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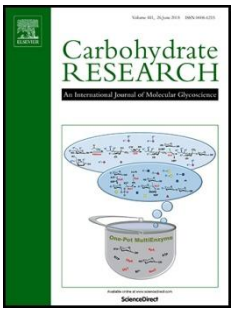

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Carbohydrate Research – Full Papers

Recovery effect of phenylpropanoid glycosides from *Magnolia obovata* fruit on alloxan-induced pancreatic islet damage in zebrafish (*Danio rerio*)

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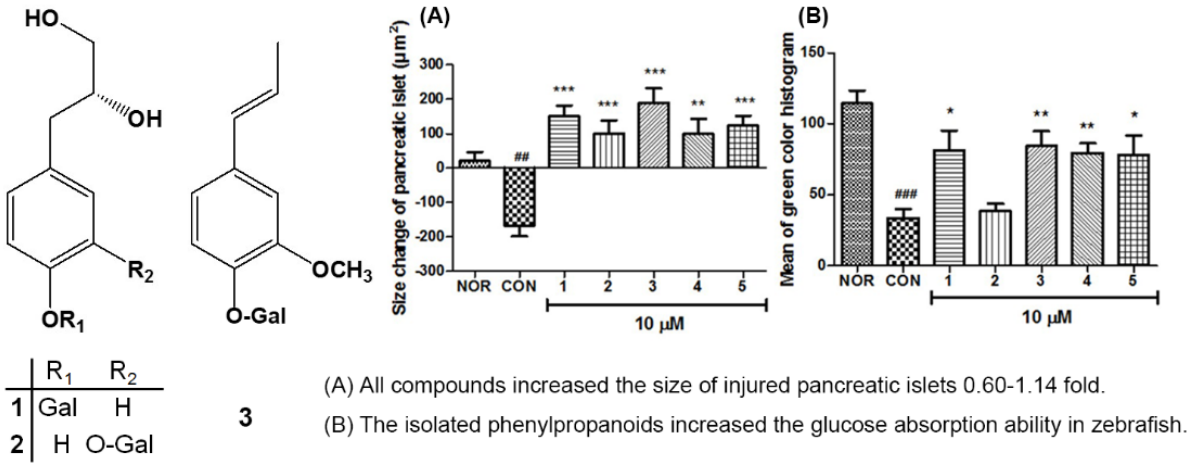
Highlights

- Three new phenylpropanoid glycosides were isolated from *Magnolia obovata* fruit.
- Recovery effect on alloxan-induced islet damage in zebrafish were evaluated.
- All compounds increased the size of injured pancreatic islets 0.60-1.14 fold.
- The isolated phenylpropanoids increased the glucose absorption ability in zebrafish.

Graphical abstract



	R_1	R_2
1	Gal	H
2	H	O-Gal

3

(A) All compounds increased the size of injured pancreatic islets 0.60-1.14 fold.
(B) The isolated phenylpropanoids increased the glucose absorption ability in zebrafish.

ABSTRACT

Investigation of phytochemicals from *Magnolia obovata* fruit led to the isolation of three novel phenylpropanoid glycosides: obovatoside A-C (**1**-**3**) and two known phenylpropanoids, syringin (**4**) and pavonisol (**5**). The structures of **1**-**5** were determined by NMR, HRMS, IR and CD spectroscopic analyses. All compounds were evaluated for their effects on recovery from alloxan-induced pancreatic islet damage in zebrafish. All compounds increased the size of the injured pancreatic islet from 0.60 to 1.14-fold. Compounds **1** and **3**-**5** significantly increased glucose absorption in zebrafish.

