Antihyperglycemic Effects of N-Containing Sugars from *Xanthocercis zambesiaca, Morus bombycis, Aglaonema treubii,* and *Castanospermum australe* in Streptozotocin-Diabetic Mice

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The aqueous MeOH extract of the leaves and root of *Xanthocercis zambesiaca* (Leguminosae) and eight structurally related nitrogen-containing sugars, fagomine (**1**), 4-*O*-*β*-D-glucopyranosylfagomine (**2**), 3-*O*-*β*-D-glucopyranosylfagomine (**3**), 3-epifagomine (**4**), 2,5-dideoxy-2,5-imino-D-mannitol (**5**), castanospermine (**6**), α-homonojirimycin (**7**), and 1-deoxynojirimycin (**8**) were evaluated for antihyperglycemic effects in streptozotocin (STZ)-diabetic mice. The insulin-releasing effects of **1** were also investigated. The blood glucose level fell after ip injection of the extract (50 mg/kg). Compounds **1**, **2**, **5**, and **6** reduced the blood glucose level after ip injection of 150 *μ*mol/kg. Compound **1** increased plasma insulin level in STZ-diabetic mice and potentiated the 8.3-mM glucose-induced insulin release from the rat isolated-perfused pancreas. The **1**-induced potentiation of insulin release may partly contribute to antihyperglycemic action.

Mulberry leaves (*Morus* spp.) have been used in traditional Chinese medicine as an antihyperglycemic.[1,2] Among its constituents are nitrogen (N)-containing sugars. Some N-containing sugars are reported to have a potent α-glucosidase inhibitory activity similar to clinically available oral antidiabetic drugs.[3,4] We have reported that fagomine (**1**), one of the N-containing sugars obtained from *Morus bombycis* Koidzumi (Moraceae), produced a potent antihyperglycemic effect in streptozotocin (STZ)-diabetic mice.[5] Recently, it has been reported that **1** occurs abundantly in the leaves and root of *Xanthocercis zambesiaca* (Bak.) Dunn, a member of the Sophoreae tribe of Leguminosae, growing in dry forest and woodland of southern Africa.[6] In the present study, we examined, in STZ-diabetic mice, the antihyperglycemic effects of an aqueous MeOH (1:1) extract prepared from *X. zambesiaca*, along with eight N-containing sugars (**1**−**8**, Chart 1) isolated from various plants including *X. zambesiaca*. In an attempt to determine the mechanism of antihyperglycemic action of **1**, its effect on the plasma insulin level in STZ-diabetic mice and the insulin-releasing effect with perfused-pancreas preparations of normal rats were also investigated.

The effects of an aqueous MeOH (1:1) extract from the leaves and roots of *X. zambesiaca* and glibenclamide, a representative antidiabetic drug, on blood glucose level were examined in STZ-diabetic mice. The extract (50 mg/kg, ip) reduced significantly the blood glucose level from 247 ± 19 mg/dL (n = 6, mean ± S.E.M.) to 154 ± 26 mg/dL and 143 ± 24 mg/dL, 4 h and 6 h, respectively, after administration (Figure 1). In the control group (n = 6), the blood glucose level was not significantly reduced 6 h after administration of saline. Glibenclamide (30 *μ*mol/kg, ip) reduced, over the same time course, the blood glucose level from 270 ± 23 mg/dL (n = 5) to 138 ± 15 mg/dL 6 h after administration. These results indicate that the extract from *X. zambesiaca* may be useful clinically in the treatment of diabetes.

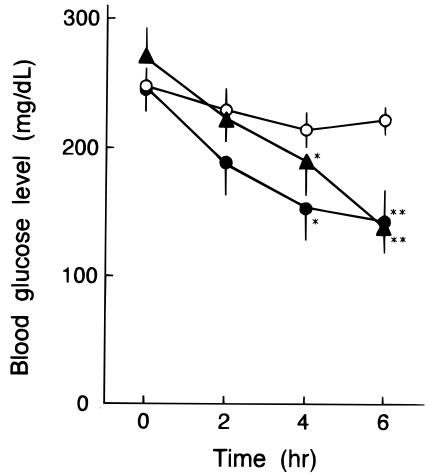

Figure 1. Time-dependent lowering of blood glucose levels induced by an aqueous MeOH (1:1) extract from *X. zambesiaca* and glibenclamide in STZ-diabetic mice. The extract (50 mg/kg, ●), glibenclamide (30 *μ*mol/kg, ▲), and saline (○) were administered intraperitoneally at the time of 0. The values are the means ± S.E.M. of 5−10 mice. *p < 0.05, **p < 0.01: Significantly different from the value at the time of 0 by one-way ANOVA and then Dunnet's multiple-range test.

