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Antihyperglycemic effects of Japanese maple *Acer amoenum* leaf extract and its constituent corilagin

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Abstract The antihyperglycemic effects of the leaves of *Acer amoenum* and purification and identification of an active compound were investigated. In screening experiments for α-glucosidase inhibitory activity, methanolic extracts of *A. amoenum* leaves showed potent inhibitory action. This extract showed antihyperglycemic effects in sucrose-loaded mice. Fractionation of the crude extract gave the active compound corilagin [β-1-*O*-galloyl-3,6-(*R*)-hexahydroxydiphenoyl-D-glucose] by spectroscopic analysis. This is the first report about the possibility of novel utilization of the Japanese maple tree as a source of compounds for prevention or treatment of diabetes mellitus.

Key words *Acer amoenum* · Diabetes mellitus · α-Glucosidase inhibitory effect · Japanese maple · Corilagin

Introduction

As a possible approach to the prevention and treatment of diabetes, decreasing postprandial hyperglycemia has been proposed.[1] Different types of antihyperglycemic drugs are conceivable.[2] In particular, by inhibiting carbohydrate hydrolysis enzymes such as α-glucosidase and α-amylase in the digestive tract, we can delay carbohydrate digestion.

We could also reduce postprandial hyperglycemia by impairing glucose production and diminishing glucose absorption from the small intestine. Such an inhibitory action has been shown to be one of the most effective approaches for preventing diabetes in recent clinical studies.[3] Thus, it is believed that an antihyperglycemic effect caused by the inhibition of α-glucosidase may be important for diabetes care. Worldwide, many kinds of herbs are used for diabetic care[4,5] and in medicinal therapy for diabetes. To identify new compounds that may be useful for preventing or treating diabetes, we screened many kinds of natural products that exert an antihyperglycemic effect. As part of these ongoing experiments, we found that an extract from the leaves of *Acer amoenum* had an antihyperglycemic effect.

Acer amoenum is a kind of Japanese maple. The Japanese maple is an aesthetic symbol in Japan and is very popular as a garden tree. The wood of the tree is also used as material for making furniture, and the leaves are used as artistic items to decoration for Japanese cuisine. Furthermore the leaves of *A. amoenum* are used as food materials in dishes such as tempura and in regional drinks in Japan.

The antioxidative effect of Japanese maple leaves and the constituent vitexin have already been reported.[6] Furthermore, orientin, iso-orientin,[7] and galloylcyanidin glycoside[8] have also been isolated from the leaves of Japanese maple.

Although these effects and ingredients of Japanese maple extract are known, antihyperglycemic effects, mechanisms, and the active compounds responsible have not been investigated. To identify the active antihyperglycemic compound from *A. amoenum* leaves, we performed the α-glucosidase inhibitory test and carbohydrate-loading test in mice.

Materials and methods

Materials

Acer amoenum leaves were collected from the grounds of Tokyo University of Marine Science and Technology in June. The methanol extract of the leaves of *A. amoenum* (AA) was obtained as described below. The leaves were extracted with a tenfold volume of methanol at room temperature for 2 days. After filtration, the extract was evaporated using a rotary evaporator. The recovery rate of AA was 9.0% versus the weight of fresh leaves.

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Animals

Male ddY mice (SLC, Shizuoka, Japan, 6–8 weeks old) were used. The mice were housed in a room maintained at 24° ± 1°C and 50% ± 10% humidity under a 12-h light/dark cycle (lights on from 8:00 A.M. to 8:00 P.M.), and the animals had free access to water and food. Animal studies were conducted according to the 2006 guidelines entitled Notification No. 88 of the Ministry of the Environment in Japan and Guidelines for Animal Experimentation of Tokyo University of Marine Science and Technology with the approval of the Animal Care and Use Committee of Tokyo University of Marine Science and Technology.