Keywords: alloxan; anti-diabetic; *Magnolia obovata*; obovatoside; pancreatic islet; zebrafish

1. Introduction

The incidence of diabetes is growing rapidly worldwide. The Republic of Korea has the fifth highest diabetes death rate of the OECD countries, behind Mexico, Turkey, Israel and Portugal.[1] Diabetes is classified into type 1 (insulin-dependent) and type 2 (non insulin-dependent). Type 1 is characterized by an absolute deficiency of insulin caused by massive β-cell necrosis of the pancreatic islets of Langerhans of the pancreas. The destruction of these cells leads to failure of the pancreas to respond to glucose and classic symptoms of insulin deficiency. Most people with diabetics have type 2, which is characterized by hyperglycemia in the context of insulin resistance and a relative lack of insulin.[2] Type 2 diabetes can result in diabetic retinopathy, nephropathy, mastophathy, cardiovascular and microvascular complications.[3,4] Diabetes is not curable, but proper control of blood glucose is possible.[5] Diabetes can be regulated with insulin preparations or hypoglycemic drugs, which can prevent morbidity and reduce diabetic mortality. Various medicinal plants have also been reported as potential sources of anti-diabetic treatments.[6-8] These plants contain secondary metabolites such as lignans, flavonoids, alkaloids, and terpenoids, that can be assessed for the ability to alleviate diabetes symptoms. Our preliminary experiments showed that a

methanol extract and solvent fractions of *Magnolia obovata* fruit significantly increased the size of alloxan-induced pancreatic islets in the zebrafish (*Danio rerio*). This study was performed to identify the anti-diabetic constituents of *M. obovata* fruit.

M. obovata (Magnoliacea) is a deciduous tree that is found throughout Korea, China, and Japan. It has been used in Chinese medicine to treat fever, headache, and diarrhea and to relieve asthma.[9] The fruit of this plant is called Hoo-Bahk-Ja in Korean traditional medicine. Previous phytochemical research isolated several phenylethanoids and phenylpropanoids from the *Magnolia* species.[10-12] However, few studies have been carried out on their phytochemical and pharmacological potential, including investigations on anti-diabetics from *M. obovata* fruits.

The zebrafish (*Danio rerio*) is a small, shoaling freshwater cyprinid fish. It is a model organism that is widely used in biomedical research because of its small size, easy maintenance in laboratories, numerous offspring, transparent embryos, and amenability to genetic and chemical screens.[13-14] Zebrafish have been treated with alloxan to damage the pancreatic islets and observe variation in islet size and glucose absorption. Flavonoids from *Morus alba* fruit were used to treat zebrafish

to examine recovery from alloxan-induced damage of pancreatic islets in zebrafish.[15] Therefore, this study isolated and identified phenylpropanoid glycosides in the fruit of *M. obovata*, and examined the anti-diabetic potential of the isolated compounds. This paper describes a procedure for isolating and determining the structure of three new phenylpropanoid glycosides and two known ones, from the fruit of *M. obovata* and for evaluating the effect on recovery from alloxan-induced pancreatic islet damages in zebrafish.

2. Results and discussion

2.1. Structural characterization of the compounds.

Methanol extracts of *M. obovata* fruit were fractionated using EtOAc, *n*-BuOH, and water. From the EtOAc and *n*-BuOH fractions, three new phenylpropanoid glycosides (**1-3**) along with two known ones (**4-5**) were isolated through repeated SiO_2, ODS, and Sephadex LH-20 column chromatography. The two known compounds were identified to be syringin (**4**) and pavonisol (**5**) on the basis of spectroscopic analysis data such as NMR, IR, MS, and CD by comparison of the data with those reported in the literatures (Figure 1). [16,17]

Compound **1** was isolated as orange needles. The IR spectrum (CaF_2, ν) showed absorption bands for a hydroxyl group (3357 cm^{-1}), CH group (2918 cm^{-1}), and olefin group (1610 cm^{-1}). The molecular formula was determined to be $C_{15}H_{22}O_8$ from the high-resolution molecular ion peak $[M]^+$ *m/z* 330.1328 (calcd. for $C_{15}H_{22}O_8$, 330.1315) in the HREIMS. The ^1H NMR spectrum (400 MHz, CD_3OD, δ) showed four olefin methine proton signals at δ 7.15 (2H, d, *J* = 8.8 Hz, H-2,6), 7.01 (2H, d, *J* = 8.8 Hz, H-3,5) from a para-disubstituted benzene ring, one oxygenated methine proton signal at δ 3.83 (1H, dddd, *J* = 7.2, 6.0, 5.6, 4.4

