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Research Paper

Constituents with dipeptidyl peptidase-IV inhibitory effect from the flower buds of *Rosa rugosa*

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ABSTRACT

Seven hydrolysable tannins, rugosin A (**1**), rugosin C (**2**), rugosin A methyl ester (**3**), casuarictin (**4**), tellimagrandin II (**5**), 1,2,3,4,6-penta-*O*-galloyl-*β*-D-glucopyranose (**6**), and 1,3-di-*O*-galloyl-4,6-*O*-hexahydroxydiphenoyl-*β*-D-glucopyranose (**7**) were isolated from the flower buds of *Rosa rugosa*. Among these constituents, compounds **1**, **2**, **3**, **5**, and **7** exhibited inhibitory activity against dipeptidyl peptidase-IV (DPP-IV), with compound **1** showing the most potent effect. Structure–activity relationship study indicated that the presence of a valoneoyl group plays a critical role in the manifestation of activity. This finding offers important insights into the development of novel DPP-IV inhibitors and antidiabetic agents.

Key words: hydrolysable tannin, rugosin A, *Rosa rugosa*, dipeptidyl peptidase-IV, antidiabetic agent

1. Introduction

Rosa rugosa Thunb., a member of the family Rosaceae, is distributed throughout East and Northeast Asia, including Japan and China. The flower buds have long been used as a traditional medicine and are also consumed routinely as herbal tea. Several hydrolyzable tannins have previously been isolated from the flower buds as major chemical constituents, along with flavonoids and other phenolic compounds (Chao et al., 2026; Hatano et al., 1990; Ochir et al., 2010; Okuda et al., 1990). Extracts and individual constituents from the flower buds or flowers have been reported to exhibit various pharmacological activities, including anti-inflammatory and antioxidative effects (Chang et al., 2019; Dong et al., 2025; Kim et al., 2024; Ng et al., 2004). Our research group has been engaged in the exploratory study of therapeutic agents for diabetes, utilizing medicinal plants as source materials. We have also investigated a variety of bioactive compounds derived from medicinal flowers and functional foods (Matsuda et al., 2023; Nakamura et al., 2013a, 2013b, 2021, 2022).

Dipeptidyl peptidase-IV (DPP-IV) inhibitors are a class of antidiabetic agents used in the treatment of type 2 diabetes. These agents inhibit the activity of DPP-IV, an enzyme responsible for degrading incretin hormones such as glucagon-like peptide-1 (GLP-1), which stimulates insulin secretion. Consequently, DPP-IV inhibitors contribute to lowering blood glucose levels. In the present study, we evaluated the DPP-IV inhibitory activity of various plant extracts as part of our exploratory research on therapeutic agents for diabetes. A methanolic (MeOH) extract from the flower buds of *R. rugosa* was found to exhibit notable DPP-IV inhibitory activity. From the MeOH extract of *R. rugosa* flower buds, we isolated seven hydrolyzable tannins: rugosin A (**1**) (Nitta et al., 2013; Okuda et al., 1982, 1990), rugosin C (**2**) (Okuda et al., 1982, 1990), rugosin A methyl ester (**3**) (Nitta et al., 2013), casuarictin (**4**) (Ashibe et al., 2017; Okuda et al., 1983), tellimagrandin II (**5**) (Hatano et al., 1990; Takeuchi et al., 2015), 1,2,3,4,6-penta-*O*-galloyl-*β*-D-glucopyranose (**6**) (Okuda et al., 1983), and 1,3-di-*O*-galloyl-4,6-*O*-hexahydroxydiphenoyl-*β*-D-glucopyranose (**7**) (Yoshida et al., 1991). In this paper, we describe the DPP-IV inhibitory effects of these isolated compounds, along with their structure–activity relationships.

