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Aldose reductase inhibitory effect by tectorigenin derivatives from *Viola hondoensis*

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Abstract—Aldose reductase (AR), the key enzyme of the polyol pathway, is known to play important roles in the diabetic complication. The inhibitors of AR, therefore, would be potential agents for the prevention of diabetic complication. The AR inhibition activity of several isoflavonoids was evaluated in vitro against rat lens. Tectoridin-4′-*O*-β-D-glucoside exhibited strong AR inhibition activity on rat lens with an IC_{50} of 0.54 μM. Similar activities were recorded for the natural tectorigenin and tectoridin. In contrast, tectoridin-4′-*O*-β-D-glucoside showed a stronger inhibitory activity than tectorigenin and tectoridin. Our results indicate that glucose conjugation position in this type of isoflavonoids may be required for the activity.

1. Introduction

Aldose reductase (AR), the key enzyme of the polyol pathway, has been demonstrated to play important roles not only in the cataract formation in the lens but also in the pathogenesis of diabetic complications such as neuropathy, nephropathy, and retinopathy. Evidence suggests that compounds which inhibit AR could be effective for the prevention of diabetic complications. A number of structurally diverse naturally occurring and synthetic AR inhibitors have been studied in vivo to clarify their effectiveness for prevention of cataract formation as well as diabetic complications in experimental animals as well as in clinical trials. It is well established that natural products are an excellent source of chemical structures with a wide variety of biological activities.[1] This has opened up new fields of investigation of potential active compounds, some of which are already widely used in diabetic complication chemotherapy. In our screening program to search for AR inhibitors from plants, a MeOH extract of the aerial parts of *Viola hondoensis* exhibited AR inhibitory activity (>70% inhibition at 25 μg/mL), which led us to investigate the AR inhibitory compounds from this plant. *V. hondoensis* belongs to Violaceae and is distributed in the southern part of Korea.[2] In traditional medicine, the herb has been used as an expectorant and an skin eruptions.[3] Previous pharmacological and phytochemical studies on *Viola* species have revealed it to be a rich source of cyclotides,[4] several flavone glycosides.[5] Despite a number of studies on the genus Violaceae, there have been no investigations regarding the chemical constituents and the AR inhibitory activity of *V. hondoensis*. Therefore, we have investigated the AR inhibitory compounds of this plant. Bioassay-guided fractionation of a MeOH extract of the whole plants of this plant led to the isolation of four isoflavonoids, tectoridin-4′-*O*-β-D-glucoside (**1**), tectorigenin (**2**), tectoridin (**3**), and tectorigenin-4′-*O*-β-D-glucoside (**4**), as the active principles. In this report, we describe the isolation and structure determination of these compounds, and the evaluation of their AR inhibitory activity. This type of tectorigenin has been reported to possess AR inhibitory activity.[6] However, to our knowledge, AR inhibitory activity of this glucose conjugation position of tectorigenin is now being reported for the first time in this study (Fig. 1).

2. Results and discussion

The AR inhibitory activity of the isolates was tested in vitro, and the results are presented in Table 1. Of

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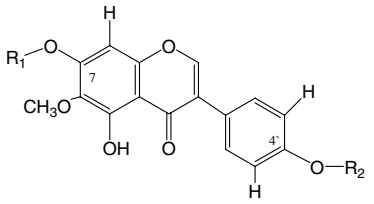

	R1	R2
1:	Glu	Glu
2:	H	H
3:	Glu	H
4:	H	Glu

Figure 1. Structure of compounds **1**–**4** isolated from *V. hondoensis*.

Table 1. Inhibitory effects of isolated compound on rat lens aldose reductase

Compounds	Inhibition IC_{50} (μM)
Tectoridin-4′-*O*-β-D-glucoside (**1**)	0.54 ± 0.02
Tectorigenin (**2**)	1.12 ± 0.08
Tectorigenin-7-*O*-β-D-glucoside (Tectoridin; **3**)	1.26 ± 0.05
Tectorigenin-4′-*O*-β-D-glucoside (**4**)	0.87 ± 0.03
TMG (Tetramethylene glutaric acid)	0.48 ± 0.05

Inhibition rates were calculated as percentages with respect to the control value.

the compounds tested, tectoridin-4′-*O*-β-D-glucoside (**1**) which possesses a group at C-4′, seven of glucose conjugation exhibited the most potent inhibitory activity (IC_{50} = 0.54 ± 0.02 μM). However, compound **2** (IC_{50} = 1.12 ± 0.08 μM) substituted a non-glucoside group at C-4′ and C-7 exhibited significantly lower activity than **1**. A similar case was observed between compounds **3** and **4**. Tectorigenin-4′-*O*-β-D-glucoside (**4**) without a glucoxyl group at C-7 was much more effective than **3** with a glucose group. These results indicate that substitution of a glucose group at C-4′ of tectorigenin increases the inhibitory activity of AR. Furthermore, we have found that the substitution of a glucose group of 4′ position of tectorigenin appeared to induce the inhibitory activity of AR. The AR inhibitory activity of isolated isoflavonoid in diabetic complication-related animal models has not been directly evaluated yet. However, our results suggest the anti-diabetic complication effect of this 4′-*O*-glucose-7-*O*-glucose type of tectorigenin warrants further investigation and optimization.