Assay for inhibitory activity against α-glucosidase

Intestinal α-glucosidase inhibitory activity was determined as described in the literature with some modification.[9] The activity of α-glucosidase was assayed with crude enzyme solution from mice small intestine, 27.7 mM maltose or 55.3 mM sucrose (final concentrations), with or without AA at different concentrations. Acarbose, which is known to be a major inhibitor of α-glucosidase, was used as a positive control. The resultant glucose levels were measured by commercial assay kit (Glucose C II-test Wako, Osaka, Japan). Each enzyme activity ratio (%) and the IC_{50} value of each sample were calculated by comparison with the control (without sample).

Carbohydrate-loading tests in mice

The mice were fasted for 20 h and divided into groups according to body weight ($n = 8$). Each sample was dissolved in water and administrated orally. In the control group, mice were given sucrose (2000 mg/kg body weight) or glucose (1000 mg/kg body weight). In the AA-treated group, mice were given a mixture of 1000 mg/kg body weight of AA and each carbohydrate. Blood from the tail vein was collected sequentially in test tubes and centrifuged at 4500 *g* for 2 min. The serum was collected and the serum glucose level was measured. The serum glucose level was measured before administration, and at 30, 60, 90, and 120 min after administration. The serum glucose level was measured by the above-mentioned commercial assay kit.

Statistical analysis

Data are presented as the mean ± SE. The significance of differences was calculated using Student's *t* test, and the results were considered statistically significant when $P < 0.05$.

Purification of the active ingredient from AA

To isolate the constituent responsible for the α-glucosidase inhibitory effect, AA was fractionated by using the intensity of the inhibitory effect against sucrase. AA (5.0 g) was partitioned between 90% methanol and *n*-hexane. The 90% methanol layer was chromatographed on an ODS column (Wakogel C-200, Wako) with a water–methanol gradient and ethyl acetate elution to give three fractions. The active fraction, Fr. 1, eluted with 40% methanol, was subjected to HPLC using an ODS column (Develosil HG-5 20 × 250 mm; Nomura, Aichi, Japan) with 40% methanol at a flow rate of 8.0 ml/min, detected at UV 215 nm, to give seven fractions. The active fraction, Fr. 1-3, was further purified by the same HPLC system using a more polar mobile phase (30% methanol) and was divided into thirteen fractions to give Fr. 1-3-7 (**1**, 23.5 mg, t_R: 9.2 min) as the active compound.

Spectroscopic analysis

To determine the chemical structures of purified compounds, NMR spectra were recorded with a 400-MHz NMR spectrometer (AV-400, Bruker) and MS spectral data were obtained on an electrospray inonization (ESI) mass spectrometry (QSTAR, Applied Biosystems).

Compound **1**, corilagin [beta-1-*O*-galloyl-3,6-(*R*)-hexahydroxydiphenoyl-D-glucopyranose] (**1**): dark reddish brown crystal: ESIMS *m/z*: 657 $[M + Na]^+$; ^{1}H NMR (D_2O, 400 MHz); δ 6.89 (2H, *s*, galloyl-H‴), 6.70 (1H, *s*, galloyl-H′), 6.54 (1H, *s*, galloyl-H″), 6.25 (1H, *brs*, H-1), 4.81 (1H, *m*, H-6a), 4.80 (1H, *m*, H-3), 4.58 (1H, *t*, $J = 9.5$ Hz, H-5), 4.41 (1H, *brs*, H-4), 4.19 (1H, *t*, $J = 9.3$ Hz, H-6b), 4.13 (1H, *brs*, H-2); ^{13}C-NMR (D_2O, 100 MHz); δ 169.4 (C-7′), 167.8 (C-7″), 166.1 (C-7‴), 144.7 (C-5′), 144.5 (C-5″), 144.4 (C-3‴ and C-5‴), 143.7* (C-3′), 143.6* (C-3″), 138.8 (C-4‴), 136.4 (C-4″), 135.3 (C-4′), 125.0** (C-2′), 123.5** (C-2″), 119.2 (C-1‴), 115.2 (C-1″), 114.3 (C-1′), 110.3 (C-2‴ and C-6‴), 110.0 (C-6″), 107.2 (C-6′), 94.7 (C-1), 73.9 (C-5), 68.6 (C-3), 65.8 (C-2), 63.5 (C-6), 60.0 (C-4). Signals with single or double asterisks may be interchanged.

