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Phenolic Compounds from the Leaves and Twigs of *Osteomeles schwerinae* That Inhibit Rat Lens Aldose Reductase and Vessel Dilation in Zebrafish Larvae

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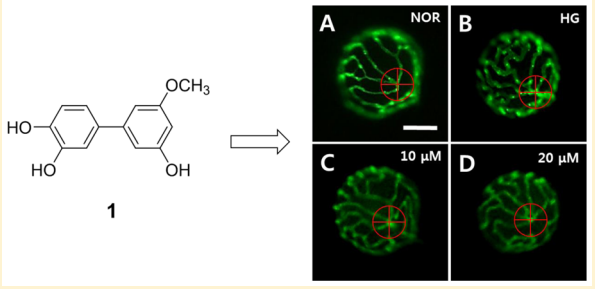
Supporting Information

ABSTRACT: Three new phenolic biphenyl derivatives (**1**−**3**) and one new lignan glycoside (**4**) were isolated from the leaves and twigs of *Osteomeles schwerinae*. The structures of the new compounds were established by spectroscopic data interpretation. The inhibitory effects of **1**−**4** on rat lens aldose reductase in vitro were examined, and compounds **1**−**3** markedly inhibited the enzyme with IC_{50} values of 3.8 to 13.8 μM. In addition, the effects of these isolates on the dilation of hyaloid-retinal vessels induced by high glucose (HG) in zebrafish larvae were investigated. Compound **1** was the most effective in reducing HG-induced dilation of hyaloid-retinal vessels.



Aldose reductase (AR) (alditol/$NADP^+$ oxidoreductase, E.C.1.1.1.21) is the first rate-limiting enzyme in the polyol pathway that catalyzes NADPH-dependent reduction of glucose to sorbitol. During hyperglycemic events, elevated glucose levels enhance AR activity by increasing glucose flux through the polyol pathway, inducing functional and morphological changes that lead to diabetic complications such as cataracts, retinopathy, neuropathy, and nephropathy.[1,2] Therefore, the development of pharmacological AR inhibitors might provide a therapeutic approach for delaying and preventing diabetic complications.[3]

Osteomeles schwerinae C. K. Schneid. is a species of deciduous to semievergreen shrub of the family Rosaceae that is indigenous to Asia and Polynesia.[4,5] It is used in traditional folk medicine in mainland China to treat various diseases, including dysentery, diarrhea, sore throat, arthritis, neuralgia, and furuncles.[6] In a preliminary study to identify effective, naturally sourced therapeutic agents for diabetic complications, it was found that the EtOH extract of the leaves and twigs of *O. schwerinae* inhibited rat lens aldose reductase (RLAR) and isolated two flavonoids, hyperoside and quercitrin, as the major active compounds of the extract.[7] Recently, a phenolic biphenyl compound purified from this plant was reported to exhibit antiglycation and antiangiogenic activities.[8] Further phytochemical study of *O. schwerinae* to search for the other active compounds related to the RLAR-inhibitory effect resulted in the isolation of three new phenolic biphenyl derivatives (**1**−**3**) and a new lignan glycoside (**4**). In this report, described are the isolation and structural elucidation of these compounds as well as the characterization of their inhibition of RLAR in vitro and vessel dilation in zebrafish larvae.

The EtOH extract of the leaves and twigs of *O. schwerinae* was suspended in H_2O and partitioned successively with *n*-hexane, EtOAc, and *n*-BuOH. The EtOAc-soluble fraction, which significantly inhibited RLAR (IC_{50} 2.5 μg/mL), was subjected to a series of chromatographic separation steps guided by RLAR-inhibitory activity, leading to the isolation of compounds **1**−**4**.

RESULTS AND DISCUSSION

Compound **1** was obtained as a brownish powder. High-resolution ESIMS analysis of **1** yielded a molecular ion peak at m/z 233.0815 $[M + H]^+$, in accordance with the molecular formula, $C_{13}H_{12}O_4$. The UV absorption maxima of **1** in MeOH at 212, 266, and 292 nm suggested the presence of aromatic ring(s). The ^1H NMR spectrum showed the typical signals for a 1,3,4-trisubstituted benzene ring [δ_H 7.00 (1H, d, J = 2.1 Hz), 6.90 (1H, dd, J = 8.4, 2.1 Hz), and 6.79 (1H, d, J = 8.4 Hz)], a 1,3,5-trisubstituted benzene ring [δ_H 6.56 (1H, d, J = 2.1 Hz), 6.55 (1H, d, J = 2.1 Hz), and 6.29 (1H, dd, J = 2.1, 2.1 Hz)], and a methoxy group (δ_H 3.77, 3H, s) (Table 1). The ^{13}C NMR spectrum (Table 1), combined with the DEPT data, showed that **1** has 13 carbons constituting a methoxy and six aromatic methine and six aromatic quaternary carbons. These spectroscopic data suggested the presence of a biphenyl structure in **1**.[9,10] The complete assignment of ^1H and ^{13}C NMR resonances was made by DEPT, ^1H−^1H COSY, HSQC, and HMBC techniques. The *ortho*-coupled protons of a 1,3,4-