The effects of eight structurally related N-containing sugars on blood glucose level were also examined in STZ-diabetic mice. Compound **1** (150 *μ*mol/kg, ip)

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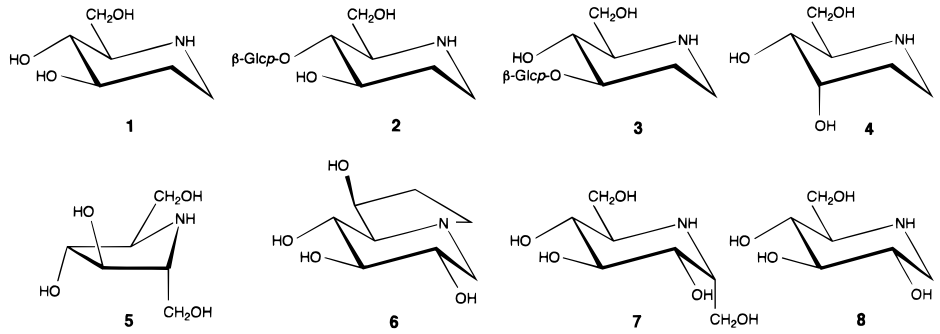
Chart 1



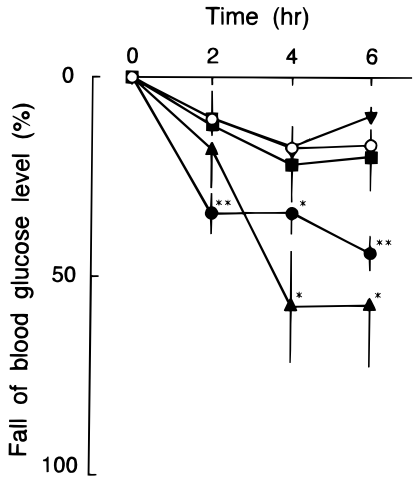


Figure 2. Time-dependent antihyperglycemic effects of compounds **1**−**4** in STZ-diabetic mice. Compounds **1** (●), **2** (▲), **3** (▼), **4** (■) and saline (○) were administered intraperitoneally at a dose of 150 μmol/kg at the time of 0. The values are the means ± S.E.M. of 5−10 mice. *p < 0.05, **p < 0.01: Significantly different from saline-control by one-way ANOVA and then Scheffe's multiple-comparison test.

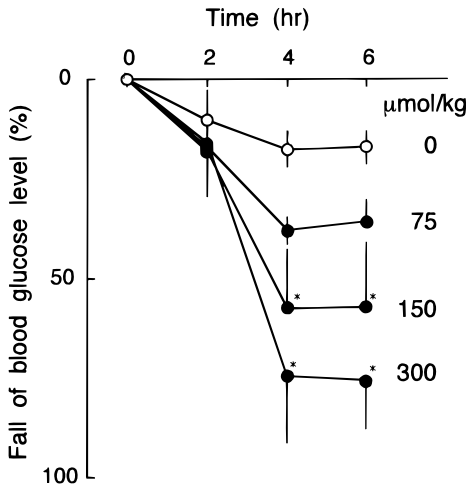


Figure 3. Dose-dependent antihyperglycemic effects of compound **2** in STZ-diabetic mice. Compound **2** (●) at the doses of 75, 150, and 300 μmol/kg and saline (○) were administered intraperitoneally at the time of 0. The values are the means ± S.E.M. of 5−10 mice. *p < 0.05: Significantly different from saline-control by one-way ANOVA and then Scheffe's multiple-comparison test.