Results

Effect of AA on α-glucosidase activity

To confirm the effects of AA on α-glucosidase activity, in vitro experiments were performed with sucrose or maltose as the substrate. The IC_{50} values of AA and acarbose against sucrase activity were 180.5 ± 5.1 and 0.8 ± 0.2 μg/ml, respectively. Against maltase activity, these values were 636.5 ± 5.0 and 0.5 ± 0.1. The effect of AA on α-glucosidase activity is also shown in Fig. 1. At 800 μg/ml of AA, sucrase and maltase activities were 74.2% and 55.3% suppressed, respectively.

Effect of AA on the serum glucose level in carbohydrate-loaded mice

Figure 2 shows the antihyperglycemic effects of AA in sucrose-loaded mice. The serum glucose level in the AA

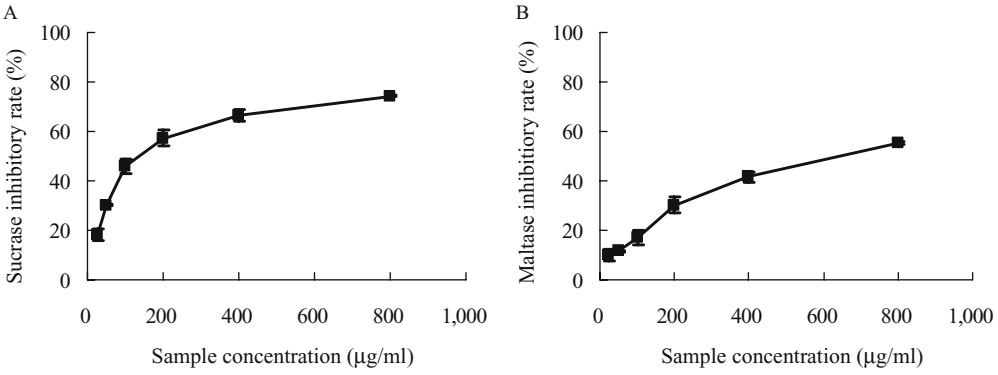

Fig. 1A,B. Inhibitory effect of methanol extract of *Acer amoenum* (AA) on the activity of α-glucosidase. Inhibitory effect of AA on the activity of murine small intestinal α-glucosidase for the hydrolysis of sucrose (**A**) and maltose (**B**) are shown. Data are presented as means ± SE ($n = 3$)