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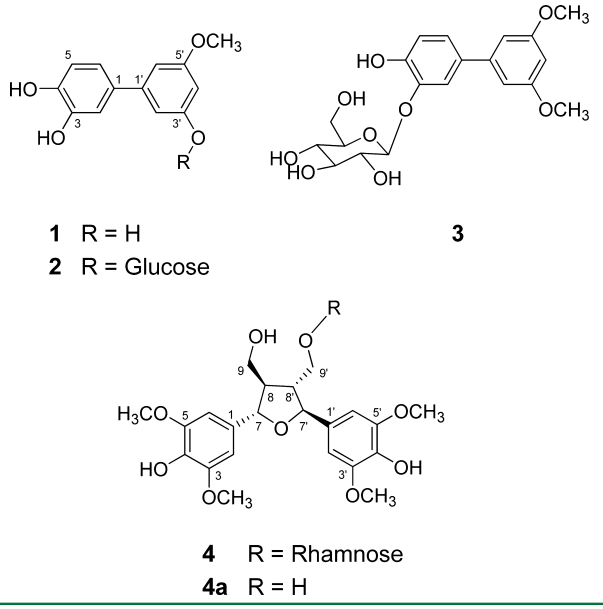

Table 1. NMR Spectroscopic Data for Compounds 1−3 in CD_3OD (700 MHz for ^1H NMR, 175 MHz for ^{13}C NMR)

	1		2		3	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	134.7		134.1		134.4	
2	115.1	7.00 d (2.1)	115.0	7.02 d (2.1)	117.8	7.46 d (2.1)
3	146.3		146.4		147.1	
4	146.5		146.5		148.8	
5	116.6	6.79 d (8.4)	116.6	6.81 d (8.4)	117.6	6.89 d (8.4)
6	119.5	6.90 dd (8.4, 2.1)	119.6	6.94 dd (8.4, 2.1)	123.5	7.17 dd (8.4, 2.1)
1′	144.8		144.7		144.4	
2′	104.9	6.56 d (2.1)	108.4	6.86 d (2.1)	105.9	6.69 d (2.1)
3′	159.8		160.5		162.7	
4′	100.5	6.29 dd (2.1, 2.1)	101.9	6.62 dd (2.1, 2.1)	99.8	6.39 dd (2.1, 2.1)
5′	162.6		162.4		162.7	
6′	107.3	6.55 d (2.1)	107.6	6.73 d (2.1)	105.9	6.69 d (2.1)
Glc-1			102.5	4.93 d (7.0)	104.7	4.83 d (7.0)
Glc-2			75.0	3.46 m	75.1	3.52 m
Glc-3			78.1	3.48 m	77.8	3.48 m
Glc-4			71.6	3.38 m	71.6	3.40 m
Glc-5			78.3	3.47 m	78.6	3.44 m
Glc-6			62.6	3.92 dd (11.9, 2.1), 3.70 dd (11.9 5.6)	62.7	3.91 dd (11.9, 2.1), 3.70 dd (11.9 5.6)
OCH_3-3′					55.9	3.81 s
OCH_3-5′	55.7	3.77 s		3.80 s	55.9	3.81 s

trisubstituted benzene ring at δ_H 6.90 and 6.79, showing HSQC cross-peaks with the carbons at δ_C 119.5 and 116.6, respectively, were assigned to H-6 and H-5 by the HMBC correlations of δ_H 6.90 with C-1 (δ_C 134.7), C-2 (δ_C 115.1), and C-4 (δ_C 146.5) and δ_H 6.79 with C-3 (δ_C 146.3) and C-4 (δ_C 146.5) (Figure 1). The remaining proton of a 1,3,4-trisubstituted benzene ring at δ_H 7.00, showing an HSQC cross-

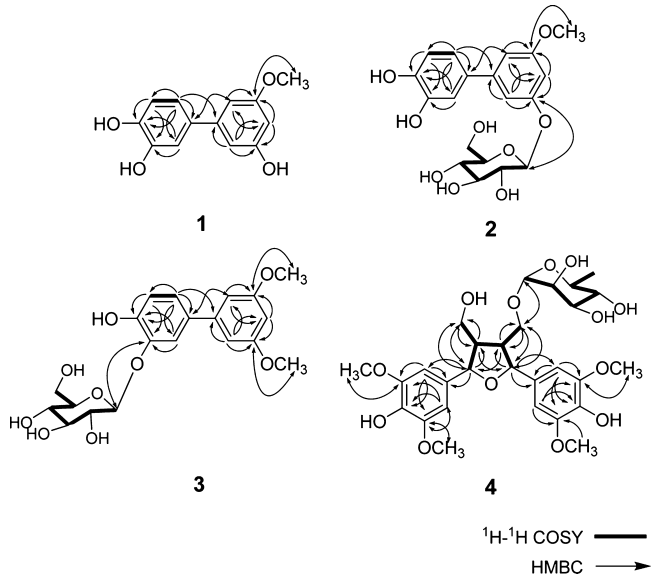

Figure 1. Key $^1H-^1H$ COSY and HMBC correlations of compounds **1**−**4**.

