 293 2619.
E-mail address: koketsu@gifu-u.ac.jp (M. Koketsu).

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.08.008

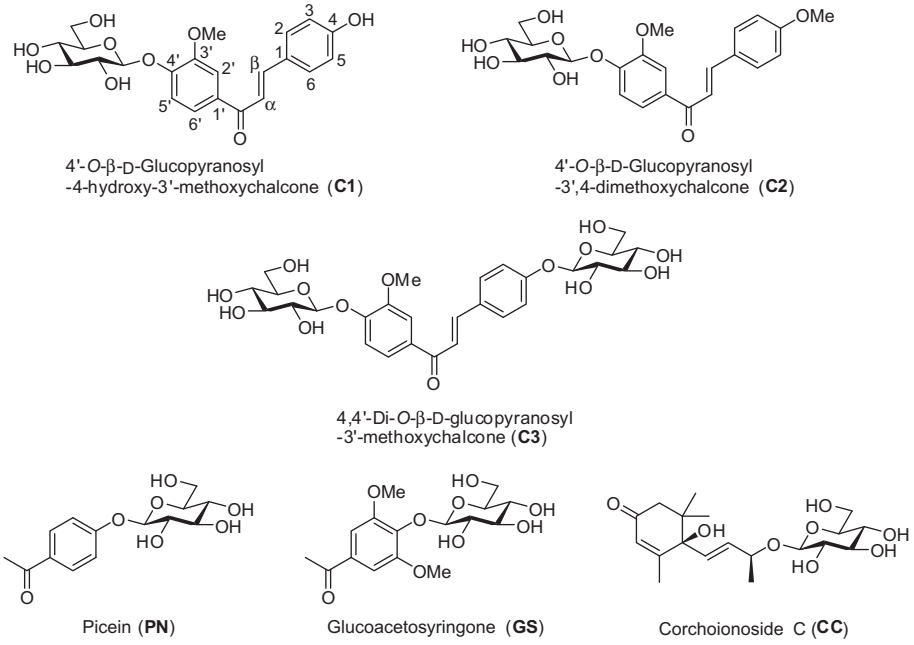

Figure 1. Structures of isolated chalcone glycosides and other glycosides from *Brassica rapa* L. 'hidabeni'.

stimulation. Here we proposed that the inhibitory mechanisms of Ag-stimulated degranulation by chalcone glycosides were mainly due to suppression of Ca^{2+} mobilization through the diminution of intracellular ROS production by NADPH oxidase (NOX) inactivation.

2. Results and discussion

2.1. Chemical structures of chalcone glycosides and other glycoside constituents

The structures of isolated compounds were elucidated on the basis of 1D-, 2D-NMR, and MS studies. Three compounds were chalcone glycosides which were 4′-*O*-β-D-glucopyranosyl-4-hydroxy-3′-methoxychalcone (**C1**), 4′-*O*-β-D-glucopyranosyl-3′,4-dimethoxychalcone (**C2**), and 4,4′-di-*O*-β-D-glucopyranosyl-3′-methoxychalcone (**C3**).[8] These compounds were 4′-glycosidized-3′-oxychalcones, the skeletons have been scarcely reported. The others were two phenolic glycosides (picein (**PN**) and glucoacetosyringone (**GS**)) and one roseoside (corchoionoside C (**CC**)).[17–19]

2.2. Chalcone glycosides suppress antigen-stimulated degranulation in RBL-2H3 cells

In type I allergy, the binding of antigen to the high affinity immunoglobulin E (IgE) receptor (FcεRI) on the surface of mast cells and basophils is the first event leading to the release of chemical mediators such as histamine, arachidonic acid metabolites, and cytokines which mainly cause asthmatic and inflammatory responses.[9–11] Firstly, we examined three chalcone glycosides (**C1**, **C2**, and **C3**), two phenolic glycosides (**PN** and **GS**) and one roseoside (**CC**) isolated from *B. rapa* L. 'hidabeni' for their effects on β-hexosaminidase release in vitro. Among them, **C1** and **C2** significantly suppressed Ag-stimulated degranulation in a dose-dependent manner (Fig. 2).