Hz, H-8), one germinal coupled oxygenated methylene proton signal at δ 3.50 (1H, dd, *J* = 11.2, 4.4 Hz, H-9a) and 3.43 (1H, dd, *J* = 11.2, 6.0 Hz, H-9b) and one germinal coupled methylene proton signal δ 2.78 (1H, dd, *J* = 14.0, 5.6 Hz, H-7a) and 2.65 (1H, dd, *J* = 14.0, 7.2 Hz, H-7b), which were assigned to the signals of a phenylpropanoid moiety with three hydroxyl groups. In addition, a anomer proton signal at δ 5.22 (1H, d, *J* = 8.0 Hz, H-1'), four oxygenated methine proton signals at δ 4.14 (1H, dd, *J* = 2.8, 2.8 Hz, H-4'), 3.77 (1H, m, H-5'), 3.61 (1H, dd, *J* = 8.8, 8.0 Hz, H-2'), 3.58 (1H, dd, *J* = 8.8, 2.8 Hz, H-3') and one oxygenated methylene proton signal at δ 3.87 (1H, dd, *J* = 12.4, 2.4 Hz, H-6'a), 3.69 (1H, dd, *J* = 12.4, 5.6 Hz, H-6'b) indicated the presence of an aldohexose moiety. Taken together, these data suggested that compound **1** was a phenylpropanoid monoglycoside. The ^{13}C-NMR spectrum showed 15 carbon signals from phenylpropanoid and hexose moieties. The multiplicity of each carbon was determined using distortionless enhancement by polarization transfer (DEPT). In the downfield region, one sp^2 oxygenated olefin quaternary carbon signal at δ 157.7 (C-4), one sp^2 olefin quaternary carbon signal at δ 133.8 (C-1) and four sp^2 olefin methine carbon signals at δ 131.2 (C-2, 6) and 117.6 (C-3, 5) from a para-disubstituted benzene ring with one hydroxyl group were observed.

In the upfield region, one oxygenated methine carbon signal at δ 74.5 (C-8), one oxygenated methylene carbon signal at δ 66.4 (C-9), and one methylene carbon signal at δ 40.1 (H-7) were observed. The sugar was identified as a *β*-galactopyranose based on ^{13}C-NMR chemical shifts such as a anomer carbon signal at δ 100.2 (C-1'), four oxygenated methine carbon signals at δ 75.6 (C-5'), 72.9 (C-3'), 72.0 (C-2'), 68.6 (C-4') and one oxygenated methylene carbon signal at δ 62.8 (C-6') and the coupling constant (*J*=8.0 Hz) of the anomer proton signal. To determine the position of the key functional groups, a heteronuclear multiple bonding connectivity (gHMBC) experiment was performed. In the gHMBC spectrum, the cross peak between the anomer proton signal of the galactopyranosyl moiety (δ_H 5.22, H-1') and the oxygenated olefin quaternary carbon signal (δ_C 157.7, C-4) indicated that the galactopyranose was linked to the hydroxyl of C-4. The structure of **1** was determined to be a new compound, 4-(2,3-dihydroxypropyl)phenyl-1-*O*-*β*-D-galactopyranoside. The absolute configuration of C-8 was confirmed as *S* by compared the specific optical rotation value of the aglycone (**1a**) ($[\alpha]_D$ -8.3°), obtained through acid hydrolysis, with the literature ($[\alpha]_D$ -4.0°).[18] As a result, the chemical structure of **1** was determined to be 4-[(2*S*)-2,3-dihydroxypropyl]phenyl *β*-D-galactopyranoside and the compound

was, named obovatoside A (**1**).