peak with the carbon at δ_C 115.1, was then assigned to H-2, supported by the ^1H NMR coupling constant and from the HMBC spectrum. The aromatic protons of a 1,3,5-trisubstituted benzene ring at δ_H 6.56, 6.55, and 6.29, showing HSQC cross-peaks with the carbons at δ_C 104.9 107.3, and 100.5, respectively, were attributed to H-2′, H-6′, and H-4′ on the basis of their splitting patterns and coupling constants as well as a 2D NMR analysis. The location of a methoxy group at C-5′ was proposed from the HMBC correlation between the methoxy protons (δ_H 3.77) and the carbon signal at δ_C 162.6 (C-5′) (Figure 1). Furthermore, the HMBC correlations of H-6′ with C-1 (δ_C 134.7) and H-6 with C-1′ (δ_C 144.8) supported the connection between two phenyl rings. Hence, the structure of **1** was established as 5′-methoxy-(1,1′-biphenyl)-3,4,3′-triol.

Compound **2** was obtained as a brownish powder and gave a molecular ion peak at m/z 417.1158 $[M + Na]^+$ in the HRESIMS, establishing the molecular formula as $C_{19}H_{22}O_9$, 162 mass units greater than that of **1**. The ^1H and ^{13}C NMR data (Table 1) of **2** were very similar to those of **1** except for the resonances of a monosaccharide unit, suggesting that **2** is a phenolic biphenyl monoglycoside with the same aglycone unit as **1**. The carbon signals at δ_C 102.5, 78.3, 78.1, 75.0, 71.6, and 62.6 and an anomeric proton signal at δ_C 4.93 of **2** were typical of a glycopyranosyl unit, which was identified as D-glucose by acid hydrolysis followed by GC analysis, in the β-configuration as evidenced by the large coupling constant (J = 7.0 Hz) of the anomeric proton. The linkage position of the β-D-glucopyranosyl unit at C-3′ was confirmed on the basis of the HMBC cross-peak of the anomeric proton signal with C-3′ (δ_C 160.5) (Figure 1). The complete assignment of the chemical shifts of this biphenyl glycoside skeleton and its substitution pattern were elucidated from the ^1H and ^{13}C NMR spectrum as well as DEPT experiments combined with various 2D NMR techniques ($^1H-^1H$ COSY, HSQC, and HMBC spectra). Thus, the structure of **2** was assigned as 5′-methoxy-(1,1′-biphenyl)-3,4,3′-triol 3′-*O*-β-D-glucopyranoside.

Compound **3** was obtained as a brownish powder, and its molecular formula, $C_{20}H_{24}O_9$, was determined on the basis of its $[M + Na]^+$ peak (m/z 431.1317) in the HRESIMS and from its NMR data. The ^1H and ^{13}C NMR spectra (Table 1) of **3**

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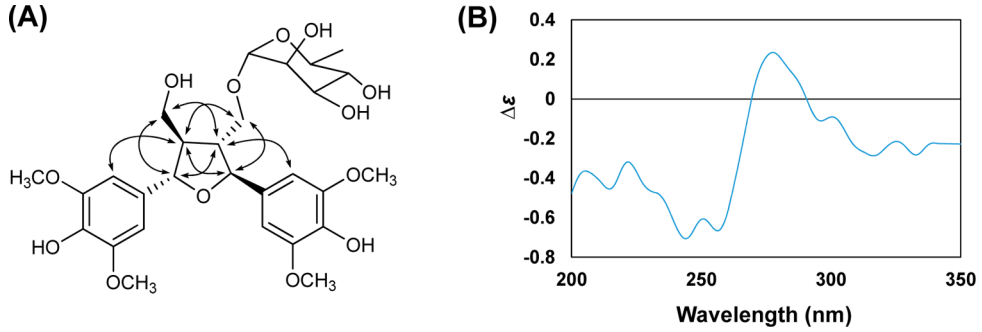

Figure 2. Key NOE correlations (A) of **4** and ECD spectrum (B) of **4a**.