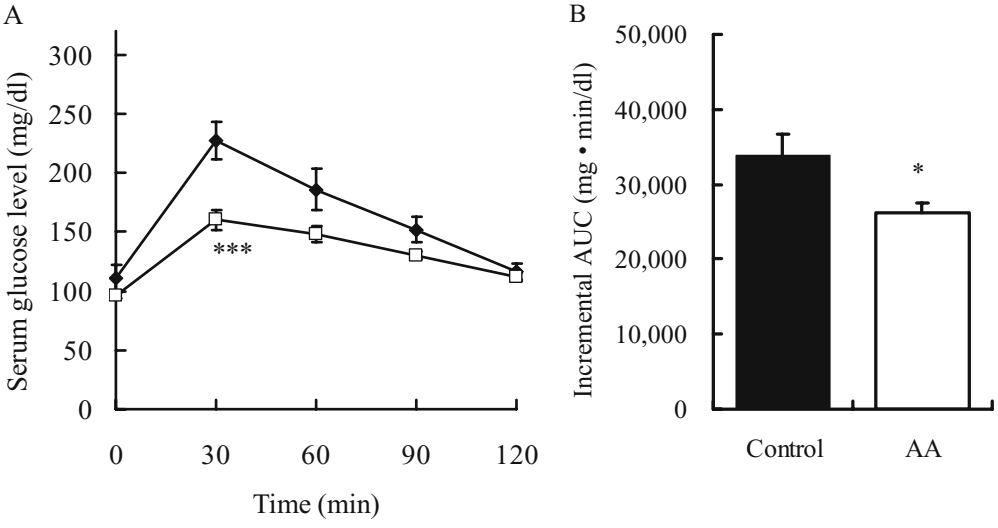

Fig. 2A,B. Effect of *Acer amoenum* extract (*AA*) on the serum glucose levels in sucrose-loaded mice. Fasted mice were given 2000 mg/kg of sucrose with (*squares*) or without (*diamonds*) 1000 mg/kg of AA, and serum glucose levels were monitored. Serum glucose levels (**A**) and the areas under the curves (*AUC*) (**B**) are shown. Data are presented as means ± SE ($n = 8$). *$P < 0.05$, ***$P < 0.005$ versus control

group was significantly lower than that in the control group. In the sucrose-loading test, the serum glucose level at 30 min after administration in the AA group was 160.2 ± 8.2 mg/dl, while in the control group the value was 227.8 ± 15.9 (Fig. 2A). The total incremental area (area under the curve: AUC) of serum glucose level over 120 min for the AA group was also significantly lower than that for control group (Fig. 2B). It was thus found that the administration of 1000 mg/kg AA had a suppressive effect on hyperglycemia in sucrose-loading tests. In contrast, AA had no significant effect in glucose-loading tests (Fig. 3).

Identification of corilagin (**1**)

Compound **1** was identified as follows. The ^{13}C-NMR spectrum revealed 27 carbon atoms and the ESIMS data ($[M + Na]^+ = 657$) suggested the molecular formula to be $C_{27}H_{22}O_{18}Na$. The ^{1}H- and ^{13}C-NMR spectra showed the presence of a sugar and three galloyl moieties. The structure of compound **1** was identified as the known galloyl glucose corilagin (Fig. 4) by further analysis of 1D and 2D NMR spectra. Our spectrum data for corilagin were compared with those in the reports of Wei et al.[10] and Gao et al.[11]

Effect of corilagin on α-glucosidase activity

Corilagin exerted an α-glucosidase inhibitory effect. The IC_{50} values of corilagin for sucrase and maltase were calculated to be 79.8 ± 5.6 and 107.5 ± 10.7 μg/ml, respectively.

Effect of corilagin on the serum glucose level in sucrose-loaded mice

Corilagin (15 mg/kg) isolated from AA was used in an in vivo experiment in sucrose-loaded mice (2000 mg/kg). As shown in Fig. 5, the serum glucose levels in the treated group were significantly lower than those in the control group at 60, 90, and 120 min (Fig. 5A). The incremental AUC of serum glucose level for the corilagin group was also significantly lower than that for the control group. It was thus found that the administration of 15 mg/kg corilagin had a suppressive effect on hyperglycemia in sucrose-loading tests.

Discussion

This report is the first to show that *Acer amoenum* extracts and its constituent corilagin exert antihyperglycemic effects

