Studies on the Constituents of Whole Plants of *Youngia japonica*

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Two new guaiane-type sesquiterpene (1, 2), 2 new phenylpropanoid derivatives (3, 4), and 5-oxo-11-hydroxy-8(*Z*)-undecenoic acid 11-*O*-glucoside (5), together with 17 known compounds have been isolated from the whole plants of *Youngia japonica* (L.) DC., which have been known to be used as folk medicines to treat people suffering from atopy. The guaiane-type sesquiterpene, grosheimin (17) exhibited strong antiallergic and antioxidant activities.

Key words *Youngia japonica*; antiallergic; guaiane; phenylpropanoid

Youngia japonica (L.) DC. belongs to the Compositae family. This plant, which is distributed throughout the temperate zone, is used as an antipyretic and for detoxification. Moreover, in the Kyushu area, this plant is used as a folk medicine for the treatment of atopy. Investigating the constituents of this plant, Miyase *et al.* reported the isolation and chemical structural determination of guaiane-type sesquiterpene glycosides[1] from the *n*-BuOH fraction of its methanolic extract. We have achieved the isolation of antiallergic compounds from the water-soluble fraction of the methanolic extract, which we examine in detail.

The whole plants of *Youngia japonica* (1230.0 g), which were collected from around Kumamoto University, were extracted with refluxing MeOH to obtain the extract (133.0 g), which was treated with water to obtain the water-soluble portion (107.0 g) and the insoluble portion (26.0 g). The former was passed through highly porous polystyrene gel eluted with water, 40% MeOH, 60% MeOH, 80% MeOH, 100% MeOH, successively, to afford the water eluate (93.0 g) and ten fractions. The fractions were repeatedly column-chromatographed on silica gel and reversed silica gel to obtain 22 compounds. The following compounds were obtained from the fractions: **12** (960 mg) and **4** (240 mg) from fraction 1 (2.3 g), **2** (9.3 mg) from fraction 2 (1.8 g), **9** (19.8 mg), **22** (13.2 mg), and **1** (9.0 mg) from fraction 4 (855.7 mg), **20** (1.6 mg), **13** (7.3 mg), **1** (6.0 mg), **19** (9.3 mg), and **5** (5.3 mg) from fraction 5 (396.7 mg), **8** (14.8 mg), **21** (51.2 mg), **12** (14.4 mg), and **7** (180.8 mg) from fraction 6 (2.5 g), **6** (309.0 mg), **15** (46.6 mg), **10** (36.4 mg), **7** (10.5 mg), **11** (19.0 mg), and **3** (61.6 mg) from fraction 7 (1.9 g), **17** (44.8 mg), **14** (3.7 mg), **16** (57.2 mg), and **18** (28.5 mg) from fraction 8 (1.9 g). Further, **6**, **7**, **8**, **10**, **11**, **9**, **15**, **16**, **17**, **18**, **19**, **13**, **12**, **14**, **20**, **21**, and **22** were identified respectively as 1) three flavonoids: luteolin 7-*O*-*β*-D-glucopyranoside,[2] luteolin 7-*O*-*β*-D-glucuronopyranoside,[3] luteolin 7-*O*-*β*-D-glucuronopyranoside 6″-*O*-methyl ester[4]; 2) three quinic acid derivatives: quinic acid 3,4-dicaffeate,[2] quinic acid 3,5-dicaffeate,[2] chlorogenic acid[5]; 3) five guaiane-type sesquiterpenoids[1]: 8-epidesacylcynaropicrin-*β*-D-glucopyranoside, glucozaluzanin, grosheimin, crepiside I, crepiside D; 4) three phenylpropanoid derivatives: dihydrosyringenin 9-*O*-*β*-D-glucopyranoside,[6] chicoric acid,[7] (*dl*)-syringaresinol-*β*-D-glucopyranoside[8]; 5) two benzyl alcohol glycosides: benzyl alcohol 7-*O*-*β*-D-glucopyranoside[9] and benzyl alcohol 7-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside; and 6) triptophan. Among these compounds the following five were previously known sesquiterpenes: **15** (8-epidesacylcynaropicrin *β*-D-glucopyranoside), **16** (glucozalzanin C), **17** (grosheimin), **18** (crepiside I), **19** (crepiside D); three known aromatics: **6** (luteolin 7-*O*-*β*-D-glucopyranoside), **10** (quinic acid 3,4-dicaffeate), **11** (quinic acid 3,5-dicaffeate). The remaining 9 compounds were isolated for the first time from *Youngia japonica*, as shown in Fig. 1.