2. Material and Methods

2.1. General

^{1}H-NMR spectra, JEOL JNM-EX 270 (270 MHz), JNM-LA 500 (500 MHz), and JNM-ECA 600 (600 MHz,) spectrometers; ^{13}C-NMR spectra, JEOL JNM-EX 270 (68

MHz), JNM-LA 500 (125 MHz,), and JNM-ECA 600 (150 MHz) spectrometers (Tokyo, Japan). The HPLC instruments used for compound isolation were a Shimadzu SPD-20A UV–VIS detector, a Shimadzu LC-6AD pump, and a Shimadzu C-R6A chromatopac (Shimadzu Corporation, Kyoto, Japan). COSMOSIL 5C18-MS-II columns (250 × 4.6 mm i.d. and 250 × 20 mm i.d.; Nakarai Tesque Co., Ltd., Kyoto, Japan) and a YMC-Pack Ph column (250 × 4.6 mm i.d.; YMC Co., Ltd., Kyoto, Japan) were used for analytical and preparative purposes. The following materials were used for chromatography: normal-phase silica gel column chromatography, Silica gel 60N (Kanto Chemical Co., Inc., 63–210 μm, Tokyo, Japan); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical Ltd., Aichi, Japan); size exclusion chromatography, Sephadex LH-20 (GE HealthCare Technologies Inc., Chicago, USA); TLC, precoated TLC plates with Silica gel $60F_{254}$ (0.25 mm, Merck KGaA, Darmstadt, Germany) (ordinary phase) and Silica gel RP-18 F_{254S} (0.25 mm, Merck KGaA, Darmstadt, Germany) (reversed phase). Detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 followed by heating.

2.2. Chemicals and reagents

Solvents and Reagents: Chemicals were purchased from Fujifilm Wako Pure Chemical Corp. (Osaka, Japan) and Nacalai Tesque Inc (Kyoto, Japan).

2.3. Plant material

Dried flower buds of *Rosa rugosa* were obtained from commercial sources. The plant materials were identified by one of the authors (H. M.).

2.4. Extraction and isolation

The dried flower buds of *Rosa rugosa* (2.0 kg) were extracted three times with methanol under reflux for 3 h. Evaporation of the solvent under reduced pressure provided a MeOH extract (350 g, 17.5% from the dried flower buds). A part of the MeOH extract (348 g) was partitioned into an EtOAc-H_2O (1:1, v/v) mixture to furnish an EtOAc-soluble fraction (60.0 g, 3.0%) and an aqueous phase (288 g). The aqueous phase was further extracted with 1-butanol to give a 1-butanol-soluble fraction (60.0 g, 3.0%) and an H_2O-soluble fraction (228 g, 11.5%). A part of the EtOAc- soluble fraction (60.0 g) was subjected to normal phase silica gel column chromatography [*n*-hexane–$CHCl_3$ (1:0 → 2:1 → 0:1, v/v) → $CHCl_3$–MeOH (50:1 → 10:1 → 5:1 → 2:1, v/v) → MeOH] to give 10 fractions [Fr.1 (1.60 g), Fr.2 (0.88 g), Fr.3 (5.97 g), Fr. 4 (2.29 g), Fr.5 (2.39 g), Fr.6 (7.27 g), Fr.7 (7.70 g), Fr.8 (27.5 g), Fr.9 (1.03 g), Fr.10 (1.29 g)]. Fraction 8 (27.5 g) was further separated by reversed phase silica gel column chromatography [CH_3CN–H_2O–CH_3COOH (5:95:1 → 10:90:1 → 20:80:1 → 30:70:1 → 40:60:1 → 50:50:1 → CH_3CN)] to give 13 fractions [Fr.8-1–Fr.8-4, Fr.8-5 (3300 mg), Fr.8-6, Fr.8-7 (2246 mg), Fr.8-8 (622 mg), Fr.8-9 (510 mg), Fr.8-10 (250 mg), Fr.8-11 (108 mg), Fr8-12 (222 mg), Fr.8-13 (101 mg)]. Fraction 8-5 (3300 mg) was further separated by Sephadex LH-20 column chromatography [CH_3CN → CH_3CN–H_2O (7:3 → 1:1, v/v)] to give seven fractions [Fr.8-5-A, Fr.8-5-B, Fr.8-5-C (288 mg), Fr.8-5-D, Fr.8-5-E, Fr.8-5-F (348 mg), Fr.8-5-G (160 mg)]. Fr.8-5-C (288 mg) was purified by HPLC [CH_3CN- H_2O (2:8, v/v), YMC-Pack Ph] to give 1,3-di-*O*-galloyl-4,6-hexahydroxydiphenoyl-*β*-D-glucose (**7**, 147 mg, 0.0073%). Fr.8-5-F (348 mg) was purified by HPLC [CH_3CN- H_2O (2:8, v/v), YMC-Pack Ph] to give tellimagrandin II (**5**, 283 mg, 0.014%). Fr.8-5-G (160 mg) was purified by HPLC [CH_3CN- H_2O (17:83, v/v), YMC-Pack Ph] to give casuarictin (**4**, 101 mg, 0.0050%). Fraction 8-7 (2246 mg) was further separated by Sephadex LH-20 column chromatography [MeOH → MeOH–H_2O (7:3 → 1:1, v/v)] to give 12 fractions [Fr.8-7-A–H, Fr.8-7-I (373 mg), Fr.8-7-J (267 mg), Fr.8-7-K, Fr.8-7-L]. Fr.8-7-I (373 mg) was purified by HPLC [CH_3CN- H_2O (1:4, v/v), COSMOSIL 5C18 MS-II] to give rugosin A methyl ester (**3**, 13.4 mg, 0.00066%), 1,2,3,4,6-penta-*O*-galloyl-*β*-D-glucopyranose (**6**, 134 mg, 0.0066%). Fr.8-7-J (267 mg) was purified by HPLC [CH_3CN- H_2O (17:83, v/v), COSMOSIL 5C18 MS-II] to give rugosin C (**2**, 10.8 mg, 0.00053%), Fr.8-7-J-2 (52 mg). Fr.8-7-J-2 (52 mg) was purified by HPLC [CH_3CN- H_2O (1:4, v/v), COSMOSIL 5C18 MS-II] to give rugosin A (**1**, 7.4 mg, 0.00037%) (Figure 1). These constituents **1**–**7** were identified by comparison of their ^{1}H-NMR and ^{13}C-NMR data with those reported in the literature.