showed characteristic resonances for a phenolic biphenyl glycoside and a close resemblance with the analogous data for **2**. The presence of a biphenyl unit in **3** was confirmed by the HMBC correlations of H-6′ with C-1 (δ_C 134.4) and H-6 with C-1′ (δ_C 144.4) (Figure 1). The main difference in the NMR spectra of **3** versus **2** was the appearance of a signal for a methoxy group [δ_H 3.80 (3H, s); δ_C 55.9], indicating replacement of one of the hydroxy groups in **2**. The placement of this methoxy group on C-3′ (δ_C 162.7) of **3** was deduced from an HMBC correlation between the proton signal of this methoxy group and C-3′ (Figure 1). The sugar moiety was elucidated as D-glucose by comparison of the NMR signals for the sugar unit of **3** with previous reports and confirmed by GC analysis after acid hydrolysis. The large coupling constant (J = 7.0 Hz) of the anomeric proton at δ_H 4.83 indicated a β-configuration of the D-glucose moiety. The β-D-glucopyranosyl unit was located at C-3, as supported by the HMBC correlation between the anomeric proton signal and C-3 (δ_C 147.1) (Figure 1). A detailed analysis of the ^{1}H and ^{13}C NMR data of **3** with the aid of the DEPT, ^{1}H−^{1}H COSY, HSQC, and HMBC spectra permitted the complete structure assignment of **3** as 3′,5′-dimethoxy-(1,1′-biphenyl)-3,4-diol 3-*O*-β-D-glucopyranoside.

Compound **4**, a white powder, exhibited a $[M + Na]^+$ peak at m/z 605.2211 in the HRESIMS, consistent with the molecular formula, $C_{28}H_{38}O_{13}$. The UV absorption maxima at 238 and 272 nm suggested the presence of aromatic ring(s). The ^{13}C NMR spectrum indicated the presence of two symmetrical parts and one sugar moiety. Acid hydrolysis of **4** yielded an aglycone (**4a**) and a monosaccharide unit. The ^{1}H NMR data suggested the aglycone unit to be a 2,5-diaryl tetrahydrofuran type of neolignan,[11,12] as a result of the benzylic proton signals [δ_H 5.04 (1H, d, J = 7.6 Hz, H-7) and 4.99 (1H, d, J = 7.6 Hz, H-7′)] and methine proton resonances [δ_H 2.49 (1H, m, H-8) and 2.34 (1H, m, H-8′)] observed. Four aromatic proton singlets [δ_H 6.73 (2H, s, H-2′, 6′) and 6.72 (2H, s, H-2, 6)] and four methoxy signals [δ_H 3.87 (6H, s, OMe-2, -6), 3.86 (6H, s, OMe-2′, -6′)] indicated that two aromatic moieties were symmetrically substituted with a 3,5-dimethoxy and 4-hydroxy substitution pattern. The ^{1}H NMR data of **4a** were identical to those of icariol A_2,[12] a 2,5-diaryl tetrahydrofuran lignan isolated from *Epimedium sagittatum*, indicating that the aglycone part of **4** has the same planar structure as icariol A_2. The large coupling constants of H-7 ($J_{H\text{-}7/H\text{-}8}$ = 7.6 Hz) and H-7′ ($J_{H\text{-}7'/H\text{-}8'}$ = 7.6 Hz) indicated each hydroxymethylene group to be oriented in a *trans* relationship to the vicinal aryl group. Thus, the configuration of **4a** was concluded to be either the all-*trans* or *meso* form. The significant NOE correlations (Figure 2A) of **4** and the negative specific rotation ($[\alpha]_D^{25}$ −15.8) of **4a** suggested a *trans*−*trans* configuration.[12] Finally, the absolute configuration of **4a** was determined by comparison of its electronic circular dichroism (ECD) spectrum with that of 7*S*,7′*S*,8*R*,8′*R*-icariol A_2.[13] Both showed Cotton effects that were negative around 240 nm and positive around 280 nm (Figure 2B). The similarity between the two ECD spectra indicated the same absolute configuration of **4a** as 7*S*,7′*S*,8*R*,8′*R*-icariol A_2. On the other hand, the six carbon signals (δ_C 102.1, 73.9, 72.6, 72.1, 70.2, and 18.1) of the sugar moiety and the small coupling constant (J = 1.0 Hz) of the anomeric proton (δ_H 4.66) suggested that **4** contained an α-L-rhamnopyranosyl unit. In the HMBC spectrum, a correlation was observed between H-1″ and C-9′ (Figure 1), indicating the rhamnosyl unit to be connected to C-9′ of the aglycone. Thus, the structure of **4** was determined as 7*S*,7′*S*,8*R*,8′*R*-icariol A_2 9′-*O*-α-L-rhamnopyranoside.

The inhibitory effects of the isolated compounds (**1**−**4**) on RLAR in vitro were examined, and the results are presented in Table 2. Of the compounds tested, the phenolic biphenyls (**1**−**3**) inhibited RLAR approximately as potently as the positive control, 3,3-tetramethyleneglutaric acid (TMG) (IC_{50} 28.7 ± 1.3 μM), with IC_{50} values of 3.8 to 13.8 μM. In contrast, compounds **4** and **4a** did not exhibit significant activity (IC_{50} > 100 μM).