In an attempt to reveal the mechanisms underlying the inhibitory effects of **C1** and **C2** on Ag-stimulated degranulation in RBL-2H3 cells, we examined early intracellular signaling events following Ag stimulation. Data presented in Figure 3 show that

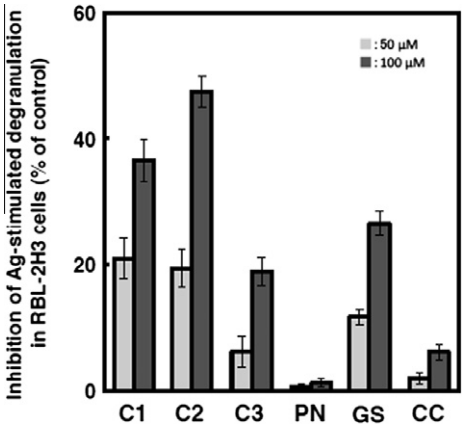

Figure 2. Effects of **C1**, **C2**, **C3**, **PN**, **GS**, and **CC** isolated from *B. rapa* L. 'hidabeni' on Ag-stimulated degranulation in RBL-2H3 cells. IgE-sensitized RBL-2H3 cells were treated with each compound (50 or 100 μM) and stimulated with antigen (DNP–BSA), and then subjected to β-hexosaminidase release assays. Values are mean ± SEM (n = 10).

both **C1** and **C2** did not affect IgE-mediated multiple signal transduction pathways, which play a crucial role in degranulation. Next, we monitored the $[Ca^{2+}]_i$ mobilization following Ag stimulation that is activated as a downstream event by Ag-induced early intracellular signaling. As shown in Figure 4, the levels of $[Ca^{2+}]_i$ immediately increased by Ag challenge. However, both treatments with **C1** and **C2** suppressed that $[Ca^{2+}]_i$ elevation. Especially, **C2** completely abolished Ag-stimulated elevation of $[Ca^{2+}]_i$. It is well known that the elevation of $[Ca^{2+}]_i$ following Ag stimulation is regulated by intracellular ROS that is generated by NADPH oxidase.[20] We further measured the levels of intracellular ROS following Ag stimulation. Both **C1** and **C2** suppressed Ag-stimulated ROS production (Fig. 5A). However, the suppression of ROS production following Ag stimulation by **C1** and **C2** were not due to their radical scavenging activities (Fig. 5B).

Treatment with DPI, a potent inhibitor of NADPH oxidase (NOX) abolished ROS production after Ag stimulation.[21,22] NOX is an enzyme complex composed of membrane-bound subunits ($gp91^{phox}$

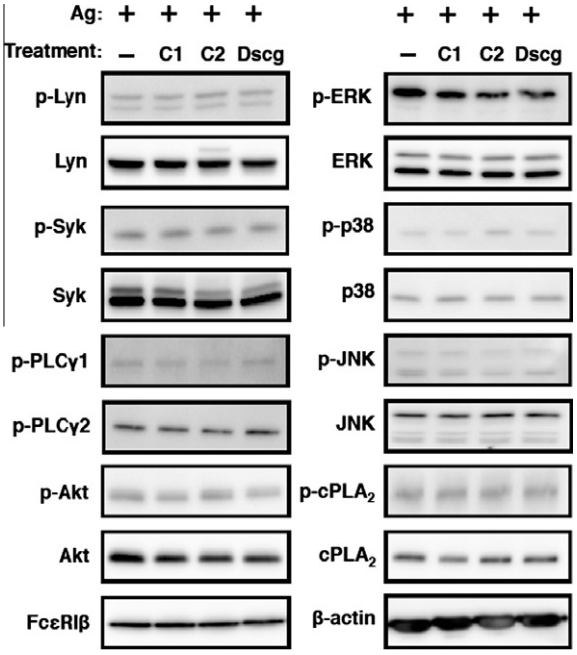

Figure 3. Effects of **C1** and **C2** on Ag-stimulated activation of intracellular signaling pathways in RBL-2H3 cells. IgE-sensitized RBL-2H3 cells were treated with or without 50 μM of **C1** or **C2** for 30 min. The cells were then stimulated with or without DNP–BSA. Cell lysates were subjected to Western blot analysis for the indicated proteins. A representative blot from three independent experiments is shown.