2.5. Protocol for inhibitory effect on human recombinant DPP-IV

The experiment was performed according to the method described in our previous reports (Hussein et al., 2011). DPP-IV solution (2 U/mL; Funakoshi, ATK-DPP0901-100) was added to a 96-well assay plate (25 μL per well; IWAKI, 3881-096), followed by the addition of 25 μL of the test sample. The DPP-IV solution was prepared in 50 mM Tris–maleate buffer containing 0.1% BSA. The plate was incubated at 37 °C for 3 min. A 40 mM GPNT solution (Gly-Pro-pNA·TOS; FUJIFILM Wako, 336-30741) prepared in DMSO was diluted 50-fold with 50 mM Tris–maleate buffer containing 0.1% BSA, and 50 μL of the diluted solution was added to each well. The mixture was incubated at 37 °C for 30 min. Absorbance at 405 nm was measured. The DPP-IV inhibitor sitagliptin (IC_{50} = 31 nM) was used as a reference compound. Furthermore, in our previous report (Hussein et al., 2011), we demonstrated that the reference compound diprotin-A (Ile-Pro-Ile) exhibits strong DPP-IV inhibitory activity (90.6 ± 0.1% inhibition at 100 μg/mL; IC_{50} of approximately 8.8 μg/mL).

Significant difference from the control group was calculated by the Dunnett test. Probability (p) values less than 0.05 were considered to be significant ($^{*}p < 0.05$, $^{**}p < 0.01$).