Next, we deal with the structures of five of the new compounds. Compound **1**, which was obtained as a white powder showing $[\alpha]_D$ −12.6° (CH_3OH), was found to have the molecular formula $C_{21}H_{33}O_9$ at *m*/*z* 429.2146 in the positive HR-FAB-MS. The ^{1}H-NMR spectrum exhibited signals due to two secondary methyl groups at δ 0.97 (3H, d, J=6.7 Hz) and 1.34 (3H, d, J=7.3 Hz), two vinyl exo-methylene protons at δ 5.06, 5.13 (each 1H, br s) and three oxygenated methine protons at δ 4.33 (1H, m), 4.14 (1H, dd, J=9.8, 11.6 Hz) and 3.58 (1H, ddd, J=4.9, 10.4, 10.4 Hz). The ^{13}C-NMR spectrum showed two secondary methyl groups at δ 8.8 and 16.3; two methylene carbons at δ 33.9, 50.9; three oxygen-bearing methine carbons at δ 76.5, 80.1, 80.4; five methine carbons at δ 38.9, 41.7, 42.9, 48.2, 58.2; two olefinic carbons at δ 114.1 and 145.4; and a carbonyl carbon at δ 181.7, together with a *β*-D-glucopyranosyl moiety at δ 103.3, 74.2, 75.4, 71.8, 78.1, 62.9. Based on the fact that **1** comprised of two secondary methyl groups, one lactone ring, one vinyl exo-methylene group and two oxygen-bearing methine carbons, **1** was estimated to be a guaiane-type sesquiterpene. The following heteronuclear multiple bond correlation (HMBC) characterized the guaiane skeleton, the location of the function groups, and the substitutions: from H-1 (m, δ 2.79) to C-6 (δ 80.1), C-2 (δ 33.9), C-5 (δ 48.2), C-10 (δ 145.4), C-14 (δ 114.1); from Ha-2 (m, δ 2.13) to C-1 (δ 41.7), C-3 (δ 80.4), C-10; from Hb-2 (m, δ 2.01) to C-1, C-5, C-10; from H-3 (m, δ 4.33) to C-15 (δ 8.8); from H-4 (m, δ 2.46) to C-15, C-2, C-1, C-3; from H-5 (m, δ 2.31) to C-15, C-4 (δ 38.9), C-1, C-6 (δ 80.1), C-3, C-10; from H-6 (dd, J=9.8,

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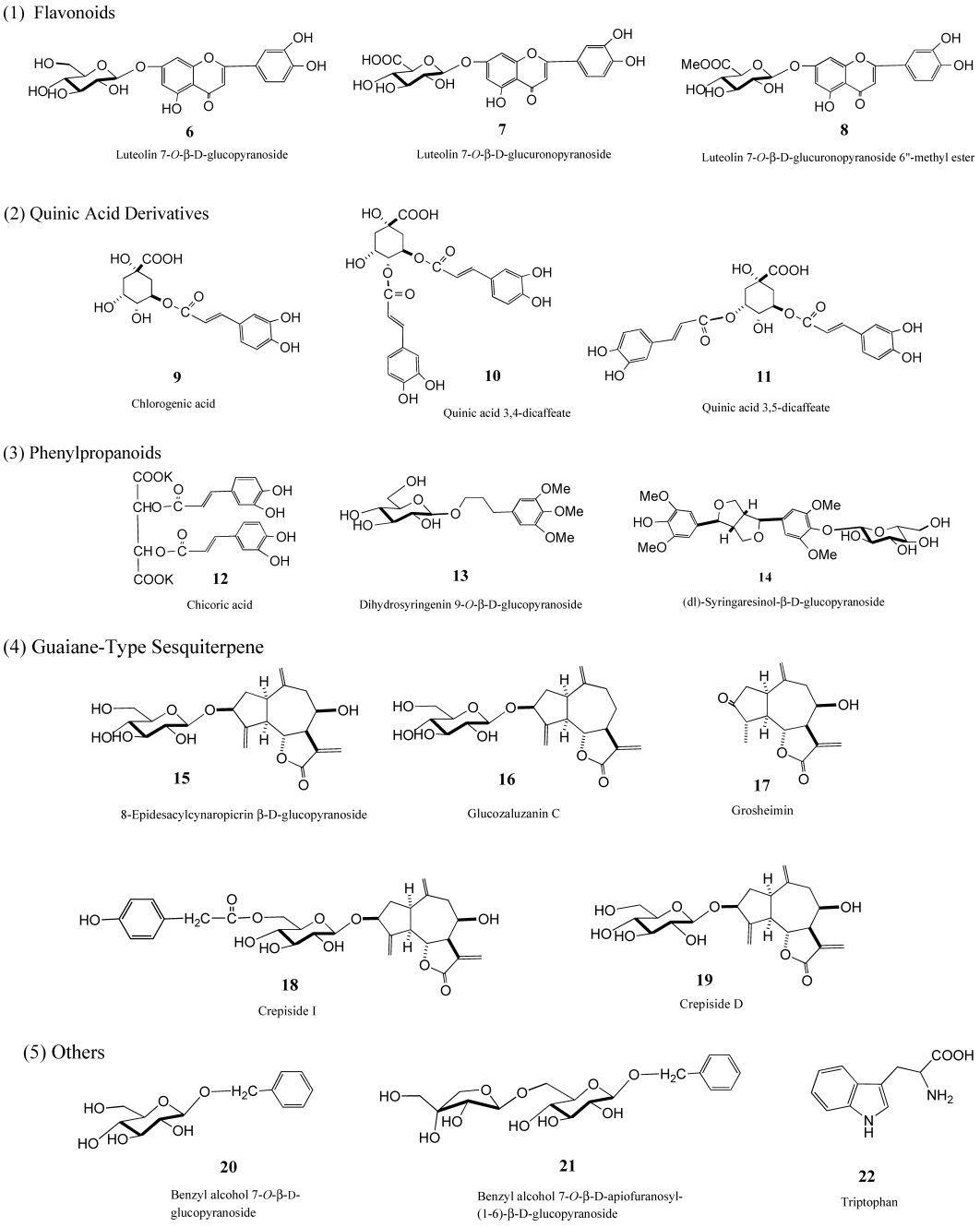