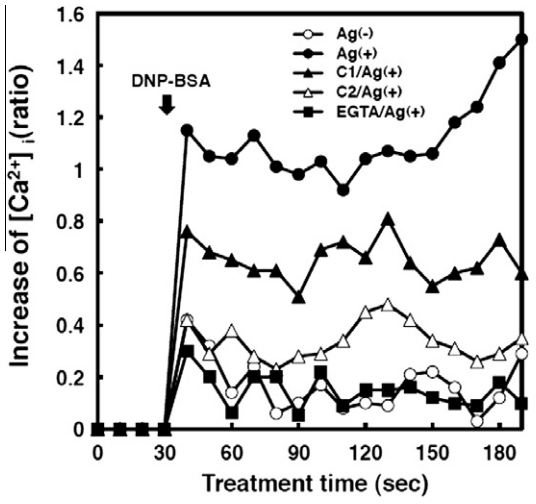

Figure 4. Effects of **C1** and **C2** on Ag-stimulated elevation of intracellular $[Ca^{2+}]_i$ in RBL-2H3 cells. IgE-sensitized RBL-2H3 cells were treated with or without 50 μM of **C1** or **C2** for 30 min. The cells were then stimulated with or without DNP–BSA, after which intracellular Ca^{2+} levels were measured. Values are mean ± SEM ($n = 12$). Arrow: time at which DNP–BSA was added, filled circle: Ag-treated cells, open circle: non-Ag-treated cells, filled triangle: Ag- and **C1**-treated cells, open triangle: filled square: Ag- and **C2**-treated cells, filled square: Ag- and EGTA-treated cells.

and p22^{phox}), cytosolic subunits (p40^{phox}, p47^{phox}, and p67^{phox}) and a monomeric GTP binding protein of Rho family Rac2.[23] Upon activation, the cytosolic subunits of NOX are translocated to the membrane, initiating the production of superoxide, which is readily converted to hydrogen peroxide.[24] Both treatments with **C1** and **C2** inhibit the translocation of all cytosolic subunits (Fig. 5C). We further observed the translocation of p47^{phox} cytosolic subunits using an immunofluorescence technique. Both **C1** and **C2** certainly inhibited the translocation of p47^{phox} cytosolic subunits (Fig. 5D). These results supporting that suppression of ROS production by **C1** and **C2** were due to their direct NOX inactivation.

Taken together, our findings suggest that the inhibitory effects of **C1** and **C2** isolated from *B. rapa* L. 'hidabeni', on Ag-stimulated degranulation are mainly due to the suppression of Ca^{2+} mobilization through the inactivation of NOX (Fig. 6). From these our findings, **C1** and **C2** would be candidates for alleviating type I allergy.

3. Materials and methods

3.1. Extraction and isolation procedures of chemical constituents

B. rapa L. 'hidabeni' was grown in Takayama City, Gifu, bought from HIDANOUSAN Co., Ltd of Hida City, Gifu, in 2008. The fresh aerial parts of *B. rapa* L. 'hidabeni' (23.2 kg) were extracted with methanol at room temperature. On removal of the solvent under reduced pressure, the methanol extract (400 g) was partitioned first with *n*-hexane, then with ethyl acetate, and finally with *n*-butanol. The *n*-butanol-soluble phase (34 g) of the aerial parts of *B. rapa* L. 'hidabeni' was separated by column chromatographies (Sephadex LH-20, Diaion HP-20, and silica gel), preparative thin-layer chromatography (PTLC) on silica gel, and reverse-phase (RP) high-performance liquid chromatography (HPLC) to give six compounds. Detail isolation procedure was carried out as described previously.[8] The spectral data of compounds **C1**, **C2**, and **C3** were mentioned in the literature.[8]

3.1.1. Picein (PN)

White amorphous powder, $[\alpha]_D^{25}$ −58.9 ($c = 0.10$, MeOH), IR (film): 3368, 1652 cm^{-1}. ESIMS: m/z 321 [M+Na]$^+$. UV (MeOH) λ_{max} (log ε): 272 (4.1), 269, 257 nm. ^{1}H NMR (600 MHz, CD_3OD): δ 7.95 (2H, d, $J = 8.7$ Hz, H-3 and H-5), 7.14 (2H, d, $J = 8.7$ Hz, H-2 and H-6), 5.01 (1H, d, $J = 7.8$ Hz, H-1′), 3.88 (1H, dd, $J = 11.9$, 2.2 Hz, H-6′α), 3.68 (1H, dd, $J = 11.9$, 5.5 Hz, H-6′β), 3.51–3.44 (3H, m, H-2′, H-3′, and H-5′), 3.42–3.35 (1H, m, H-4′), 2.54 (3H, s, 4-COCH_3). ^{13}C NMR (150 MHz, CD_3OD): δ 198.1 (C=O), 161.7 (C-1), 131.3 (C-4), 130.3 (C-3 and C-5), 115.9 (C-2 and C-6), 100.2 (C-1′), 77.0 (C-5′), 76.6 (C-3′), 73.5 (C-2′), 69.9 (C-4′), 61.1 (C-6′), 25.1 (4-COCH$_3$).