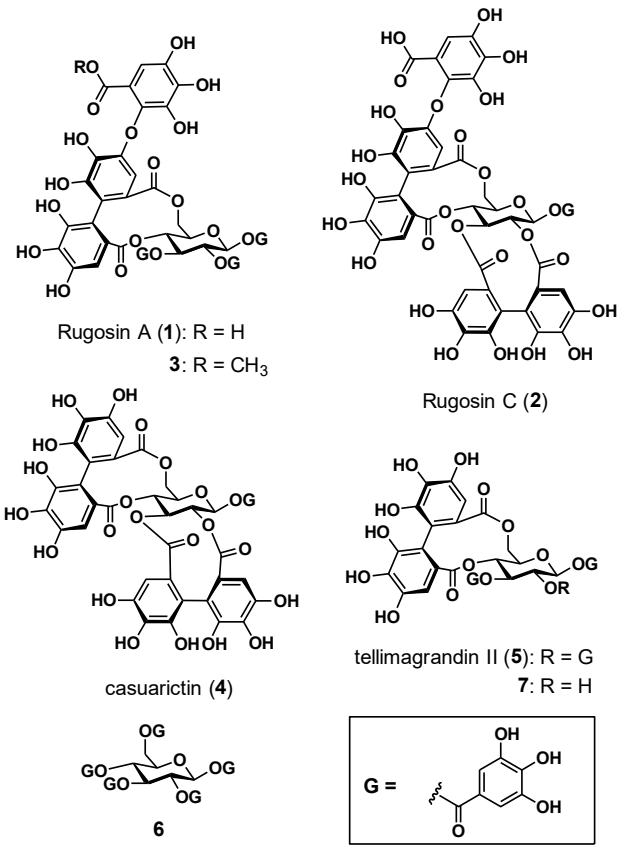

Figure 1. Chemical Structures of 1–7.

The inhibition rate (%) was calculated using the following equation:

Inhibition (%) = [(A-B) / A] ×100

where A is the absorbance of the control (without test sample) and B is the absorbance in the presence of the test sample.

3. Results

Our research group has been engaged in the exploration of functional constituents derived from medicinal foods and medicinal plants. In particular, as part of our efforts toward the development of antidiabetic agents, we have investigated DPP-IV inhibitory constituents using medicinal plants as materials. As a result, we reported that chalconaringenin 20-*O*-*β*-D-glucopyranoside (IC_{50} = 23.1 μM) and aureusidin 6-*O*-*β*-D-glucopyranoside (IC_{50} = 24.3 μM), isolated from the flowers of *Helichrysum arenarium*, exhibited DPP-IV inhibitory activity (Morikawa et al., 2015). In the present study, we conducted exploratory research on constituents from medicinal plants with DPP-IV inhibitory activity and found that the extract of *R. rugosa* flower buds exhibited significant inhibitory effects [inhibition (%) at 100 μg/mL = 77.7]. It has previously been reported that the flower buds of *R. rugosa* contain hydrolyzable tannins (Hatano et al., 1990; Ochir et al., 2010; Okuda et al., 1990). Therefore, we isolated tannins from the flower parts and further investigated their DPP-IV inhibitory activity. Namely, the methanol extract was partitioned with EtOAc–H_2O (1:1, v/v), yielding an EtOAc-soluble fraction (3.0%) and an aqueous phase. The aqueous phase was further partitioned (1:1, v/v) with 1-butanol to afford a 1-butanol-soluble fraction (3.0%) and a water-soluble fraction (11.5%). Bioassay-guided fractionation revealed that the EtOAc-soluble and butanol-soluble fractions exhibited DPP-IV inhibitory activities [inhibition (%) at 100 μg/mL = 90.3, 78.7, respectively], whereas the water-soluble fraction showed no notable activity. Subsequently, EtOAc-soluble fraction exhibiting DPP-IV inhibitory activity was subjected to normal-phase silica gel and reversed-phase ODS column chromatography, followed by HPLC, for isolation and purification. As a result, seven hydrolysable tannins, rugosin A (**1**), rugosin C (**2**), rugosin A methyl ester (**3**), casuarictin (**4**), tellimagrandin II (**5**), 1,2,3,4,6-penta-*O*-galloyl-*β*-D-glucopyranose (**6**), and 1,3-di-*O*-galloyl-4,6-*O*-hexahydroxydiphenoyl-*β*-D-glucopyranose (**7**) were obtained. Finally, the isolated compounds were evaluated for their DPP-IV inhibitory activity. Among the constituents, compounds **1**, **2**, **3**, **5**, and **7** showed inhibitory effects against DPP-IV [inhibition (%) at 30 μM = 86.3 for **1**, 43.8 for **2**, 16.7 for **3**, 21.0 for **5**, 37.5 for **7**, respectively] (Table 1).