Fig. 1. Isolated Compounds

11.6 Hz, δ 4.14) to C-4, C-8 (δ 76.5); from H-7 (m, δ 1.99) to C-13 (δ 16.7), C-5, C-8, C-6, C-9 (δ 50.9); from Ha-9 (m, δ 2.01) to C-14, C-10, C-8; from Hb-9 (m, δ 2.79) to C-7 (δ 58.2), C-14, C-10; from H-11 (dq, J=6.7, 7.3 Hz, δ 2.66) to C-13, C-5, C-8, C-12 (δ 181.7); from H_3-13 (d, J=7.3 Hz, δ 1.34) to C-11 (δ 42.9), C-7 (δ 58.2), C-12; from Ha-14 (br s, δ 5.06) to C-1, C-9, C-8; from Hb-14 (br s, δ 5.13) to C-1, C-9, C-8, C-10; from H_3-15 (d, J=6.7 Hz, δ 0.97) to C-4, C-5, C-3. Furthermore, the nuclear Overhauser effect spectroscopy (NOESY) between H-1 and H-5, Ha-2 and Ha-14, H-3 and H-4, H-3 and H-5, H-4 and H-5, H-6 and H-8, H-8 and H-11, H-6 and H_3-15, H-7 and H_3-13, Ha-9 and Hb-14 revealed the stereochemistry of **1**. The location of the sugar linkage was decided by the HMBC from the glucosyl anomeric proton at δ 4.34 (1H, d, J=6.7 Hz) to the aglycone C-3 at δ 80.4. Consequently, the structure of **1** was characterized as shown in Fig. 2.

Compound **2**, which was obtained as a white powder showing $[\alpha]_D$ +35.0° (pyridine), was found to have a molecular formula $C_{26}H_{37}NO_{11}$ at *m/z* 539.2367 in the negative HR-FAB-MS. The ^{1}H-NMR spectrum displayed signals due to a single tertiary methyl group at δ 1.58 (3H, s), vinyl protons at δ 4.90 (1H, br s) and 5.01 (br s), a characteristic Hβ-6 at δ 3.93 (1H, t-like, J=9.8 Hz), and an anomeric proton at δ 4.55 (1H, d, J=7.9 Hz). The ^{13}C-NMR spectrum showed signals with chemical shifts at δ 11.8 (C-15), 33.6 (C-2), 42.2 (C-11), 44.4 (C-1), 45.4 (C-9), 52.2 (C-7), 54.1 (C-5), 60.7 (glc C-6), 69.8 (glc C-4), 73.2 (C-8), 73.3 (glc-2), 76.6 (glc-3), 76.9 (glc C-5), 80.8 (C-6), 100.2 (glc C-1), 110.7 (C-14), 114.3 (C-4), 144.8 (C-10), 150.4 (C-3), 172.9 (C-12), 176.3