3. Experimental

3.1. Isolation and identification of the examined compounds

The whole plants of *V. hondoensis* (Violaceae) were collected in April 2004 at Ullung island, Korea. The botanical identification was made by Dr. Joongku Lee. A voucher specimen of this raw material has been deposited at the herbarium of the Seoul National University (SNU-03-10-01). DL-Glyceraldehyde, sodium phosphate, and adenine dinucleotide phosphate reduced form (NADPH) were purchased from Sigma Chem. Co. (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade. The air-dried aerial parts from *V. hondoensis* (430 g) were extracted with MeOH at 80 °C (3 × 1 L for 4 h). The MeOH extract (53 g, IC_{50} = 22.8 μg/mL) was suspended in water and successively partitioned with *n*-hexane (3 × 3 L, 12 g), $CHCl_3$ (3 × 3 L, 3 g), EtOAc (3 × 3 L, 7 g), and *n*-BuOH (3 × 3 L, 13 g) to obtain hexane-soluble fraction (IC_{50} = 15.4 μg/mL), EtOAc-soluble fraction (IC_{50} = 6.4 μg/mL), BuOH-soluble fraction (IC_{50} = 77.2 μg/mL), and H_2O-soluble fraction (IC_{50}> 92.3 μg/mL), respectively. Since the EtOAc-soluble fraction showed the strongest AR inhibitory activity, this fraction (6.5 g) was further purified by silica gel column chromatography (3 × 50 cm; 63–200 μm particle size) using a stepwise gradient of CH_3Cl/MeOH (from 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 3:1, 1:1 to 0:1; 500 mL for each step) in low air pressure, to yield six fractions (Fr. 1–Fr. 6). Of these, Fr. 1, Fr. 2, and Fr. 3 showed the most potent AR inhibitory activity (75%, 82%, and 73% inhibition at 10 μg/mL). Fr. 2 [eluted with CH_3Cl/MeOH (8:1), 1.4 g] was chromatographed over silica gel (2 × 35 cm; 63–200 μm particle size) using a mixture of $CHCl_3$/MeOH (7:2), to yield five subfractions (Fr. 1-1–Fr. 1-5). Further purification of Fr. 1-1 (0.3 g) by preparative MPLC [LiChroprep® Si 60 column (25 × 310 mm; 40–63 μm particle size); mobile phase $CHCl_3$/MeOH (9:1); flow rate 5 mL/min] resulted in the isolation of compounds **2** (2.3 mg), **3** (3.2 mg), and **4** (4.9 mg). Another active fraction, Fr. 3 [eluted with CH_2Cl_2/MeOH (7:1), 800 mg], was subjected to silica gel column chromatography using a stepwise gradient of $CHCl_3$/MeOH (from 10:1, 9:1, 8:2, 7:3, 6:4 to 1:1; 200 mL for each step) to yield three subfractions (Fr. 3-1–Fr. 3-3). Fr. 3-2 [eluted with $CHCl_3$/MeOH (from 8:2 to 7:3), 130 mg] was purified by preparative MPLC [LiChroprep® Si 60 column (25 × 310 mm; 40–63 μm particle size); mobile phase $CHCl_3$/MeOH/H_2O (15:1:0.5); flow rate 5 mL/min], to afford compound **1** (7 mg). The structures of the isolated compounds **1**–**4** were identified by analyses of MS and NMR data, and comparison with those in the literature.[7]

3.2. Determination of aldose reductase inhibition in vitro

Rat lenses were removed from Sprague–Dawley rats weighing 250–280 g and frozen until use. The supernatant fraction of the rat lens homogenate was prepared according to the procedures of Hayman and Kinoshita.[8] The concentrations of the inhibitors producing 50% inhibition of the enzyme activity (IC_{50}) were calculated from the least-squares regression line of the logarithmic concentrations plotted against the remaining activity. All the isolated isoflavonoids were dissolved in DMSO to obtain a stock solution of 5 mM, and appropriate dilutions were made before the enzyme assay (final DMSO concentration <3%, and control activity was not affected by this concentration).

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