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Flavonoid Glycosides Inhibit Sortase A and Sortase A-Mediated Aggregation of *Streptococcus mutans*, an Oral Bacterium Responsible for Human Dental Caries

Woo-Young Yang[1], Chang-Kwon Kim[2], Chan-Hong Ahn[1], Heegyu Kim[1], Jongheon Shin[2*], and Ki-Bong Oh[1*]

[1]*Department of Agricultural Biotechnology, College of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea*
[2]*Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea*

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*Corresponding authors
J.S.
Phone: +82-2-880-2484;
Fax: +82-2-762-8322;
E-mail: shinj@snu.ac.kr
K.-B.O.
Phone: +82-2-880-4646;
Fax: +82-2-873-3112;
E-mail: ohkibong@snu.ac.kr

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Three flavonoids were isolated from dried flowers of *Sophora japonica* using repetitive column chromatography and high-performance liquid chromatography. The flavonoids were identified as rutin (**1**), quercetin-3′-*O*-methyl-3-*O*-α-L-rhamnopyranosyl(1 → 6)-β-D-glucopyranoside (**2**), and quercetin (**3**) on the basis of spectroscopic analysis and comparison of values reported in the literature. These compounds inhibited the action of sortase A (SrtA) from *Streptococcus mutans*, a primary etiologic agent of human dental caries. The onset and magnitude of inhibition of saliva-induced aggregation of *S. mutans* treated with compound **1** was comparable to that of untreated *S. mutans* with a deletion of the *srtA* gene.

Keywords: *Sophora japonica*, flavonoids, *Streptococcus mutans*, sortase A, cell aggregation

Many surface proteins from gram-positive bacteria are covalently anchored to bacterial cell wall peptidoglycans, through a general sorting mechanism catalyzed by a superfamily of membrane-associated transpeptidases known as sortases [3]. Surface proteins with a C-terminal sorting signal are covalently linked to the cell wall by sortase A (SrtA), which is involved in the pathogenesis of gram-positive bacterial infections. The sorting signal is composed of a LPXTG motif, a hydrophobic domain, and a tail of positively charged residues. *Streptococcus mutans* forms part of the commensal microflora and is deemed to be the major pathogen responsible for the generation of dental caries [13]. SrtA and sorting signal-containing surface proteins control *S. mutans* adherence, biofilm formation, and aggregation ability [1]. *S. mutans* with a knockout of the *srtA* gene had a decreased ability to colonize oral mucosa and teeth and reduced biofilm biomass [9]. Therefore, SrtA could be a future target for the prevention of dental caries. In our search for bioactive compounds from Korean folk medicine, we screened a few hundred commercially available specimens and found that the organic extract from dried flowers of *Sophora japonica* (Huaihua) significantly inhibited SrtA derived from *S. mutans* strain OMZ65, isolated from the human oral cavity. From the polar chromatographic fraction of *S. japonica* extract, we recently identified two maltol derivatives and two flavonol glycosides as SrtA inhibitors [16]. However, nuclear magnetic resonance data and SrtA inhibitory activity tests revealed the presence of additional flavonoid metabolites in these fractions. Here, we report the isolation and biological activity of three flavonoid glycosides from *S. japonica*.

Dried flowers from *S. japonica* (3 kg) were macerated and extracted three times with MeOH (10 L) and CH_2Cl_2 (10 L). The combined crude extracts (352 g) were successively partitioned between H_2O and organic solvents, including *n*-hexane, EtOAc, and BuOH. The EtOAc fraction (8 g) was separated by C_{18} reversed-phase vacuum flash chromatography using a sequential mixture of MeOH and H_2O as the eluent