Compound **2** was isolated as a pale yellow viscous oil. The IR spectrum (CaF_2, ν) showed absorption bands for the hydroxyl group (3352 cm^{-1}), CH group (2923 cm^{-1}), and olefin group (1601 cm^{-1}). The high-resolution pseudomolecular ion peak $[M+Na]^+$ *m/z* 369.1251 (calcd. for $C_{15}H_{22}O_9Na$, 369.1264) in the HRFABMS, which was 16 amu higher than **1**, indicated that **2** had one more hydroxyl group than **1**. The 1D and 2D NMR assignments of **2** were based on ^1H and ^{13}C NMR, DEPT, gHSQC and gHMBC and comparison with results for **1**. The NMR data of **2** were similar to **1** with exception of the aglycone moiety. The NMR spectrum (400 MHz, CD_3OD, δ) showed three olefin methine proton signals at δ 7.09 (1H, d, *J* = 2.0 Hz, H-2), 6.79 (1H, dd, *J* = 8.0, 2.0 Hz, H-6), and 6.75 (1H, d, *J* = 8.0 Hz, H-5) and two oxygenated olefin quaternary carbon signals at δ_C 146.6 and 146.7 from a 1,2,4-trisubstituted benzene ring with two hydroxyl groups. The position of the galactopyranosyl group was determined be an glycosidation shift in the ^{13}C-NMR spectrum and gHMBC results. Shifts of the two oxygenated olefin quaternary carbon signals of C-3 and C-4 (δ_C 146.6 and 146.7) were 1.5 ppm downfield and 1.7 ppm upfield, respectively; the shift of the olefin quaternary carbon signal of C-1 (δ_C 133.2) 5.6 ppm upfield compared to

demethyleugenol *β*-D-glucopyranoside.[19] Confirmation came from the cross peak between the anomer proton signal (δ_H 5.12, H-1') and the oxygenated olefin quaternary carbon signal (δ_C 146.6, C-3) in the gHMBC spectrum. The structure of **2** was determined to be a new compound, 4-(2,3-dihydroxypropyl)-2-hydroxyphenyl-2-*O*-*β*-D-galactopyranoside. The absolute configuration of C-8 was confirmed as *S* by compared the optical rotation value of the aglycone (**2a**) ($[\alpha]_D$ -13.6°), obtained through acid hydrolysis, with the literature ($[\alpha]_D$ -4.0°).[18] The chemical structure of **2** was determined to be 4-[(2*S*)-2,3-dihydroxypropyl]-2-hydroxyphenyl *β*-D-galactopyranoside and the compound was, named obovatoside B (**2**).

Compound **3** was isolated as a pale brown viscous oil. The IR spectrum (CaF_2, ν) showed absorption bands for the hydroxyl group (3383 cm^{-1}), CH group (2919 cm^{-1}), and olefin group (1586 cm^{-1}). The molecular formula was determined to be $C_{16}H_{22}O_7$ from the high-resolution molecular ion peak $[M]^+$ *m/z* 326.1299 (calcd. for $C_{16}H_{22}O_7$, 326.1365) in the HREIMS. The ^1H NMR spectrum (600 MHz, CD_3OD, δ) showed the proton signals of a 1,2,4-trisubstituted benzene ring (δ_H 7.07, 1H, d, *J* = 8.4 Hz, H-5; 6.97, 1H, d, *J* = 1.8 Hz, H-2; 6.86, 1H, dd, *J* = 8.4, 1.6 Hz, H-6) and a *trans*-double bond (δ_H 6.34, 1H, d, *J* = 15.6 Hz, H-7; 6.20, 1H,

dq, *J* = 15.6, 6.6 Hz, H-8). In the upfield region, one methoxy proton signal (δ_H 3.85, 3H, s) and one allyl methyl proton signal (δ_H 1.84, 3H, d, *J* = 6.6 Hz, H-9) were assigned to a phenylpropenyl moiety with one hydroxyl and one methoxy group. In addition, a anomer proton signal at δ 5.21 (1H, d, *J* = 8.4 Hz, H-1'), four oxygenated methine proton signals at δ 4.14 (1H, dd, *J* = 3.0, 3.0 Hz, H-4'), 3.82 (1H, m, H-5'), 3.61 (1H, dd, *J* = 9.6, 8.4 Hz, H-2'), 3.59 (1H, dd, *J* = 9.6, 3.0 Hz, H-3') and one oxygenated methylene proton signal at δ 3.68 (1H, dd, *J* = 12.0, 6.0 Hz, H-6a'), 3.61 (1H, dd, *J* = 12.0, 3.6 Hz, H-6b') indicated the presence of a aldohexose moiety. These data suggested that compound **3** was a phenylpropenyl monoglycoside. The ^{13}C-NMR spectrum showed 16 carbon signals for phenylpropenyl, hexose and methoxy (δ_C 56.8) moieties. In the downfield region, two oxygenated olefin quaternary carbon signals at δ_C 151.0 (C-3) and δ_C 147.4 (C-4) one olefin quaternary carbon signal at δc 134.7 (C-1) and five olefin methine carbon signals at δc 131.9 (C-7), δc 125.3 (C-8), δc 120.1 (C-6), δc 118.0 (C-5), and δc 111.1 (C-2) were observed. In the upfield region, a methoxy carbon signal and one allyl methyl carbon signal at δ_C 18.6 (C-9) were observed. The sugar was identified as a *β*-galactopyranose from chemical shifts such as a anomer carbon signal at δ 100.9 (C-1'), four oxygenated methine carbon signals at