Table 2. Inhibitory Effects of Compounds 1−4 and 4a from *O. schwerinae* against RLAR[a]

compound	IC_{50}[b] (μM)
1	13.8 ± 1.3
2	5.6 ± 0.7
3	3.8 ± 0.2
4	>100[c]
4a	>100
TMG[d]	28.7 ± 1.3

[a]Results are expressed as means ± SEM (n = 3). [b]IC_{50} indicates the concentration (μM) at which the inhibition percentage of the RLAR activity was 50%, and the values were determined by regression analysis. [c]IC_{50} cutoff values were set to 100 μM (>100 μM was considered inactive). [d]3,3-Tetramethyleneglutaric acid (TMG) was used as a positive control.

Zebrafish have been used extensively for drug screening and to analyze early and late diabetic complications.[14] Thus, those compounds found (**1**−**3**) that potently inhibited RLAR in vitro were examined for their potential effects on retinopathy in vivo in a diabetic zebrafish. The change in hyaloid-retinal vessel dilation was assessed in transgenic zebrafish embryos that express the enhanced green fluorescent protein (EGFP) in the vasculature (*flk*:EGFP) under high-glucose (HG) (130 mM)

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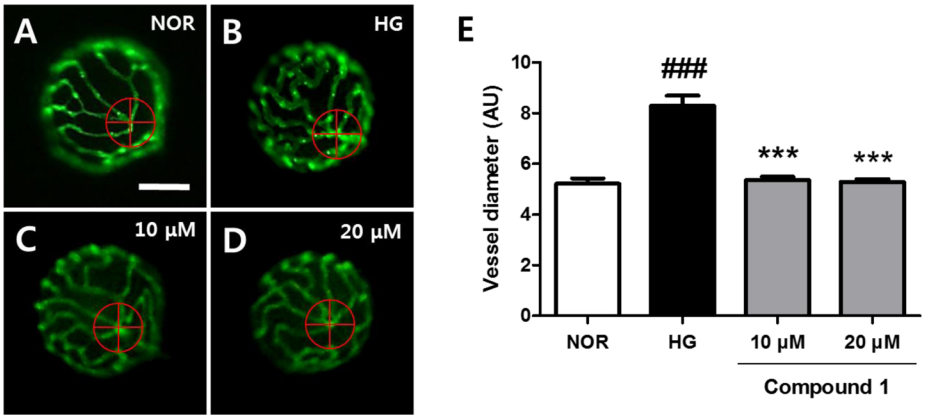

Figure 3. Effect of compound **1** on dilation of hyaloid-retinal vessels from a high-glucose (HG)-induced diabetic retinopathy model. (A–D) Hyaloid-retinal vessels of *flk*:EGFP transgenic zebrafish. (A) Untreated normal group (NOR). (B) HG-treated control group (HG). (C) 10 μM and (D) 20 μM compound **1** in the HG-treated group. (E) Data are displayed as mean artificial units (AU) for vessel diameters. The diameters of hyaloid-retinal vessels were measured at locations proximal to the optic disc (red circle). Scale bar = 50 μm. The hyaloid vessel diameter of each lens was measured three times, and the experiment was performed in triplicate. $^{###}p < 0.001$ vs NOR, $^{***}p < 0.001$ vs HG.

conditions. The toxic effects of **1**–**3** on zebrafish were examined initially, and the results indicated a lack of cytotoxicity for these compounds at concentrations up to 20 μM (data not shown). Thus, to evaluate the effects of **1**–**3** on the HG-induced dilation of hyaloid-retinal vessels, *flk:EGFP* transgenic zebrafish embryos were treated with 10 and 20 μM of **1**–**3**. As shown in Figure 3, only compound **1**, with no sugar moiety, significantly reduced the HG-induced dilation of hyaloid-retinal vessels. This compound reduced the diameters of HG-induced hyaloid-retinal vessels by about 95% and 99% at 10 and 20 μM, respectively, versus the HG-treated control group.

Development and investigation of AR inhibitors, especially natural anti-AR agents with few side effects, might provide a therapeutic approach to delay and prevent diabetes-related complications. Previous studies on natural products that inhibit AR have found certain flavonoids and stilbenes to be potent AR inhibitors.[15−17] Our results have shown three phenolic biphenyl components (**1**–**3**) to contribute to the inhibitory effect of the leaves and twigs of *O. schwerinae* on RLAR. Furthermore, the new phenolic biphenyl, 5′-methoxy-(1,1′-biphenyl)-3,4,3′-triol (**1**), showed both inhibitory effects on RLAR in vitro and vessel dilation in zebrafish larvae in vivo and thus represents a promising agent for the prevention or treatment of diabetic complications and other related diseases.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-2000 digital polarimeter. ECD spectra were measured on a JASCO J-715 spectrometer. All 1D (^{1}H and ^{13}C) and 2D (^{1}H–^{1}H COSY, HMBC, HSQC, and NOESY) NMR spectra were obtained using a Bruker Avance 700 NMR spectrometer with TMS as an internal standard. HRESIMS were recorded on a Shimadzu LCMS-IT-TOF spectrometer. Column chromatography was performed using silica gel (70–230 mesh, Merck) and Sephadex LH-20 (Amersham Pharmacia Biotech). Thin-layer chromatography (TLC) was performed on precoated silica gel 60 F_{254} (0.25 mm, Merck) and RP-18 F_{254s} plates (0.25 mm, Merck). Spots were detected under UV light (254 nm) and by spraying with 10% H_2SO_4 followed by heating.