reduced significantly the blood glucose level 2 h after administration, and the antihyperglycemic activity was sustained over a 2- to 6-h period (Figure 2). Compound **1** (300 μmol/kg, ip) had no effect on blood glucose levels during the fasting state in normal mice (data not shown). Compound **2** reduced the blood glucose at a later time (4 h) than **1** post dosing, and the antihyperglycemic effects were produced in a dose-dependent manner (75 μmol/kg to 300 μmol/kg, ip) (Figures 2 and 3). Compound **2** may be metabolized to **1** by β(1−4)-glucosidase in vivo, because there was a time lag in the occurrence of blood glucose lowering when compared with **1**. Intraperitoneal administration of 150 μmol/kg of **3** or **4** had no effect on the blood glucose levels in STZ-diabetic mice (Figure 2). The free hydroxyl group at C-3 in **1** may play an important role in its efficacy. Compounds **1** and **2** are present in concentrations of 8.6−11.2% and 0.3−1.0%, respectively, in the aqueous MeOH (1:1) extract of the dry leaves and the root of *X. zambesiaca*.[6] Therefore, unidentified compounds contained in the plant may also contribute to the antihyperglycemic activity induced by this extract, although **1** and **2** appear to be the primary active ingredients.

Of the four other related compounds, **5** and **6** significantly reduced blood glucose 4 h, and 4−6 h, respectively, following ip administration (150 μmol/kg, Figure 4). Intraperitoneal administration of 150 μmol/kg of **7** and **8** had no effect on blood glucose levels in STZ-

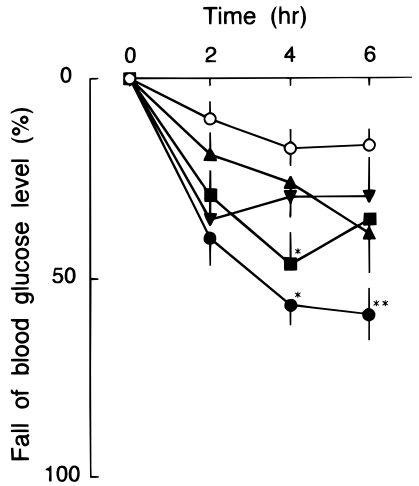


Figure 4. Time-dependent antihyperglycemic effects of compounds **5**−**8** in STZ-diabetic mice. Compounds **5** (●), **6** (■), **7** (▲), **8** (▼) and saline (○) were administered intraperitoneally at a dose of 150 μmol/kg at the time of 0. The values are the means ± S.E.M. of 5−10 mice. *p < 0.05, **p < 0.01: Significantly different from saline-control by one-way ANOVA and then Scheffe's multiple-comparison test.

diabetic mice. These results indicate that a hydroxylation at C-2 of **1** markedly reduces the **1**-induced antihyperglycemic activity. We have already reported that 2-*O*-α-D-galactopyranosyl-DNJ produced a potent