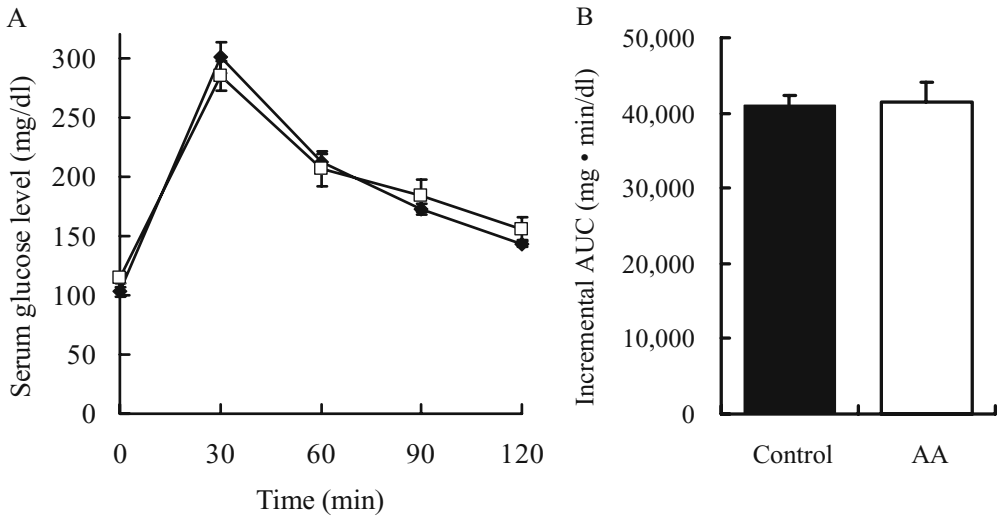

Fig. 3A,B. Effect of AA on the serum glucose level in glucose-loaded mice. Fasted mice were given 1000 mg/kg of glucose with (*squares*) or without (*diamonds*) 1000 mg/kg of AA and serum glucose levels were monitored. Serum glucose levels (**A**) and the areas under the curves (**B**) are shown. Data are presented as means ± SE ($n = 8$)

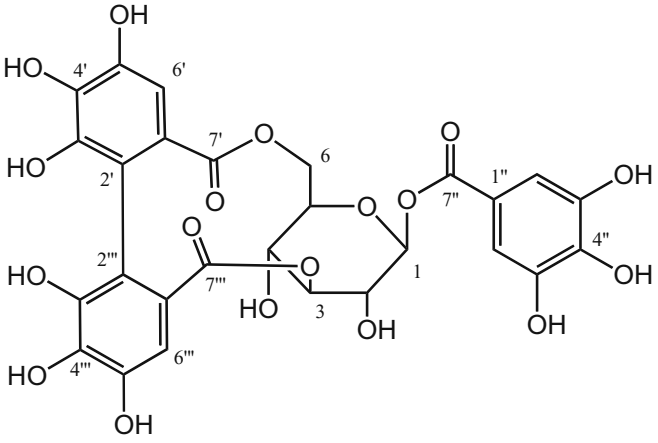

Fig. 4. Chemical structure of corilagin (**1**) from *Acer amoenum*

through a mechanism that involves the inhibition of α-glucosidase. As revealed in Fig. 2, AA exhibited an anti-hyperglycemic effect in a sucrose-loading test; however, in Fig. 3, it was shown that AA did not have a similar effect in a glucose-loading test. Thus, the antihyperglycemic effect of AA is due to an inhibitory effect on α-glucosidase, which is a disaccharide hydrolysis enzyme; an in vitro test showed such an effect in Fig. 1. In fact, both in vivo and in vitro, AA showed the same degree of antihyperglycemic effect and α-glucosidase-inhibitory effect as an extract of guava (*Psidium guajava*) leaves,[12] which is a medicinal herb that is used to treat diabetes (data not shown), whereas acarbose showed more effective inhibition in vitro than they did. Some side effects of medicinal drugs, including α-glucosidase inhibitors, have been reported;[13] however, many kinds of natural product have been used for the prevention or treatment of diabetes for many centuries.[4,5]

We purified an active ingredient from AA and a spectroscopic analysis of its structure revealed that the compound was corilagin (**1**), as shown in Fig. 4. In Fig. 5, corilagin demonstrated an antihyperglycemic effect in a sucrose-loading test. The AUC values of AA and corilagin were 22.4% and 12.9%, respectively, lower than those of the control.

By comparing the IC_{50} values of AA and corilagin, it was found that the sucrase- and maltase-inhibitory effects of corilagin were 2.3- and 5.9-fold more effective than AA, respectively; however, the recovery rate of corilagin purified from AA was 0.47%. Therefore corilagin may not be the only active compound, and other active compounds may also contribute to the inhibitory effect of AA on α-glucosidase.