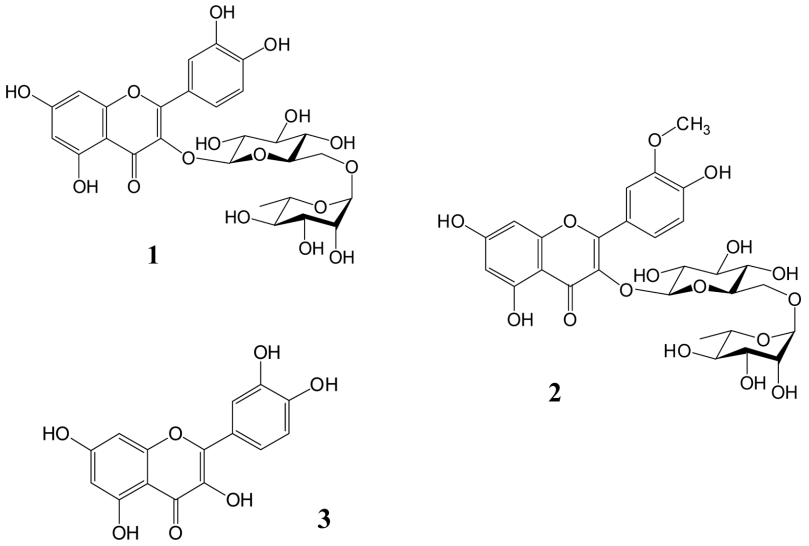

Fig. 1. Structures of compounds **1–3** from the flowers of *Sophora japonica*.
Rutin (quercetin-3-*O*-α-L-rhamnopyranosyl(1 → 6)-β-D-glucopyranoside) (**1**), quercetin-3′-*O*-methyl-3-*O*-α-L-rhamnopyranosyl(1 → 6)-β-D-glucopyranoside (**2**), and quercetin (**3**).

(six fractions in the gradient, H_2O–MeOH, from 90:10 to 0:100), acetone, and finally EtOAc. Based on SrtA inhibitory activity test results, the fraction eluted with H_2O–MeOH (70:30) (1.1 g) was chosen for separation. This fraction was separated by reversed-phase high-pressure liquid chromatography (Zorbax 5 μm Eclipse-XDB-C_{18} column, 250 × 4.6 mm; H_2O–MeOH, 50:50) to yield, in order of elution, compounds **1**, **2**, and **3** as amorphous solids. A total of 10.2, 8.3, and 12.8 mg of compounds **1**, **2**, and **3** were purified and identified to be rutin (quercetin-3-*O*-α-L-rhamnopyranosyl(1 → 6)-β-D-glucopyranoside) [2], quercetin-3′-*O*-methyl-3-*O*-α-L-rhamnopyranosyl(16)-β-D-glucopyranoside [12, 15], and quercetin (**3**) [12], respectively, by combined spectroscopic analyses (Fig. 1). The spectroscopic data for these compounds were in good agreement with the literature.

Recombinant SrtA derived from *S. mutans* strain OMZ65 was purified from an *Escherichia coli* extract using metal chelate-affinity chromatography [7]. An increase in fluorescence intensity upon cleavage of a synthetic peptide substrate containing the LPETG motif was used to measure recombinant SrtA enzyme activity [14]. The inhibitory activities (IC_{50} values) of compounds **1**, **2**, and **3** toward *S. mutans* SrtA are shown in Table 1 and are compared with those of the known SrtA inhibitors, curcumin (IC_{50} = 91.5 μM) and berberine chloride (IC_{50} = 113.8 μM) [4, 11]. Among the isolated compounds, compound **3** showed weak inhibitory activity, with an IC_{50} value of 210.8 μM. In contrast, compounds **1** and **2**, which contain *O*-rhamnoglucoside at the C-3 position, were more active, with IC_{50} values of 134.1 and 185.9 μM, respectively. In addition, a methoxy group at the C-3′ position affected SrtA inhibitory activity because compound **1** had greater inhibitory activity than compound **2**. These results show that the co-occurrence of a hydroxyl group at C-3′ and *O*-rhamnoglucoside at C-3 is essential for compound **3** to exhibit strong SrtA inhibitory activity. It is well known that SrtA inhibitors act as antibacterial agents and disrupt the pathogenesis of bacterial infections without affecting microbial viability [10]. Therefore, to eliminate the effects of test compounds on *S. mutans* cell aggregation due to inhibition of cell growth, the minimum inhibitory concentration (MIC) of compounds **1–3** was determined by the microtiter broth dilution method [4]. As shown in Table 1, compounds **1–3** did not display antibacterial activity against *S. mutans* strain OMZ65.

It was expected that inhibitors of SrtA would block SrtA-

Table 1. Inhibitory effects of compounds **1–3** on the activity of SrtA enzyme and cell growth of *S. mutans* strain OMZ65.

Compounds	SrtA IC_{50} (μM)	MIC (μM)
1	134.1 ± 1.6	>419.4
2	185.9 ± 3.4	>409.9
3	210.8 ± 2.9	>847.0
Curcumin	91.5 ± 1.7	>694.9
Berberine chloride	113.8 ± 2.3	>344.1

Curcumin and berberine chloride were used as reference inhibitors of SrtA. IC_{50} values are means ± SD (n = 3).