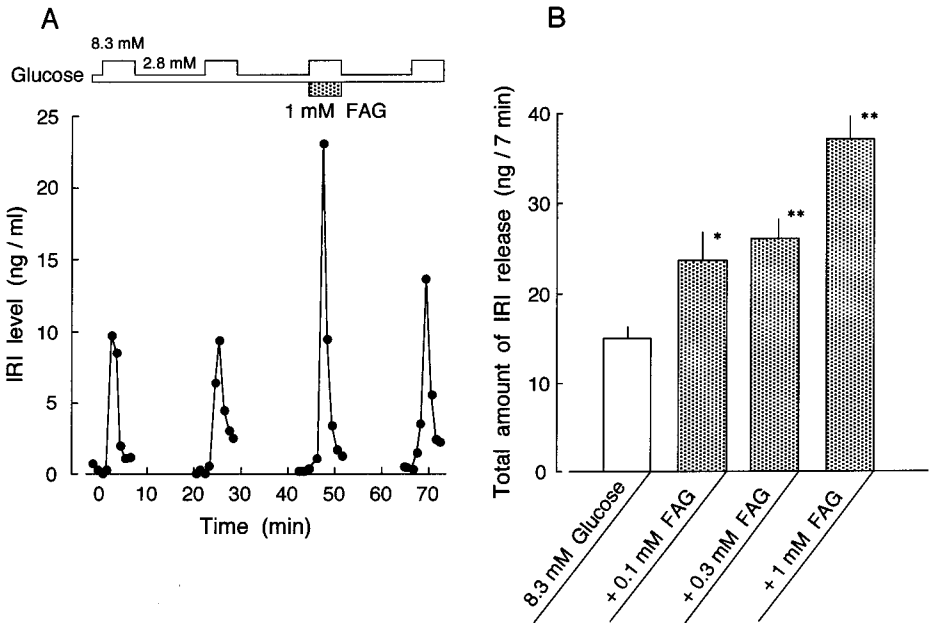


Figure 5. Potentiating effect of compound **1** (FAG) on 8.3-mM glucose-induced immunoreactive insulin (IRI) release from perfused pancreas of normal rat. (**A**) Typical data of time course of increase in IRI level induced by 8.3 mM glucose and the potentiating effect of 1 mM FAG. (**B**) Concentration-dependent potentiating effects of FAG in the total amount of IRI release for 7 min at 8.3 mM glucose. An open column and dotted columns show the total amount of IRI release without and with FAG (0.1−1 mM), respectively. The values are the means ± S.E.M. of 4−16 rats. $*p < 0.05$, $**p < 0.01$: Significantly different from the amount at 8.3 mM glucose alone by one-way ANOVA and then Scheffe's multiple-comparison test.

antihyperglycemic effect in STZ-diabetic mice.[5] This suggests that galactosidation at C-2 of **8** elicits antihyperglycemic activity.

The effect of **1** on plasma insulin levels in STZ-diabetic mice was examined. In five mice, compound **1** (300 μmol/kg, i.p.) increased significantly ($*p < 0.05$) the plasma insulin levels to 454 ± 12 pg/mL and 560 ± 48 pg/mL 30 min and 2 h, respectively, after ip administration, when compared with those before (385 ± 24 pg/mL). In the other five mice, saline (ip) did not increase significantly plasma insulin levels until 2 h (462 ± 34 pg/mL) after ip administration when compared with those before (403 ± 18 pg/mL). These results suggest that **1** may stimulate the insulin release in vivo in STZ-diabetic mice to cause blood glucose lowering.

It is well known that some N-containing sugars have an α-glucosidase inhibitory activity.[3,7−9] Compound **8** has been shown to be a potent inhibitor of all types of mammalian α-glucosidases.[10,11] In the present study, it was shown that **8** did not elicit an antihyperglycemic action after ip administration. Compounds **1** and **5**, which are weaker than **4** as α-glucosidase inhibitors,[12] were more potent than **4** as antihyperglycemics. Therefore, α-glucosidase inhibition does not play a key role in the antihyperglycemic effect of N-containing sugars by ip administration.

The effects of **1** and **2** on immunoreactive insulin (IRI) release from perfused pancreas of normal rat were also examined. The amount of IRI released into the perfusate was increased by changing the concentration of glucose from 2.8 mM to 8.3 mM (Figure 5A). The 8.3-mM glucose-induced IRI release was increased markedly in the presence of 1 mM of **1**. The effect of **1** was reversible as the IRI release response returned to normal after wash out. Compound **1** (0.1−1 mM), in a concentration-dependent manner, enhanced significantly the total amount of IRI that was released for 7 min by 8.3-mM glucose stimulation (control; 15.1 ± 1.3 ng, $n = 16$) (Figure 5B). Compound **1** had no effect on IRI release in the perfusate containing 2.8 mM of glucose at a concentration of 1 mM (data not shown). Glibenclamide also potentiated glucose-induced insulin release from isolated perfused pancreas of normal rats.[13] These results indicate that **1** potentiates glucose-stimulated insulin release in perfused rat pancreas similarly to glibenclamide. Compound **2** had no effect on the 8.3-mM glucose-induced IRI release at a concentration of 1 mM (Figure 6). The lack of potentiating effects of **2** on insulin release may support the hypothesis that **3** is hydrolyzed over time to the active **1**.

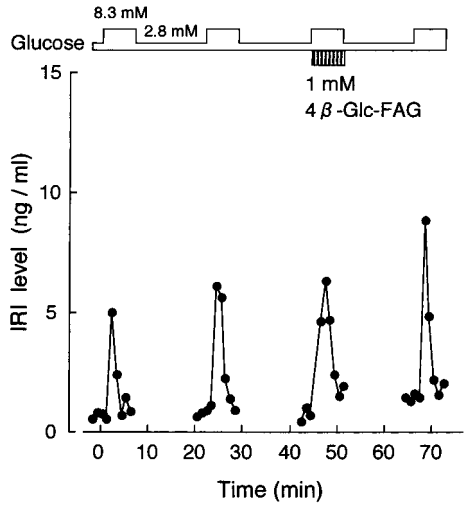


Figure 6. Effect of compound **2** (4β-Glc-FAG) on 8.3-mM glucose-induced immunoreactive insulin (IRI) release from perfused pancreas of normal rat. 4β-Glc-FAG (1 mM) did not affect a 8.3-mM glucose-induced increase in IRI level.