3.1.2. Glucoacetosyringone (GS)

White amorphous powder, $[\alpha]_D^{25}$ −3.9 ($c = 0.10$, MeOH), IR (film): 3381, 1640 cm^{-1}. ESIMS: m/z 382 [M+Na]$^+$, 739 [2M+Na]$^+$. UV (MeOH) λ_{max} (log ε): 276 (4.1) nm. ^{1}H NMR (600 MHz, CD_3OD): δ 7.30 (2H, s, H-3 and H-5), 5.08 (1H, d, $J = 7.5$ Hz, H-1′), 3.89 (6H, s, 2- and 6-OMe), 3.74 (1H, dd, $J = 12.3$, 2.7 Hz, H-6′α), 3.62 (1H, dd, $J = 12.3$, 5.5 Hz, H-6′β), 3.47 (1H, t, $J = 7.5$ Hz, H-2′), 3.42–3.36 (2H, m, H-3′ and H-4′), 3.20 (1H, dq, $J = 5.5$, 2.8 Hz, C-5′), 2.57 (3H, s, 4-COCH_3). ^{13}C NMR (150 MHz, CD_3OD): δ 198.0 (C=O), 152.9 (C-2 and C-6), 139.3 (C-1), 133.0 (C-4), 106.3 (C-3 and C-5), 103.1 (C-1′), 77.1 (C-5′), 76.5 (C-3′), 74.4 (C-2′), 70.0 (C-4′), 61.2 (C-6′), 55.8 (2- and 6-OMe), 25.2 (4-COCH$_3$).

3.1.3. Corchoionoside C (CC)

White amorphous powder, $[\alpha]_D^{25}$ −87.4 ($c = 0.10$, MeOH), IR (film): 3382, 1649 cm^{-1}. ESIMS: m/z 409 [M+Na]$^+$, 795 [2M+Na]$^+$. UV (MeOH) λ_{max} (log ε): 241, 236 (4.0), 230 nm. ^{1}H NMR (600 MHz, CD_3OD): δ 6.06 (1H, d, $J = 15.1$ Hz, H-7), 5.80 (1H, dd, $J = 15.1$, 6.9 Hz, H-8), 5.78 (1H, d, $J = 1.4$ Hz, H-4), 4.52 (1H, quint, $J = 6.9$ Hz, H-9), 4.32 (1H, d, $J = 8.3$ Hz, H-1′), 4.18 (1H, br s, 6-OH), 3.81 (1H, d, $J = 8.9$ Hz, H-6′α), 3.62 (1H, dd, $J = 11.7$, 5.5 Hz, H-6′β), 3.35–3.29 (2H, m, H-3′ and H-4′), 3.22–3.17 (2H, m, H-2′ and H-5′), 2.58 (1H, d, $J = 16.5$ Hz, H-2α), 2.12 (1H, d, $J = 16.5$ Hz, H-2β), 1.92 (3H, d, $J = 1.4$ Hz, 13-Me), 1.23 (3H, d, $J = 6.9$ Hz, 10-