There have been some reports on the compounds present in plants of the *Acer* genus. These have included flavonoid structures, such as kaempferol,[14] afzelin, quercitrin,[15] and anthocyanins.[16] Some flavonoids have been reported to have an antihyperglycemic effect through the inhibition of α-glucosidase.[17,18]

Moreover, some reports have shown that plants of the *Acer* genus contain galloyl derivatives, such as methyl gallate,[19] gallotannins,[20] and flavonol glycoside gallate ester.[21] In addition, a galloylcyanidin glycoside has been purified from Japanese maple.[8] It has been reported that some galloyl derivatives also showed α-glucosidase-inhibitory activity, such as epigallocatechin gallate from tea (*Camellia sinensis*)[22] and ellagitannins from clove (*Syzygium aromaticum*).[23] Tannins show high affinity for sugars,[24] and tannin and sugars may form hydrogen bonds through the galloyl part of these compounds in the small intestine, and this may prevent the absorption of sugars. In addition, the galloyl part may inhibit enzymes through a high affinity for proteins.[25]

Corilagin is a member of the tannin family that has been discovered in medicinal plants such as *Terminalia catappa*,[26] *Terminalia chebula*,[11] *Dimocarpus longan*,[27] and *Phyllanthus niruri*.[10] This compound has also been isolated from the bark of *Acer nikoense*.[28] It has been reported that corilagin has some interesting bioactivities, such as antioxidative,[26] anti-inflammatory,[29] antihypertensive,[30] and antimicrobial effects.[31] These results suggest the possibility of the novel utilization of the Japanese maple to provide compounds for prevention or treatment of diabetes mellitus.

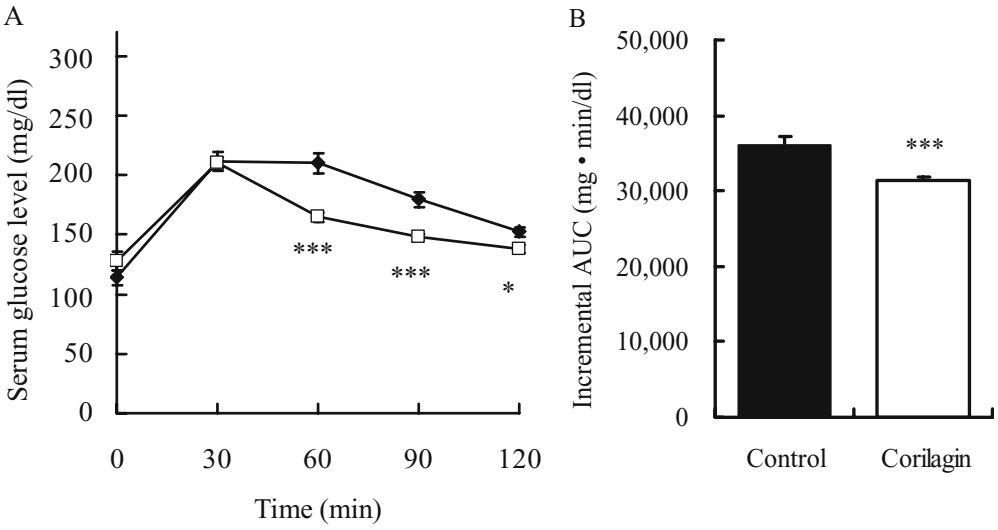

Fig. 5A,B. Effect of corilagin on the serum glucose levels in sucrose-loaded mice. Fasted mice were given 2000 mg/kg of sucrose, with (*squares*) or without (*diamonds*) 15 mg/kg of corilagin, and serum glucose levels were monitored. Serum glucose levels (**A**) and the areas under the curves (**B**) are shown. Data are presented as means ± SE ($n = 8$). *$P < 0.05$, ***$P < 0.005$ versus control

Conclusions

An extract from *Acer amoenum* had an antihyperglycemic effect and an α-glucosidase inhibitory effect. The active compound was purified and identified as corilagin. It is possible that compounds present in *A. amoenum* may be useful for prevention or treatment of diabetes mellitus.

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