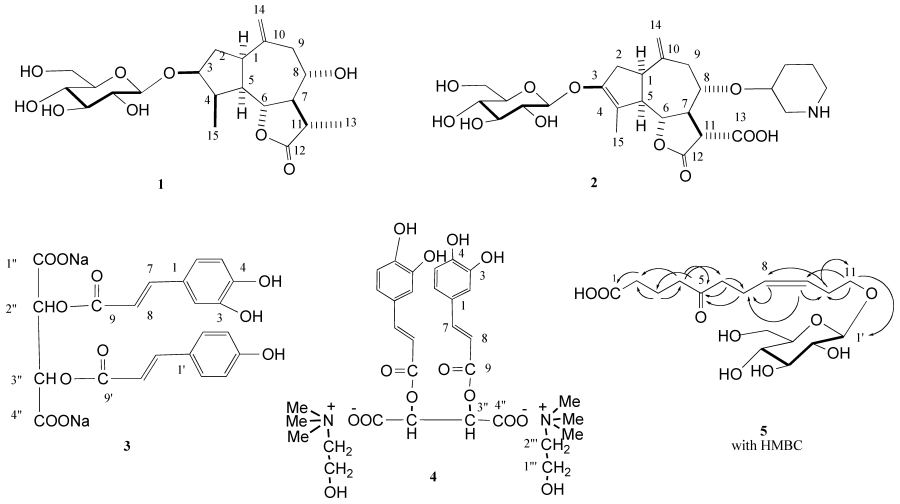

Fig. 2. Structures of Isolated New Compounds

(C-13), which were approximately identical to those of **19**. Additionally, the signals due to C-11—C-13 and the signals that newly appeared at δ 23.3 (–CH_2–), 28.8 (–CH_2–), 52.8 (–CH_2–N–), 54.0 (–CH_2–N–), 66.9 (–CH–O–) indicated the presence of one mole of a piperidine derivative. The signals at δ 42.2, 172.3, and 176.3 were assigned to C-11, C-12, and C-13, respectively. Further, the occurrence of carboxylic acid at C-13 was substantiated. The glucosyl linkage was suggested to be at C-3 of the aglycone by the HMBC from glucosyl anomeric proton at δ 4.55 (1H, d, J=7.9 Hz) to the C-3 at δ 150.4. The bonding of the aglycone and the piperidine moiety was regarded to be between C-8-OH of the aglycone and β-carbon position to the NH in piperidine, of which ^{13}C-NMR signals displayed two nitrogen-bearing methylene signals at δ 52.8 and 54.0. On the basis of the above observation, the structure of **2** could be estimated as a guaiane-type sesquiterpene carrying a β-D-glucopyranosyl moiety at C-3-OH and the piperidine derivative at C-8α-OH, as shown in Fig. 2.

Compound **3**, which was obtained as a pale yellow powder showing $[\alpha]_D$ +211.7° (H_2O), indicated a molecular ion at m/z 501 $[M+2Na-2H]^-$, 479 $[M+Na-H]^-$ in the negative FAB-MS, whose molecular mass number is lesser than that of **12** (chicoric acid) by 16 mass units. The ^{1}H-NMR spectrum showed signals due to H-2″ (s) and H-3″ (s) of tartaric acid moiety at δ 5.61, H-7 (1H, d, J=15.9 Hz) at δ 6.49, H-7′ (1H, d, J=15.9 Hz) at δ 6.52, H-5 (1H, d, J=8.0 Hz) at δ 6.88, H-6 (1H, dd, J=1.8, 8.0 Hz) at δ 7.05, H-2 (1H, d, J=1.8 Hz) at δ 7.17, H-8 (1H, d, J=15.9 Hz) at δ 7.68, H-3′, 5′ (each 1H, d, J=8.6 Hz) at δ 6.89, H-2′, 6′ (each 1H, d, J=8.6 Hz) at δ 7.54, and H-8′ (1H, dd, J=15.9 Hz) at δ 7.75. Therefore, the structure of **3** was found to be represented as 2-caffeoyl, 3-*p*-hydroxycinnamoyl (*S*,*S*)-tartaric acid, as shown in Fig. 2. The ^{13}C-NMR signals also confirmed this deduced structure.

Compound **4**, which was obtained as a pale yellow powder showing $[\alpha]_D$ +228.2° (H_2O), was found to have the molecular formula $[C_{32}H_{44}N_2O_{14}+H]^+$ at m/z 681.2856 in the positive HR-FAB-MS. The ^{1}H-NMR spectrum resembled that of **12** (chicoric acid); new signals due to 6×N–CH_3 at δ 2.95 (18H, s) and the signals due to 4H (t, J=4.9 Hz) at δ 3.25 and 4H (m) at δ 3.83 were observed. Based on these signals and the result of MS, the presence of choline was suggested. Comparing the ^{13}C-NMR spectrum of **4** with that of **12**, methylene signals at δ 54.1 and 65.9 and a methyl signal at δ 52.3 were uniquely observed in **4**. However, the other signals were almost identical to those obtained for chicoric acid; therefore, it was deduced that 2 mol of choline attach to chicoric acid to form **4**. Furthermore, judging from the results of the MS, **4** was estimated to be a substance with ion bonding between chicoric acid and choline, as shown in Fig. 2.