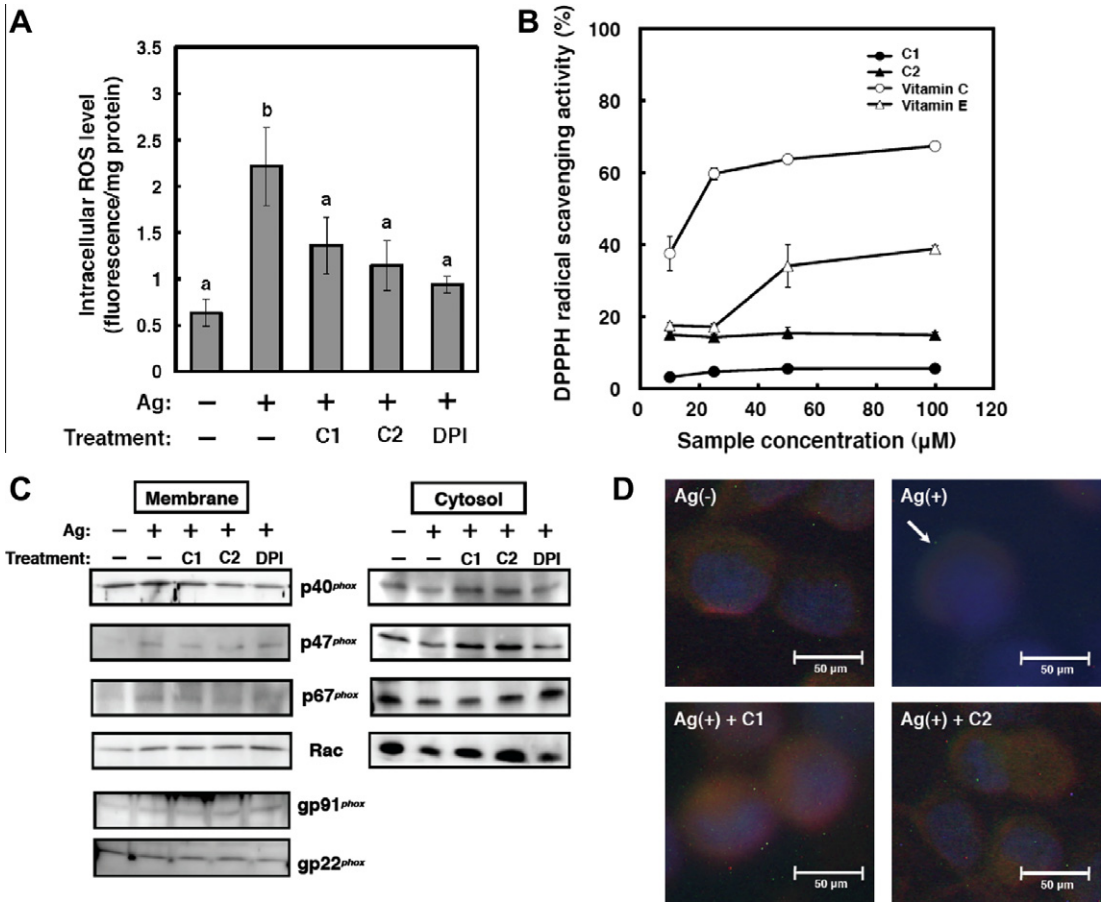

Figure 5. Effects of **C1** and **C2** on the intracellular ROS production in Ag-stimulated RBL-2H3 cells. (A) Suppressions of intracellular ROS production by **C1** and **C2** in Ag-stimulated mast cell. These data expressed 10 min after Ag stimulation. Means values with different letters are significantly different (p <0.05, one-way analysis of variance followed by Fisher's multiple range test). (B) The radical scavenging activity of **C1** and **C2**. Vitamins C and E were used as positive control. All data are expressed as mean ± SEM (n = 12) of the inhibition of DPPH radical (% inhibition). (C) Inhibitory effect of **C1** and **C2** on translocation of cytosolic subunits of NADPH oxidase (p40^{phox}, p47^{phox}, p67^{phox}, and Rac) to the membrane. A representative blot from three independent experiments is shown. (D) Immunofluorescence analysis of the translocation to the membrane of p47^{phox} cytosolic subunits in Ag-stimulated RBL-2H3 cells. Blue (Hoechst33342): nuclei, Red (Cell Mask Orange plasma membrane): cell membrane, Green (Alexa Fluor-488 Rabbit IgG): p47^{phox} cytosolic subunits. p47^{phox} cytosolic subunit was translocated following Ag stimulation (white arrow). Photographs shown represent three independent experiments.

Me), 1.04 (3H, s, 12-Me), 1.02 (3H, s, 11-Me). ^{13}C NMR (150 MHz, CD_3OD): δ 196.8 (C-3), 163.0 (C-5), 132.3 (C-7), 132.2 (C-8), 126.2 (C-4), 100.3 (C-1′), 78.7 (C-6), 77.5 (C-3′), 76.8 (C-2′), 74.0 (C-5′), 72.9 (C-9), 70.9 (C-4′), 62.2 (C-6′), 49.6 (C-2), 41.0 (C-1), 23.8 (C-12), 22.7 (C-11), 20.7 (C-10), 18.3 (C-13).

3.2. Bioassay methods

3.2.1. Reagents and materials

Mouse anti-dinitrophenol (DNP) monoclonal IgE was purchased from Yamasa (Tokyo, Japan). The 25× Complete®, a mixture of protease inhibitors, was from Roche (Penzberg, Germany). The phosphatase Inhibitor Cocktail® 1 and 2 were from Sigma (MO, USA). The antibodies to Akt, phospho-Akt, p44/42 MAP kinase (ERK), phospho-p44/42 MAP kinase (Thr202/Tyr204) (p-ERK), SAPK/JNK (JNK), phospho-SAPK/JNK (Thr183/Tyr185) (p-JNK), p38 MAP kinase (p38), phospho-p38 MAP kinase (Thr180/Tyr182) (p-p38), Lyn, phospho-Lyn, cPLA$_2$, phospho-cPLA$_2$, phospho-PLCγ1, phospho-PLCγ2, and phospho-Syk were from Cell Signaling Technology (MA, USA). The antibody to Syk was from Santa Cruz Biotechnology (CA, USA). Anti-β-actin antibody was from Sigma (MO, USA). The FcεRIβ antibody was kindly provided by Dr. J. Rivera (NIH, Molecular Immunology and Inflammation Branch). Anti-rabbit and -mouse antibodies conjugated with horseradish peroxidase and the ECL chemiluminescence kit were obtained from GE Healthcare (NJ, USA).