Table 1. DPP-IV Inhibitory Activity of Constituents 1–7.

Conc. (μM)	Inhibition (%) for DPP-IV					
	0	1	3	10	30	100
rugosin A (**1**)	0.0 ± 0.2	14.3 ± 0.3**	38.6 ± 0.1**	71.7 ± 0.6**	86.3 ± 0.2**	92.6 ± 0.2**
rugosin C (**2**)	0.0 ± 1.3	3.2 ± 2.2	6.9 ± 0.8**	23.4 ± 2.0**	43.8 ± 0.7**	63.7 ± 0.6**
3	0.0 ± 0.8	2.1 ± 3.2	2.7 ± 0.8	5.3 ± 1.4	16.7 ± 0.9**	47.2 ± 1.0**
casuarictin (**4**)	0.0 ± 0.6	3.2 ± 4.7	–2.0 ± 1.9	–1.7 ± 2.1	4.6 ± 3.0	13.2 ± 3.4**
tellimagrandin II (**5**)	0.0 ± 0.6	–0.7 ± 0.4	1.6 ± 2.3	8.6 ± 0.7**	21.0 ± 1.5**	31.6 ± 1.0**
6	0.0 ± 0.8	–2.9 ± 2.0	–3.3 ± 1.9	–5.7 ± 1.1	–12.6 ± 2.8	–18.5 ± 1.1
7	0.0 ± 0.4	2.9 ± 1.5	3.7 ± 0.4	11.6 ± 5.0**	37.5 ± 0.8**	83.3 ± 0.3**

Sitagliptin (IC_{50} = 31 nM) was used as a reference compound (postive control).
Each value represents the mean ± S.E.M. (n = 4). Significantly different from the control **p < 0.01.

4. Discussion

Based on the obtained results, structure–activity relationship analyses were conducted. It was demonstrated that the presence of galloyl, hexahydroxydiphenoyl and valoneoyl groups had a substantial impact on the manifestation of activity. Compounds **2** (IC_{50} = 40.5 μM) and **4**, which contain a hexahydroxydiphenoyl group, exhibited weaker activity compared with compounds **1** (IC_{50} = 4.3 μM) and **5**, in which the hexahydroxydiphenoyl group is replaced by two galloyl groups. Furthermore, compounds **1** and **2**, bearing valoneoyl group (an additional trihydroxybenzoic acid unit attached to the hexahydroxydiphenoyl moiety), showed enhanced activity. In contrast, comparison of compounds **1** and **3** suggested that substitution of the galloyl carboxyl group (–COOH) with a methyl ester ($–COOCH_3$) resulted in diminished activity (Figure 2).

Previously, Kato et al. isolated hydrolyzable tannin constituents from *Rosa gallica*, a species belonging to the same genus as *Rosa rugosa*, and evaluated their DPP-IV inhibitory activity (Kato et al., 2016). They reported that compound **1** and ruginosin B, which contains a valoneoyl group, exhibited strong inhibitory activity. They also concluded that retention of the carboxyl group is crucial for activity, as esterification or decarboxylation of the valoneoyl carboxyl group led to reduced activity. In the present study, compounds **1** and **2** also showed higher activity than the other compounds, consistent with the findings of Kato et al. that the additional galloyl unit in the valoneoyl group contributes to enhanced activity. Moreover, comparison of compounds **1** and **3** confirmed that replacement of the galloyl carboxyl group (–COOH) with a methyl ester ($–COOCH_3$) reduces activity, showing a trend similar to the previously reported decrease in activity upon esterification (ruginosin B → ruginosin B ethyl ester). Taken together, these results demonstrate that the additional galloyl unit in the valoneoyl group, the number and arrangement of galloyl groups, the presence or absence of an HHDP group, and the retention of the carboxyl group all have significant effects on DPP-IV inhibitory activity. These structure–activity relationships were consistently observed in both the previous report by Kato et al. and the present study. Furthermore, by including compounds **2**, **3**, **4**, and **6**—which were not examined in the study by Kato et al.—the present work enabled a more systematic elucidation of the relationship between DPP-IV inhibitory activity and the chemical structures of hydrolyzable tannins.