Compound **5**, which was obtained as an amorphous powder showing $[\alpha]_D$ −55.1° (MeOH), indicated a molecular ion peak at m/z 387 $[M-H]^-$ in the negative FAB-MS. The ^{13}C-NMR spectrum showed a total of 17 carbon signals, which included a ketone carbonyl at δ 223.6, a carboxylic acid carbonyl at δ 180.0, a two-substituted double bond at δ 130.0 and 129.5, an oxygenated methylene at δ 71.1, and 6 methylenes at δ 43.3, 40.6, 39.6, 29.8, 29.2, 27.2, together with signals due to a single β-D-glucopyranosyl moiety at δ 105.1, 75.9, 78.9, 72.5, 78.7, 63.6. In the ^{1}H-NMR spectrum, two olefinic protons (each 1H, ddd, J=7.3, 11.0, 11.0 Hz) at δ 5.44 and 5.48 suggested that the geometry of the double bond was *Z*. The heteronuclear multiple quantum coherence (HMQC) and HMBC revealed that the structure of **5** was 5-oxo-11-hydroxy-8(*Z*)-undecenoic acid 11-*O*-β-D-glucopyranoside. Figure 2 shows the structure of **5** and the HMBC results.

Next, we investigated the antiallergic and antioxidant activities of some of the specimens that we obtained. Specifically, we examined the release of hexosaminidase by the compound 48/80 (5 μg/ml) using the mast cell of rat abdomen[10] and by the antigen 2,4-dinitrophenyl-bovine serum albumin (DNP-BSA) (10 μg/ml) using rat basophilic leukemic-2H3 cells (RBL-2H3 cells).[11] In the test of the release of hexosaminidase by compound 48/50 (5 μg/ml), compounds **6**, **12**, **4**, **15**, **3**, **16** and **17** reduced it in 104, 85, 85, 101, 54, 93, 10%, respectively. In the test of the release of hexosaminidase by the antigen DNP-BSA (10 μg/ml), compounds **6**, **7**, **15**, **16**, **17**, **18** and **19** reduced it in 161, 123, 101, 99, 9, 99, 103%, respectively. Compound **17** showed strong activity in controlling the release of histamine. On the other hand, strong antioxidant activities[12] were exhibited by **4**, which has

Table 1. Antioxidative Activities

Incubation time (d)	1	2	4	5	6
12	0.001	0.006	0.014	0.017	0.020
4	0.000	0.000	0.003	0.005	0.006
3	0.000	0.002	0.005	0.006	0.008
17	0.008	0.013	0.0018	0.021	0.021
BHA	0.004	0.020	0.076	0.100	0.137
α-Tocopherol	0.042	0.080	0.184	0.235	0.287
Control	0.153	0.407	1.283	1.573	1.959

O.D. 500 nm.

ion-bonding between chicoric acid and choline, and **3** of the chicoric acid derivative, which bonds with a caffeoyl group and a *p*-hydroxycinnamoyl group. Compound **12** (chicoric acid) showed lesser significant activity than **4** and **3**. Compound **17** exhibited stronger activity in controlling the release of histamine than 3-*tert*-butyl-4-hydroxyanisole (BHA) and α-tocopherol as listed in Table 1.

Experimental

General Optical rotations were performed with a JASCO DIP-1000 KYU digital polarimeter (JASCO, Tokyo). MS were recorded on a JEOL JMS-700. ^{1}H- and ^{13}C-NMR spectra were recorded with a JEOL alpha 500 spectrometer at 500 and 125 MHz, respectively, chemical shifts were given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. Column chromatographies were carried out on MCI gel CHP-20P (75—150 mm, Mitsubishi Chem. Ind.), silica gel 60 (230—400 mesh, Merck), and Chromatorex octadecyl silica (ODS) (30—50 μm, Fuji Silysia Chem., Ltd.) and TLC was performed on a precoated silica gel 60 F_{254} (Merck).

Plant Material The whole plants of *Youngia japonica* (L.) DC. were collected from around Kumamoto University. A voucher specimen was deposited in the Herbarium of Kumamoto University.