The extract of *X. zambesiaca* in clinical treatment of diabetes may be useful because it contains N-containing

sugars having both an α-glucosidase inhibitory activity and an insulin-releasing action. In conclusion, **1** and **2** may play an important role in the antihyperglycemic action of the extract from *X. zambesiaca*, and the **1**-induced potentiation of insulin release may partly contribute to that action.

Experimental Section

Plant Materials. *X. zambesiaca* (Bak.) Dunn. was grown in the Institute of Grassland and Environmental Research, Aberystwyth, U. K. The specimen was grown from seeds collected in Zimbabwe (Zhou National Area), and a herbarium specimen is maintained at the Herbarium Botanic Garden in Harare (Lundi K. Gona 2131 B4 1982). Whole plants of *Aglaonema treubii* (Araceae) were collected in July 1995, at the Medicinal Plant Garden of Hokuriku University, Kanazawa, Japan. A voucher specimen representing this collection (NA 9601) has been deposited at the herbarium of the Institute of Grassland and Environmental Research, Aberystwyth, U.K. The leaves of *M. bombycis* (Moraceae) were obtained from a commercial source.

Compounds Used. The aqueous MeOH (1:1) extract from *X. zambesiaca* was obtained by extraction (4 °C, 24 h) from the dry leaves and the root separately homogenized in aqueous MeOH (1:1), following filtration and concentration. The isolation procedures used for **1**−**4** from the extract were as reported.[6] The isolation procedures used for **5** and **8** from *M. bombycis* (Moraceae) and for **7** from whole plants of *A. treubii* (Araceae) were as reported.[12,14] Compound **6** was a gift from Prof. R. J. Nash (Institute of Grassland and Environmental Research, Aberystwyth, U. K.). Glibenclamide (Research Biochemicals Inc., MA) was suspended in saline and used as a positive control.

Animals. Male ddY mice (4 weeks of age, 18−23 g) and male Wistar rats (6−7 weeks of age, 200−250 g) were purchased from Japan SLC (Shizuoka, Japan). Mice were injected with 150 mg/kg of STZ (Sigma, St. Louis, MO) into the tail vein and were used 4 weeks after dosing. These mice were used after fasting for 12−14 h before each experiment.

Determination of Blood Glucose Levels. Under anesthesia with ether, the blood sample (20 μL) was obtained from the orbital venous plexus using capillary glass tubes prior to and 2, 4, and 6 h after injection. The blood glucose levels were measured by the glucose oxidase method[15] using a glucose analyzer II (Beckman Instruments, Inc., CA).

Evaluation of Antihyperglycemic Activity. The antihyperglycemic activity of test compounds in STZ-diabetic mice was estimated, as previously reported.[16] It was evaluated as the percentage of one value (before − after) against another (before − 85), where before and after represent a blood glucose level before and after injection of test compounds, respectively, and 85 (mg/dL) represents the mean blood glucose level in fasting state of normal mice.

Determination of Plasma Insulin Levels in STZ-Diabetic Mice. Under anesthesia with ether, the blood samples were obtained from the orbital venous plexus using capillary glass tubes before and 30 min and 2 h after injection. The blood insulin levels were determined by an insulin ELISA kit (Seikagaku Corporation, Tokyo, Japan).

Determination of Immunoreactive Insulin (IRI) Release from Perfused Rat Pancreas. Rat pancreas preparation was made as previously described.[17] Rats were anesthetized with sodium pentobarbital (Nembutal, Abbott Labs, IL; 50 mg/kg, ip), and the pancreas was isolated along with the stomach, duodenum, and spleen in order to keep the pancreas intact. All vessels leaving the pancreas were ligated, except for the portal vein. Perfusion was carried out through the celic artery with a basal medium of Krebs−Ringer bicarbonate buffer (pH 7.4) saturated with a gas mixture of 95% O_2 and 5% CO_2, containing 0.5% bovine serum albumin, 2% dextran (T-70, Pharmacia, Uppsala, Sweden), and 2.8 mM D-glucose. The flow rate was kept at 1 mL/ min. Every 1 min, 1 mL of perfusate was collected from a portal vein catheter. The levels of IRI in perfusate were determined by radioimmunoassay.

Statistical Analysis. Significant differences between mean values before and after administration of a compound or between mean values of saline control and treatment were statistically analyzed by one-way ANOVA and then Dunnet's or Scheffe's multiple-comparison tests. All data are the mean ±S.E.M.

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