3.2.2. Cell culture

RBL-2H3 cells were obtained from Health Science Research Resource Bank (Tokyo, Japan). Cells were grown in Eagle's minimum essential medium (Gibco, MD, USA) containing 10% heat-inactivated fetal bovine serum, 100 U/ml of penicillin and 100 μg/ml of streptomycin in a humidified atmosphere of 5% CO_2 at 37 °C.

3.2.3. Degranulation assay

We used β-hexosaminidase as a marker of degranulation. Protocol of β-hexosaminidase release assay is described in a previous report.[25]

3.2.4. Measurement of intracellular Ca^{2+} concentrations

Intracellular Ca^{2+} levels were determined with Calcium Kit-Fluo 3™ (Dojindo Laboratories, Kumamoto, Japan). RBL-2H3 cells (5×10^4 cells/well) were seeded onto 96-well microplates and incubated for 1 h. Then, cells were treated with anti-DNP IgE (0.45 μg/ml) and incubated for 24 h. After washing twice with PBS, 100 μl of loading buffer containing Fluo-3AM (Calcium Kit-Fluo 3™) was added to culture medium. One hour later, cells were washed with PBS and incubated in 90 μl of loading buffer contain-

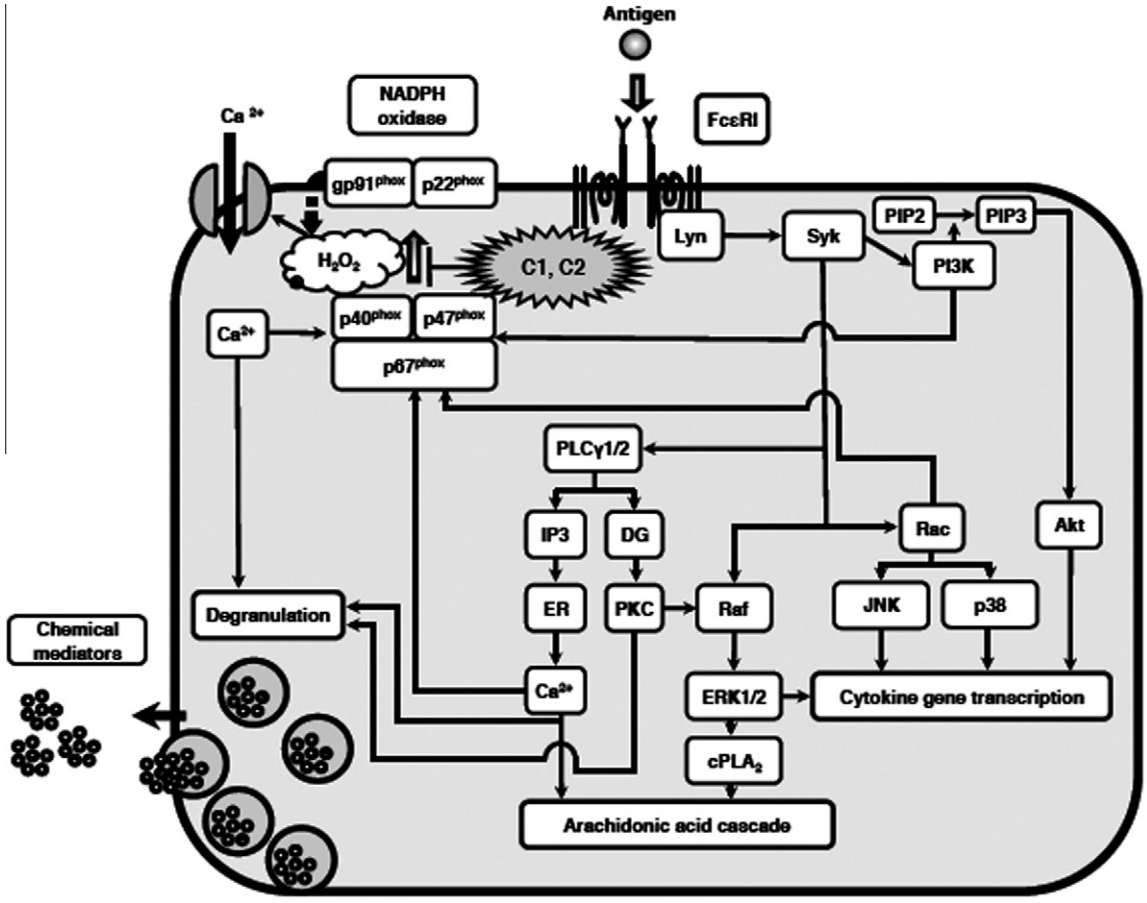

Figure 6. A scheme showing the inhibitory effects of **C1** and **C2** on Ag-stimulated degranulation in RBL-2H3 cells. **C1**and **C2** treatments inhibit Ag-stimulated degranulation through the inhibition of NOX.