Extraction and Isolation Whole plants of *Y. japonica* (L.) DC. (1230.0 g) were extracted with hot MeOH, and the extract (133.0 g) was shaken with water to obtain a soluble portion (107.0 g) and an insoluble portion (26.0 g). The water-soluble portion was subjected to CC on MCI gel CHP-20P eluting sequentially with (H_2O, H_2O–MeOH=60 : 40, H_2O–MeOH=40 : 60, H_2O–MeOH=20 : 80, MeOH 100%) to afford the water eluate (93.0 g) and ten fractions (fr. 1—10). Fr. 1 [2.3 g, H_2O–MeOH=60 : 40 eluate] was subjected to Chromatorex ODS (10 to 100% MeOH) to give **12** (chicoric acid), 960.0 mg, and **4**, 240.0 mg. Fr. 2 [1.8 g, H_2O–MeOH=60 : 40 eluate] was subjected to Chromatorex ODS (10—20% MeOH) to provide 12 subfractions (fr. 2-1 to fr. 2-12). Fr. 2-8 was subjected to various CC on silica gel ($CHCl_3$–MeOH–H_2O=6 : 4 : 1) and Chromatorex ODS (20% MeOH) to give **2**, 9.3 mg. Fr. 4 [855.7 mg, H_2O–MeOH=60 : 40 eluate] was subjected to Chromatorex ODS (20% MeOH) to provide 6 subfractions (fr. 4-1 to fr. 4-6). Fr. 4-2 (49.8 mg) was subjected to silica gel ($CHCl_3$–MeOH–H_2O=6 : 3.5 : 0.8) to give **9** (chlorogenic acid), 19.8 mg. Fr. 4-5 (119.5 mg) was subjected to silica gel ($CHCl_3$–MeOH–H_2O=6 : 4 : 1) and Chromatorex ODS (15—20—25% MeOH) to give **22** (triptophan), 13.2 mg and **1**, 9.0 mg. Fr. 5 [396.7 mg, H_2O–MeOH=60 : 40 eluate] was subjected to silica gel ($CHCl_3$–MeOH–H_2O=8 : 2 : 0.2→7 : 3 : 0.5→6 : 4 : 1) and Chromatorex ODS (20—30—40% MeOH) to provide **20** (benzyl alcohol 7-*O*-β-D-glucopyranoside), 1.6 mg, **13** (dihydrosyringenin 9-*O*-β-D-glucopyranoside), 7.3 mg, **1**, 6.0 mg, **19** (crepiside D), 9.3 mg, and **5**, 5.3 mg. Fr. 6 [2.5 g, H_2O–MeOH=40 : 60 eluate] was subjected to silica gel ($CHCl_3$–MeOH–H_2O=6 : 4 : 1) to give 10 subfractions (fr. 6-1 to fr. 6-10). Fr. 6-3 was recrystallized from MeOH to give **8** [luteolin 7-*O*-β-D-glucuronopyranoside 6″-*O*-methyl ester] 14.8 mg. Fr. 6-7 was subjected to Chromatorex ODS (H_2O) to give **21** (benzyl alcohol 7-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside), 51.2 mg. Fr. 6-9 was subjected to Chromatorex ODS (10—15—30% MeOH) to give **12** (chicoric acid), 14.4 mg, and **7** (luteolin 7-*O*-β-D-glucuronopyranoside), 180.8 mg. Fr. 7 [1.9 g, H_2O–MeOH=40 : 60 eluate] was subjected to silica gel ($CHCl_3$–MeOH–H_2O=7 : 3 : 0.5→6 : 4 : 1→6 : 4.5 : 1.2) to give **6** (luteolin 7-*O*-β-D-glucopyranoside), 309.0 mg and 9 subfractions (fr. 7-1 to fr. 7-9). Fr. 7-4 was subjected to Chromatorex ODS (40% MeOH) to give **15** (8-epidesacylcynaropicrin β-D-glucopyranoside), 46.6 mg. Fr. 7-8 was recrystallized from MeOH to give **10** (quinic acid 3,4-dicaffeate), 36.4 mg as crystal and the mother liquor was subjected to Chromatorex ODS (20—40—55% MeOH) to give **7** (luteolin 7-*O*-β-D-glucuronopyranoside), 10.5 mg, and **11** (quinic acid 3,5-dicaffeate), 19.0 mg. Fr. 7-9 was subjected to Chromatorex ODS (10—20% MeOH) to give **3**, 61.6 mg. Fr. 8 [1.9 g, H_2O–MeOH=20 : 80 eluate] was subjected to silica gel ($CHCl_3$–MeOH–H_2O=9 : 1 : 0.1) and Chromatorex ODS (40—45% MeOH) to give 14 subfractions (fr. 8-1 to fr. 8-14). Fr. 8-1 was subjected to silica gel ($CHCl_3$–MeOH–H_2O=9 : 1 : 0.1) to give **17** (grosheimin), 44.8 mg. Fr. 8-2 was subjected to silica gel ($CHCl_3$–MeOH–H_2O=9 : 1 : 0.1) and Chromatorex ODS (35—40% MeOH) to give **14** ((*dl*) syringaresinol-β-D-glucopyranoside) 3.7 mg. Fr. 8-9 was **16** (glucozaluzanin C), 57.2 mg. Fr. 8-13 was subjected to silica gel ($CHCl_3$–MeOH–H_2O=9 : 1 : 0.1) to give **18** (crepiside I) 28.5 mg.