Plant Material. The leaves and twigs of *O. schwerinae* were collected from Kunming, Yunnan Province, People's Republic of China, in April 2011, and identified by Prof. J.-H. Kim, Gachon University, Republic of Korea. A voucher specimen (No. DiAB-141) has been deposited in the Herbarium of the Diabetic Complications Research Team, Korea Institute of Oriental Medicine, Republic of Korea.

Extraction and Isolation. The dried leaves and twigs of *O. schwerinae* (2.0 kg) were extracted three times with EtOH (each 20 L) at room temperature for 2 days each with maceration and filtering and then concentrated to give an EtOH-soluble extract (65 g). The EtOH extract (60 g) was suspended in H_2O (2 L) and partitioned successively with *n*-hexane (3 × 2 L), EtOAc (3 × 2 L), and *n*-BuOH (3 × 2 L) to afford *n*-hexane- (6 g), EtOAc- (25 g), and *n*-BuOH-soluble fractions (10 g), respectively. The EtOAc-soluble fraction (20 g), which significantly inhibited rat lens aldose reductase (IC_{50} 2.5 μg/mL), was subjected to silica gel column chromatography (70–230 mesh, 40 × 9.5 cm), eluting with a gradient solvent system consisting of $CHCl_3$–MeOH (100:1 → 0:1), to afford nine fractions (A–I). Of these, the fraction that most significantly inhibited RLAR, fraction H (2.5 g, IC_{50} 0.14 μg/mL), was further investigated. Fraction H (2.3 g) was chromatographed on a silica gel column (70–230 mesh, 50 × 5 cm) using a stepwise gradient of $CHCl_3$–MeOH (50:1 → 0:1) to yield three subfractions (H1–H3). These three subfractions inhibited RLAR by 38%, 72%, and 48%, respectively, at 10 μg/mL. Thus, subfraction H2 was further purified by preparative RP-HPLC [Gilson Trilution system; YMC-pack Pro C_{18} (250 × 10 mm, i.d.) column; acetonitrile–H_2O (25:75, v/v); UV detection, 254 nm; flow rate, 2.5 mL/min] to afford **1** (4.2 mg, t_R 22.27 min), **2** (8.5 mg, t_R 15.15 min), **3** (6 mg, t_R 14.05 min), and **4** (4.5 mg, t_R 4.74 min). In addition, two previously reported compounds, quercitrin (12 mg) and hyperoside (7 mg), were isolated from subfraction H3 using a Sephadex LH-20 column (60 × 2.5 cm), eluting with a MeOH–H_2O gradient (40:60 → 70:30).

*5′-Methoxy-(1,1′-biphenyl)-3,4,3′-triol (**1**):* brownish powder; UV (MeOH) λ_{max} (log ε) 212 (4.4), 266 (3.9), 292 (3.8) nm; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 233.0815 $[M + H]^+$ (calcd for $C_{13}H_{13}O_4$, 233.0814).

*5′-Methoxy-(1,1′-biphenyl)-3,4,3′-triol 3′-O-β-D-glucopyranoside (**2**):* brownish powder; $[\alpha]_D^{25}$ −33.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.5), 266 (4.1), 292 (4.0) nm; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 417.1158 $[M + Na]^+$ (calcd for $C_{19}H_{22}O_9Na$, 417.1162).

*3′,5′-Dimethoxy-(1,1′-biphenyl)-3,4-diol 3-O-β-D-glucopyranoside (**3**):* brownish powder; $[\alpha]_D^{25}$ −35.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.4), 267 (4.0), 294 (3.9) nm; ^{1}H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 431.1317 $[M + Na]^+$ (calcd for $C_{20}H_{24}O_9Na$, 431.1318).