ing 50 μM of sample. After incubation for 30 min, cells were stimulated by DNP–BSA (10 μg/ml) and the fluorescence was measured using the fluorometric imaging plate reader (excitation; 490 nm, emission; 530 nm).

3.2.5. Measurement of intracellular ROS levels

Intracellular ROS levels were measured using a CM-H_2DCF-DA. IgE-sensitized RBL-2H3 cells (5×10^4 cells/well) were incubated with 10 μM CM-H_2DCF-DA for 30 min at 37 °C. After washing twice, cells were incubated with 50 μM of sample for 30 min. Then, cells were stimulated by DNP–BSA (10 μg/ml) and the fluorescence was measured using the fluorometric imaging plate reader (excitation; 490 nm, emission; 530 nm).

3.2.6. Measurement of DPPH radical scavenging activity

To measure in vitro antioxidant activity, DPPH radical scavenging assay was carried out as described previously.[26] Briefly, 0.1 ml of 0.5 mM DPPH radical solution, 0.8 ml of 99% ethanol and 0.1 ml of sample were rapidly mixed and the decrease in absorbance at 517 nm was monitored. DPPH free radical scavenging activity (%) was calculated using the following formula: $[(A_{517\ nm\ of\ control} - A_{517\ nm\ of\ sample})/A_{517\ nm\ of\ control}] \times 100$. Vitamin C, E, and Trolox, potent antioxidants, were used as positive controls.

3.2.7. Immunoblot analysis

Cell lysates were prepared as described previously.[27] Protein samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE), and then transferred onto PVDF membranes. After blocking for 1 h in 5% nonfat milk, the membrane was incubated with a primary antibody at 4 °C overnight, followed by incubation with a horseradish peroxidase-conjugated secondary antibody at room temperature for 1 h. Immunoreactive proteins were detected with the enhanced ECL kit and the chemiluminescence detector (LAS-4000, Fujifilm, Japan).

3.2.8. Immunofluorescence staining

The cells were washed twice with PBS and then fixed with 4% paraformaldehyde for 15 min at room temperature. Fixed cells were washed twice with PBS containing 10 mM glycine (PBS-G) and then treated with PBS containing 0.1% Triton X-100 (Sigma) (PBS-T) for 5 min at room temperature. Subsequently, the cells were blocked with 3% BSA for 10 min at room temperature. After incubation, treated cells were incubated with the primary antibody (anti-$p47^{phox}$), which was diluted with PBS-G for 1 h at room temperature. After washing with PBS-(−) containing 0.1% BSA, cells were incubated with secondary antibody (Alexa Fluor-488 Rabbit IgG, Invitrogen) for 30 min at room temperature. The nuclei and cell membranes of treated cells were further stained with Hoechst33342 (Invitrogen) and Cell Mask Orange plasma membrane stain solution (Invitrogen) for 30 min. The cells were mounted with a drop of mounting medium (Dako cytomation fluorescent mounting medium, Dako, CA, USA) and then sealed with a coverslip. Photomicrographs of mounted cells were taken with a fluorescent microscope (KEYENCE BZ-8000, Osaka, Japan).

4. Conclusions

In present study, we performed a chemical study of *B. rapa* L. 'hidabeni', and identified three chalcone glycosides along with

other glycoside constituents. We demonstrated the inhibitory effects of 4′-*O*-β-D-glucopyranosyl-4-hydroxy-3′-methoxychalcone (**C1**) and 4′-*O*-β-D-glucopyranosyl-3′,4-dimethoxychalcone (**C2**) on antigen (Ag)-stimulated degranulation in rat basophilic leukemia RBL-2H3 cells. The inhibition of Ag-mediated degranulation by **C1** and **C2** was mainly due to inactivation of NOX. Our findings suggest that **C1** and **C2** isolated from *B. rapa* L. 'hidabeni' would be effective agents for alleviating allergic symptoms.