Compound 1 A white powder $[\alpha]_D^{18}$ −12.6° (c=0.53, CH_3OH). Positive HR-FAB-MS *m/z*: 429.2146 $[M+H]^+$ (Calcd for $C_{21}H_{33}O_9$: 429.2125), ^{1}H-NMR (in CD_3OD) δ: 0.97 (3H, d, J=6.7 Hz, H_3-15), 1.34 (3H, d, J=7.3 Hz, H_3-13), 1.99 (1H, m, H-7), 2.01 (1H, m, H-2), 2.01, 2.79 (each 1H, m, H_2-9), 2.31 (2H, m, H_2-5), 2.46 (1H, m, H-4), 2.66 (1H, dq, J=6.7, 7.3 Hz, H-11), 2.79 (1H, m, H-1), 3.58 (1H, ddd, J=4.9, 10.4, 10.4 Hz, H-8), 4.14 (1H, dd, J=9.8, 11.6 Hz, H-6), 4.33 (1H, m, H-3), 5.06, 5.13 (each 1H, br s, H_2-14). ^{13}C-NMR (in CD_3OD) δ: aglycone moiety C-1—15, 41.7, 33.9, 80.4, 38.9, 48.2, 80.1, 58.2, 76.5, 50.9, 145.4, 42.9, 181.7, 16.7, 114.1, 8.8; glucosyl moiety C-1—6, 103.3, 74.2, 75.4, 71.8, 78.1, 62.9.

Compound 2 A white powder $[\alpha]_D^{24}$ +35.0° (c=0.93, pyridine). Negative FAB-MS *m/z*: 538 $[M-H]^-$, ^{1}H-NMR (in DMSO-d_6) δ: 1.58 (3H, s, H_3-15), 3.93 (1H, t, J=9.8 Hz, H-6), 4.55 (1H, d, J=7.9 Hz, glc H-1), 4.90, 5.01 (each 1H, br s, H_2-14), ^{13}C-NMR (in DMSO-d_6) δ: aglycone moiety C-1—15, 44.4, 33.6, 150.4, 114.3, 54.1, 80.8, 52.2, 73.2, 45.4, 144.8, 42.2, 172.3, 176.3, 110.7, 11.8; piperidine moiety, C-2′—6′, 52.8, 66.9, 23.3, 28.8, 54.0; glucosyl moiety C-1″—6″, 100.2, 73.3, 76.6, 69.8, 76.9, 60.7.

Compound 3 A pale yellow powder $[\alpha]_D^{27}$ +211.7° (c=0.59, H_2O). Negative FAB-MS (*m/z*) 501 $[M+2Na-2H]^-$, 479 $[M+Na-H]^-$, ^{1}H-NMR (in D_2O) δ: 5.61 (2H, s, H-2″, 3″), 6.49 (1H, d, J=15.9 Hz, H-7), 6.52 (1H, d, J=15.9 Hz, H-7′), 6.88 (1H, d, J=8.0 Hz, H-5), 6.89 (each 1H, d, J=8.6 Hz, H-3′, 5′), 7.05 (1H, dd, J=1.8, 8.0 Hz, H-6), 7.17 (1H, d, J=1.8 Hz, H-2), 7.54 (each 1H, d, J=8.6 Hz, H-2′, 6′), 7.68 (1H, d, J=15.9 Hz, H-8), 7.75 (1H, d, J=15.9 Hz, H-8′). ^{13}C-NMR (in D_2O) δ: caffeic moiety C-1—9, 128.5, 117.3, 146.4, 149.2, 116.3, 123.9, 147.8, 115.8, 169.9; *p*-hydroxy cinnamic acid moiety C-1′—9′, 128.0, 131.8, 117.3, 160.6, 117.3, 131.8, 148.7, 115.7, 169.9; 2,3-dihydroxy-butanedioic acid moiety C-1″, 4″, 174.6, C-2″, 3″, 76.5.