5. Conclusion

The DPP-IV inhibitory activity of seven hydrolyzable tannins isolated from the flower buds of *R. rugosa* was evaluated, and rugosin A (**1**) exhibited the strongest inhibitory effect. Furthermore, the correlation between the structures of the hydrolyzable tannins and their activity was partially elucidated. Compounds **1** and **2** are known to be present in the flower buds of *R. rugosa*, which are used as a food ingredient. Therefore, foods containing these active

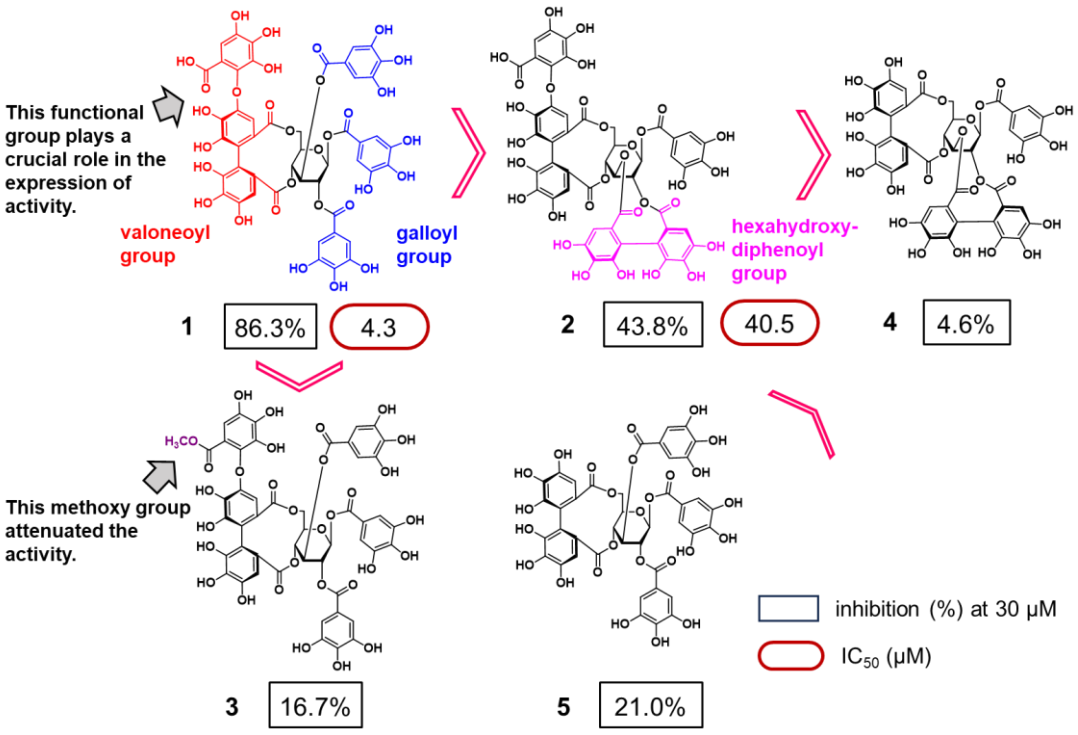

Figure 2. Structure–Activity Relationship of Compounds 1–5.

compounds or structurally related constituents may have potential for future application as functional foods. On the other hand, this study has several limitations. First, the quantity of the isolated compounds was limited, which restricted the scope of the biological assays and resulted in the study being based solely on in vitro experiments. Consequently, no in vivo data are available to support the biological relevance of the findings. Second, the active compounds exhibit relatively weak potency, and in their current form they are not suitable for use as pharmaceutical agents. To advance the development of antidiabetic agents based on the findings of this study, in vivo validation, expanded structure–activity relationship analyses, and detailed elucidation of the underlying mechanisms of action will be required.

Conflict of Interest

The authors declare no conflict of interest.

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