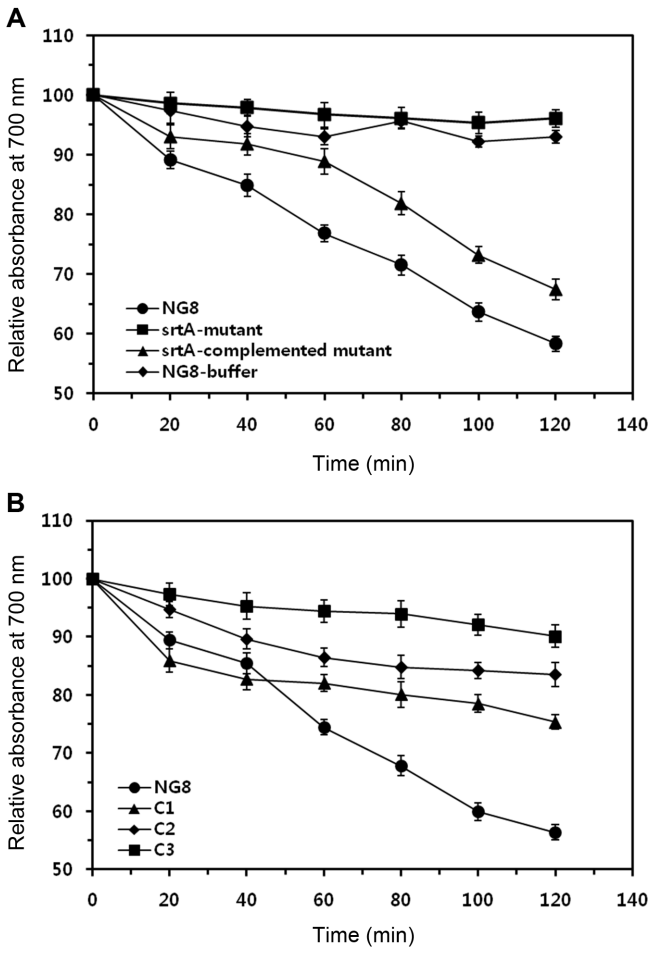

Fig. 2. Effects of compound **1** on saliva-induced aggregation of *S. mutans* NG8 and mutants.

(**A**) Saliva-induced aggregation of *S. mutans* NG8 and mutants without compound **1**. NG8-buffer refers to the aggregation assay performed with *S. mutans* NG8 in the absence of saliva. (**B**) Saliva-induced aggregation of *S. mutans* NG8 treated with compound **1**. C1, C2, and C3 refer to the aggregation assay performed with *S. mutans* NG8 in the presence of 67.1 (1/2 × IC_{50}), 134.1 (1 × IC_{50}), and 268.2 μM (2 × IC_{50}) compound **1**, respectively.

mediated protein anchoring and *S. mutans* cell aggregation. Based on these findings, we next investigated the effect of compound **1** on saliva-induced aggregation of wild-type *S. mutans* strain NG8, as well as its isogenic knockout mutants [8]. Sequence alignments showed that strain NG8 (GenBank Accession No. AF542085) had 100% identity with strain OMZ65 over its entire length (data not shown). Aggregation was assessed as a reduction in optical density at 700 nm. Both NG8 and a *srtA*-complemented mutant, but not the *srtA*-deletion mutant, aggregated upon incubation with saliva (Fig. 2A). As expected, treatment of NG8 with compound **1** significantly reduced bacterial cell aggregation in a dose-dependent manner (Fig. 2B). It is important to note that the onset and magnitude of inhibition of aggregation of NG8 treated with compound **1** (2 × IC_{50}) (Fig. 2B) was comparable to that of an untreated *srtA*-deletion mutant (Fig. 2A). This result was consistent with the observation that compound **1** is a strong inhibitor of SrtA.

In this study, three flavonoids were isolated from the flowers of *S. japonica*, and their inhibitory activities toward SrtA from *S. mutans*, an oral bacterium responsible for human dental caries, were investigated. These studies led to the identification of compounds **1–3** as SrtA inhibitors. Saliva-induced aggregation studies showed that the compound **1** might be a future treatment for *S. mutans* infection by inhibiting SrtA activity. It has been reported that *S. japonica* contains five main flavonoids: rutin, quercetin, isorhamnetin, genistein, and kaempferol [5]. Some of these flavonoids showed antiplatelet aggregating, antioxidant, anti-inflammatory, anti-obesity, and anti-osteoporosis activities [6]. Because sortases are not found in mammals, our findings provide the foundation for future structure–function studies and in vivo studies on this class of sortase inhibitors.