Compound 4 A pale yellow powder $[\alpha]_D^{28}$ +228.2° (c=0.59, H_2O). Positive FAB-MS (*m/z*) 681 $[M+H]^+$, positive HR-FAB-MS *m/z*: 681.2856 $[M+H]^+$ (Calcd for $C_{32}H_{45}N_2O_{14}$: 681.2871), ^{1}H-NMR (in D_2O) δ: 2.95 (18H, s, N-Me×6), 3.25 (4H, t, J=4.9 Hz, H-2‴, 2⁗), 3.83 (4H, m, H-2-1‴, 1⁗), 5.47 (2H, s, H-2″, 3″), 6.22 (2H, d, J=15.9 Hz, H-7, 7′), 6.74 (2H, d, J=8.6 Hz, H-5, 5′), 6.84 (2H, br d, J=8.6 Hz, H-6, 6′), 6.92 (2H, d, J=1.8 Hz, H-2, 2′), 7.49 (2H, d, J=15.9 Hz, H-8, 8′). ^{13}C-NMR (in D_2O) δ: caffeic moiety C-1—9, 125.3, 114.6, 142.7, 145.7, 113.6, 121.4, 145.2, 112.3, 167.0; 2,3-dihydroxy-butanedioic acid moiety C-1″, 4″, 170.0, 2″, 3″, 73.2; choline moiety C-1‴, 1⁗, 65.9, C-2‴, 2⁗, 54.1, N–CH_3 (52.3).

Compound 5 An amorphous powder $[\alpha]_D^{24}$ −55.1° (c=0.79, CH_3OH). Negative FAB-MS (*m/z*) 387 $[M-H]^-$, ^{1}H-NMR (in CD_3OD) δ: 2.20 (1H, m, Ha-2), 2.26 (1H, dd, J=4.9, 14.6 Hz, Hb-2), 1.53, 2.23 (each 1H, m, H_2-3), 2.06 (1H, ddd, J=8.5, 10.9, 18.9 Hz, Ha-4), 2.30 (1H, m, Hb-4), 2.23, 2.30 (each 1H, m, H_2-6), 2.42 (2H, m, H_2-7), 5.44 (1H, ddd, J=7.3, 11.0, 11.0 Hz, H-8), 5.48 (1H, ddd, J=7.3, 11.0, 11.0 Hz, H-9), 2.38 (2H, m, H_2-10), 3.56 (1H, ddd, J=7.2, 7.2, 9.2 Hz, Ha-11), 3.88 (1H, m, Hb-11), 4.38 (1H, d, J=7.9 Hz, glc H-1). ^{13}C-NMR (in CD_3OD) δ: aglycone moiety C-1—11, 180.0, 43.3, 29.2, 39.6, 223.6, 40.6, 27.2, 130.0, 129.5, 29.8, 71.1; glucosyl moiety C-1—6, 105.1, 75.9, 78.9, 72.5, 78.7, 63.6.

Assay of Anti-allergic Activities[10,11] RBL-2H3 cells were grown in RPMI1640 supplement with 10% FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin in a 37 °C humidified incubator with an atmosphere of 95% air and 5% CO_2. A 96-well plate of the cultured cell (5×10^4 cells/0.1 ml per well) was used for the degranulation assay. After sensitization of RBL-2H3 cells with 0.5 μg/ml of DNP specific IgE and washing out, cells were incubated with various concentration of the respective specimens as indicated in the text, and stimulated with antigen.

DNP-specific IgE treatment was omitted for the case of compound 48/80 as a stimulant agent. Secretion was determined by the amount of hexosaminidase release, a granule constituent. Hexosaminidase was subsequent assayed in the medium and its secreation used as a marker of degranulation. Briefly, 0.5 μl sample of the medium and 0.5 μl of the substrate (5 mM *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide in 0.2 M citrate, pH 4.5) were incu-

bated in a 96-well plate to yield the chromophore, *p*-nitrophenol. The absorbance of the colored product was assessed at 405 nm using microplate reader. Values for hexosaminidase release were expressed as a percentage of the total hexosaminidase as determined by the celle lysed in 0.1% triton X-100.

DNP specific IgE, *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide, and compound 48/80 were from Sigma (St. Louis, MO, U.S.A.). Statistical analysis of the different treatment groups was carried out by one-way analysis of variance (ANOVA) followed by Student's *t*-test.

Assay of Antioxidative Activity[12] A mixture of 2.51% linoleic acid–EtOH solution (0.80 ml), 0.05 M phosphate buffer (pH 7.0, 1.60 ml), EtOH (0.60 ml) and H_2O (0.80 ml) were added to 0.4% or 10 mM EtOH solution (0.20 ml) of each sample in vial with a cap and placed in darkness at 40 °C to accelerate oxidation. After the 5th day of incubation, this assay solution (0.05 ml) was diluted with 75% EtOH (4.85 ml), which was followed by adding 30% ammonium thiocyanate (0.05 ml). Precisely 3 min after the addition of 0.02 M ferrous chloride in 3.5% HCl (0.05 ml) to the reaction mixture, the absorbance was measured at 500 nm. The control sample was prepared in the same manner by mixing all the same chemicals and ingredients and by excluding the test compounds. α-Tocopherol and BHA were used as standard samples.

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