*7S,7′S,8R,8′R-Icariol A$_2$ 9′-O-α-L-rhamnopyranoside (**4**):* white powder; $[\alpha]_D^{25}$ −51.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 238 (4.0), 271 (3.6) nm; ^{1}H NMR (700 MHz, CD_3OD) δ_H 6.73 (2H, s, H-2′, 6′), 6.72 (2H, s, H-2, 6), 5.04 (1H, d, *J* = 7.6 Hz, H-7), 4.99 (1H,

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d, J = 7.6 Hz, H-7′), 4.66 (1H, d, J = 1.0 Hz, H-1″), 3.87 (6H, s, OMe-2, 6), 3.86 (6H, s, OMe-2′, 6′), 3.78 (1H, m, H-9′a), 3.75 (1H, dd, J = 11.2, 4.2 Hz, H-9a), 3.66 (1H, dd, J = 11.2, 4.2 Hz, H-9b), 3.54 (1H, dd, J = 10.5, 4.9 Hz, H-9′b), 2.49 (1H, m, H-8), 2.34 (1H, m, H-8′), 1.24 (3H, d, J = 6.3 Hz, H-6″); ^{13}C NMR (175 MHz, CD_3OD) δ_C 149.4 (C-3, 5, 3′, 5′), 136.3 (C-4′), 136.2 (C-4), 134.6 (C-1) 134.1 (C-1′), 105.0 (C-2′, 6′), 104.7 (C-2, 6), 102.1 (C-1″), 85.1 (C-7′), 84.4 (C-7), 73.9 (C-4″), 72.6 (C-2″), 72.1 (C-3″), 70.2 (C-5″), 67.7 (C-9′), 61.5 (C-9), 56.9 (OMe-3, 5, 3′, 5′), 54.5 (C-8), 51.6 (C-8′), 18.1 (C-6″); HRESIMS m/z 605.2211 $[M + Na]^+$ (calcd for $C_{28}H_{38}O_{13}Na$, 605.2210).

Compound 4a: white powder; $[\alpha]_D^{25}$ −15.8 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 238 (4.1), 271 (3.6) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 242 (−0.70), 278 (+0.23) nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 6.78 (4H, s, H-2, 6, 2′, 6′), 5.02 (1H, d, J = 7.6 Hz, H-7, 7′), 3.85 (12H, s, OMe-2, 6, 2′, 6′), 3.80−3.60 (4H, m, H_2-9, H_2-9′), 2.42 (1H, m, H-8, 8′); ESIMS m/z 459.1 $[M + Na]^+$.

Acid Hydrolysis of 2−4. Compounds **2**−**4** (2 mg each) in 10% HCl−dioxane (1:1, 1 mL) were heated separately at 80 °C for 3 h in a water bath. The mixture was neutralized with Ag_2CO_3, filtered, and then extracted with EtOAc (20 mL). The aqueous layer was evaporated, and the residue was treated with L-cysteine methyl ester hydrochloride (2 mg) in pyridine (0.5 mL) at 60 °C for 1 h. After the reaction was completed, the solution was treated with acetic anhydride (3 mL) at 60 °C for 1 h. Authentic samples were prepared using the same procedure. The acetate derivatives were then subjected to gas chromatography (GC) analysis. GC conditions: GC-2010 (Shimadzu) instrument; detector, FID; column, TC-1 capillary column (0.25 mm × 30 m; GL Sciences, Inc.); column temperature, 230 °C; programmed increase, 38 °C/min; carrier gas, N_2 (1 mL/min); injection and detector temperature, 270 °C. D-Glucose (t_R 16.02 min) was detected from **2** and **3**, and L-rhamnose (t_R 10.08 min) was detected from **4**.

RLAR Inhibitory Assay. All experimental protocols for animal care and use were approved by the Korea Institute of Oriental Medicine Institutional Animal Care and Use Committee (approval number 12-018), and animal husbandry and procedures were performed according to institutional guidelines. Rat lenses were removed from the eyes of 8-week-old Sprague−Dawley rats (Dae-Han Bio Link Co., Umsung, Korea) weighing 100 to 150 g and homogenized in 12 volumes of a 135 mM Na, K-phosphate buffer (pH 7.0) containing 0.5 mM phenylmethylsulfonyl fluoride and 10 mM 2-mercaptoethanol. The homogenate was centrifuged at 100000g for 30 min, and the supernatant fluid was used as the crude RLAR. The incubation mixture contained 135 mM Na, K-phosphate buffer (pH 7.0), 100 mM lithium sulfate, 0.03 mM NADPH, 1 mM DL-glyceraldehyde as a substrate, and 50 μL of enzyme fraction, with or without 25 μL of sample solution, in a total volume of 1.0 mL. The reaction was initiated by the addition of NADPH at 37 °C and stopped by the addition of 0.3 mL of 0.5 M HCl. Then, 1 mL of 6 M NaOH containing 10 mM imidazole was added, and the solution was heated at 60 °C for 10 min to convert NADP to a fluorescent product. Fluorescence was measured using a spectrofluorometric detector (Shimadzu RF-5301PC, Japan, excitation: 360, emission: 460 nm). The concentration of each test sample giving 50% inhibition (IC_{50}) was estimated from the least-squares regression line of the logarithmic concentration plotted against the remaining activity. 3,3-Tetramethyleneglutaric acid (Sigma-Aldrich) was used as a positive control.