Acknowledgment

The authors thank Dr. Juan Rivera (NIH, molecular immunology and inflammation branch) for providing FcεRIβ antibody.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.08.008. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Sultana, B.; Anwar, F. *Food Chem.* **2008**, *108*, 879.
2. Sousa, C.; Taveira, M.; Valentão, P.; Fernandes, F.; Pereira, J. A.; Estevinho, L.; Bento, A.; Ferreres, F.; Seabra, R. M.; Andrade, P. B. *Food Chem.* **2008**, *110*, 953.
3. Taveira, M.; Fernandes, F.; de Pinho, P. G.; Andrade, P. B.; Pereira, J. A.; Valentão, P. *Microchem. J.* **2009**, *93*, 140.
4. Francisco, M.; Moreno, D. A.; Cartea, M. E.; Ferreres, F.; García-Viguera, C.; Velasco, P. *J. Chromatogr., A* **2009**, *1216*, 6611.
5. Fernandes, F.; Valentão, P.; Sousa, C.; Pereira, J. A.; Seabra, R. M.; Andrade, P. B. *Food Chem.* **2007**, *105*, 1003.
6. Pereira, D. M.; Noites, A.; Valentão, P.; Ferreres, F.; Pereira, J. A.; Vale-Silva, L.; Pinto, E.; Andrade, P. B. *J. Agric. Food Chem.* **2009**, *57*, 483.
7. Choi, H. J.; Han, M. J.; Baek, N. I.; Kim, D. H.; Jung, H. G.; Kim, N. J. *Saengyak Hakhoechi* **2006**, *37*, 258.
8. Ninomiya, M.; Efdi, M.; Inuzuka, T.; Koketsu, M. *Phytochem. Lett.* **2010**, *3*, 96.
9. Beaven, M. A.; Metzger, H. *Immunol. Today* **1993**, *14*, 222.
10. Turner, H.; Kinet, J. P. *Nature* **1999**, *402*, 24.
11. Mekori, Y. A.; Metcalfe, D. D. *Immunol. Rev.* **2000**, *173*, 131.
12. Metcalfe, D. D.; Baram, D.; Mekori, Y. A. *Physiol. Rev.* **1997**, *77*, 1033.
13. Matsuda, H.; Tewtrakul, S.; Morikawa, T.; Yoshikawa, M. *Bioorg. Med. Chem.* **2004**, *12*, 4871.
14. Watanabe, J.; Shinmoto, H.; Tsushida, T. *Biosci., Biotechnol., Biochem.* **2005**, *69*, 1.
15. Han, E. H.; Park, J. H.; Kim, J. Y.; Chung, Y. C.; Jeong, H. G. *Food Chem. Toxicol.* **2009**, *47*, 1069.
16. Oka, T.; Fujimoto, M.; Nagasaka, R.; Ushio, H.; Hori, M.; Ozaki, H. *Phytomedicine* **2010**, *17*, 152.
17. Dommisse, R. A.; van Hoof, L.; Vlietinck, A. J. *Phytochemistry* **1986**, *25*, 1201.
18. Ina, H.; Yamauchi, M.; Iida, H. *Planta Med.* **1983**, *47*, 253.
19. Achenbach, H.; Waibel, R.; Raffelsberger, B.; Addae-Mensah, I. *Phytochemistry* **1981**, *20*, 1591.
20. Yoshimaru, T.; Suzuki, Y.; Matsui, T.; Yamashita, K.; Ochiai, T.; Yamaki, M.; Shimizu, K. *Clin. Exp. Allergy* **2002**, *32*, 612.
21. Suzuki, Y.; Yoshimaru, T.; Matsui, T.; Inoue, T.; Niide, O.; Nunomura, S.; Ra, C. *J. Immunol.* **2003**, *171*, 6116.
22. Swindle, E. J.; Metcalfe, D. D. *Immunol. Rev.* **2007**, *217*, 186.
23. Sarfstein, R.; Gorzalczany, Y.; Mizrahi, A.; Berdichevsky, Y.; Molshanski-Mor, S.; Weinbaum, C.; Hirshberg, M.; Dagher, M. C.; Pick, E. *J. Biol. Chem.* **2004**, *279*, 16007.
24. Bedard, K.; Krause, K. H. *Physiol. Rev.* **2007**, *87*, 245.
25. Itoh, T.; Oyama, M.; Takimoto, N.; Kato, C.; Nozawa, Y.; Akao, Y.; Iinuma, M. *Bioorg. Med. Chem.* **2008**, *17*, 3189.
26. Itoh, T.; Umekawa, H.; Furuichi, Y. *Biosci., Biotechnol., Biochem.* **2005**, *69*, 448.
27. Itoh, T.; Ohguchi, K.; Iinuma, M.; Nozawa, Y.; Akao, Y. *Bioorg. Med. Chem.* **2008**, *16*, 4500.