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References

1. Ajdić D, McShan WM, McLaughlin RE, Savić G, Chang J, Carson MB, *et al*. 2002. Genome sequence of *Streptococcus mutans* UA159, a cariogenic dental pathogen. *Proc. Natl. Acad. Sci. USA* **99:** 14434-14439.
2. Colombo PS, Flamini G, Fico G. 2014. *Primula latifolia* Lapeyr and *Primula vulgaris* Hudson flavonoids. *Nat. Prod. Res.* **28:** 1641-1644.
3. Hendrickx AP, Budzik JM, Oh SY, Schneewind O. 2011. Architects at the bacterial surface – sortases and the assembly of pili with isopeptide bonds. *Nat. Rev. Microbiol.* **9:** 166-176.
4. Hu P, Huang P, Chen WM. 2013. Curcumin inhibits the sortase A activity of the *Streptococcus mutans* UA159. *Appl. Biochem. Biotechnol.* **171:** 396-402.
5. Kim JM, Yun-Choi HS. 2008. Anti-platelet effects of flavonoids and flavonoid-glycosides from *Sophora japonica*. *Arch. Pharm. Res.* **31:** 886-890.

6. Kite GC, Veitch NC, Boalch ME, Lewis GP, Leon CJ, Simmonds MS. 2009. Flavonol tetraglycosides from fruits of *Styphnolobium japonicum* (Leguminosae) and the authentication of Fructus Sophorae and Flos Sophorae. *Phytochemistry* **70:** 785-794.
7. Lee KY, Shin DS, Yoon JM, Kang H, Oh KB. 2002. Expression of a transpeptidase for cell wall sorting reaction, from sortase, *Staphylococcus aureus* ATCC 6538p in *Escherichia coli*. *J. Microbiol. Biotechnol.* **12:** 530-533.
8. Lee SF, Boran TL. 2003. Roles of sortase in surface expression of the major protein adhesin P1, saliva-induced aggregation and adherence, and cariogenicity of *Streptococcus mutans*. *Infect. Immun.* **71:** 676-681.
9. Lévesque CM, Voronejskaia E, Huang YC, Mair RW, Ellen RP, Cvitkovitch DG. 2005. Involvement of sortase anchoring of cell wall proteins in biofilm formation by *Streptococcus mutans*. *Infect. Immun.* **73:** 3773-3777.
10. Mazmanian SK, Liu G, Jensen ER, Lenoy E, Schneewind O. 2000. *Staphylococcus aureus* sortase mutants defective in the display of surface proteins and in the pathogenesis of animal infections. *Proc. Natl. Acad. Sci. USA* **97:** 5510-5515.
11. Oh KB, Oh MN, Kim JG, Shin DS, Shin J. 2006. Inhibition of sortase-mediated *Staphylococcus aureus* adhesion to fibronectin via fibronectin-binding protein by sortase inhibitors. *Appl. Microbiol. Biotechnol.* **70:** 102-106.
12. Sang S, Lapsley K, Jeong WS, Lachance PA, Ho CT, Rosen RT. 2002. Antioxidative phenolic compounds isolated from almond skins (*Prunus amygdalus* Batsch). *J. Agric. Food Chem.* **50:** 2459-2463.
13. Selwitz RH, Ismail AI, Pitts NB. 2007. Dental caries. *Lancet* **369:** 51-59.
14. Ton-That H, Liu G, Mazmanian SK, Faull KF, Schneewind O. 1999. Purification and characterization of sortase, the transpeptidase that cleaves surface proteins of *Staphylococcus aureus* at the LPXTG motif. *Proc. Natl. Acad. Sci. USA* **96:** 12424-12429.
15. Xu X, Xie H, Hao J, Jiang Y, Wei X. 2011. Flavonoid glycosides from the seeds of *Litchi chinensis*. *J. Agric. Food Chem.* **59:** 1205-1209.
16. Yang WY, Won TH, Ahn CH, Lee SH, Yang HC, Shin J, Oh KB. 2015. *Streptococcus mutans* sortase A inhibitory metabolites from the flowers of *Sophora japonica*. *Bioorg. Med. Chem. Lett.* **25:** 1394-1397.