Measurement of Vessel Dilation in Zebrafish Larvae. All experimental protocols for animal care and use were approved by the Korea Institute of Oriental Medicine Institutional Animal Care and Use Committee (approval number 12-020), and animal husbandry and procedures were performed according to institutional guidelines. Adult zebrafish were maintained under standard conditions at 28.5 °C under a 14 h light/10 h dark cycle. Embryos were obtained from crosses between *flk*:EGFP Tg (transgenic) fish and raised in egg water (sea salt, 0.06 g/L). Three-day *flk*:EGFP Tg embryos were placed into 24-well plates (five embryos per well) and maintained in 2 mL volumes of egg water with 130 mM glucose. HG-induced embryos were treated with 10 or 20 μM solutions of compounds from 3 days postfertilization (dpf) to 6 dpf. At 6 dpf, HG-treated embryos were fixed with 4% paraformaldehyde and stored overnight at 4 °C. They were then washed in distilled water, and lenses containing hyaloid-retinal vessels were isolated using 3% trypsin (0.2 M Tris-HCl, pH 7.8) for 80 min at 37 °C. Images of hyaloid-retinal vessels were obtained using an Olympus stereomicroscope (SZX16, Japan). The diameters of hyaloid vessels were measured in three to four main branches of the optic disc to the first sub-branch using ImageJ software. All experiments were performed in triplicate using 10 larvae per group.

Statistical Analysis. The results are expressed as means ± standard error of the mean (SEM) from three independent experiments. Statistical significance was assessed using one-way analysis of variance (ANOVA) and Dunnett's multiple comparison tests with GraphPad 5.0 Prism software (GraphPad, San Diego, CA, USA).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00469.

^{1}H NMR, ^{13}C NMR, ^{1}H−^{1}H COSY, HMBC, HSQC, and HRESIMS spectra for **1**−**4** and NOESY spectrum for **4** (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Kinoshita, J. H. *Exp. Eye Res.* **1990**, *50*, 567−573.
(2) Morrison, A. D.; Clements, R. S.; Winegrad, A. I. *J. Clin. Invest.* **1972**, *51*, 3114−3123.
(3) Yabe-Nishimura, C. *Pharmacol. Rev.* **1998**, *50*, 21−33.
(4) Hsieh, C. F.; Chaw, S. M. *Bot. Bull. Acad. Sin.* **1996**, *37*, 281−285.
(5) Cuizhi, G.; Spongberg, S. A. *Flora China* **2003**, *9*, 117−119.
(6) The Editorial Board of Chinese Materia Medica of State Administration of Traditional Chinese Medicine of the People's Republic of China. *Chinese Materia Medica*, Vol. *4*; Shanghai Scientific and Technical Publishers: Shanghai, 1999; p 166.
(7) Lee, J.; Jang, D. S.; Yoo, N. H.; Lee, Y. M.; Kim, J. H.; Kim, J. S. *J. Sep. Sci.* **2010**, *33*, 582−586.
(8) Lee, Y. M.; Kim, J.; Kim, C. S.; Jo, K.; Yoo, N. H.; Sohn, E.; Kim, J. S. *Eur. J. Pharmacol.* **2015**, *760*, 172−178.
(9) Rukachaisirikul, V.; Tadpetch, K.; Watthanaphanit, A.; Saengsanae, N.; Phongpaichit, S. *J. Nat. Prod.* **2005**, *68*, 1218−1221.
(10) Dai, Y.; Zhou, G. X.; Kurihara, H.; Ye, W. C.; Yao, X. S. J. *J. Nat. Prod.* **2006**, *69*, 1022−1024.
(11) Urzua, A.; Freyer, A. J.; Shamma, M. *Phytochemistry* **1987**, *26*, 1509−1511.
(12) Matsushita, H.; Miyase, T.; Ueno, A. *Phytochemistry* **1991**, *30*, 2025−2027.
(13) Yamauchi, H.; Kakuda, R.; Yaoita, Y.; Machida, K.; Kikuchi, M. *Chem. Pharm. Bull.* **2007**, *55*, 346−347.
(14) Jörgens, K.; Hillebrands, J. L.; Hammes, H. P.; Kroll, J. *Exp. Clin. Endocrinol. Diabetes* **2012**, *120*, 186−187.

DOI: 10.1021/acs.jnatprod.5b00469
J. Nat. Prod. 2015, 78, 2249−2254

(15) Varma, S. D.; Mikuni, I.; Kinoshita, J. H. *Science* **1975**, *188*, 1215−1216.
(16) Okuda, J.; Miwa, I.; Inagaki, K.; Horie, T.; Nakayama, M. *Biochem. Pharmacol.* **1982**, *31*, 3807−3822.
(17) Matsuda, H.; Morikawa, T.; Toguchida, I.; Yoshikawa, M. *Chem. Pharm. Bull.* **2002**, *50*, 788−795.

DOI: 10.1021/acs.jnatprod.5b00469
J. Nat. Prod. 2015, 78, 2249−2254