δ 76.0 (C-5'), 73.0 (C-3'), 72.3 (C-2'), 68.8 (C-4') and one oxygenated methylene carbon signal at δ 63.0 (C-6') as well the coupling constant (*J*=8.4 Hz) of the anomer proton signal. In the gHMBC spectrum, the cross peak between the anomer proton signal of the galactopyranosyl moiety (δ_H 5.21, H-1') and the oxygenated olefin quaternary carbon signal (δ_C 147.4, C-4), and between the methoxy proton signal (δ_H 3.85, -OCH_3) and the oxygenated olefin quaternary carbon signal (δ_C 151.0, C-3) indicated that galactopyranose and the methoxy were linked to the hydroxyls of C-4 and C-3, respectively. Compound **3** was determined to be a new compound, 4-(1*E*-prop-1-enyl)-2-methoxyphenyl *β*-D-galactopyranoside and named obovatoside C (**3**).

The isolated compounds **1**-**5** were evaluated for their effect on recovery from alloxan-induced pancreatic islet damage in zebrafish. The zebrafish is a good model for studying pancreatic change *in vivo*, because the zebrafish embryo has a single pancreatic islet during the first stage of development, which allows rapid analysis of organ changes. Alloxan damage the pancreatic islet and diabetogenic, decreasing *β*-cell mass in pancreatic islet.[16] Alloxan treatment significantly decreased pancreatic islet size by 7.88-fold (p<0.01) compared to the normal group. Treatment with compound **1** led to a significant increase in the size of the

injured pancreatic islet by 0.90-fold (p<0.001) compare to the alloxan-induced group. Compounds **2-5** significantly increased the size of the pancreatic islet by 0.60-fold (p<0.001), 1.14-fold (p<0.001), 0.60-fold (p<0.01), and 0.74-fold (p<0.001), respectively, compared to the alloxan treated group (Figure 2 A, C). The ability to absorb glucose was assessed using 2-[*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxyglucose (2-NBDG), which is a recently introduced fluorescent derived from glucose modified with a 2-[*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino] group at the C-2 position.[20] It is used in diabetes studies to measure cellular ability to absorb glucose. Fluorescence intensity of the pancreatic islet was analyzed by histograms using Image J software, which indicates pixel intensity (green) from 0 to 255. Fluorescence intensity of the alloxan-treated group (pixel mean = 33.70) significantly decreased compared to the normal group (pixel mean = 114.38), (p<0.001). Although the group treated with compound **2** (pixel mean = 38.48) showed no increase in the fluorescence intensity of the pancreatic islet compared to group treated with alloxan (Figure 2 B, C), groups treated with compounds **1** (pixel mean = 81.28, p<0.05), **3** (pixel mean = 84.42, p<0.01), **4** (pixel mean = 79.71, p<0.01) and **5** (pixel mean = 78.09, p<0.05) significantly increased fluorescence intensity compared to the alloxan-

induced group.

3. Conclusion

Three new and two known phenylpropanoids were isolated from *M. obovata* fruits. All compounds increased the size of the injured pancreatic islet, and compounds **1** and **3-5** significantly increased glucose absorption in zebrafish. Therefore, these results demonstrate the potential for the biomedical use of these compounds and plant extracts as anti-diabetic agents.

4. Experimental

4.1. General methods

The resins used for column chromatography (c.c.) were SiO_2 (Kiesel gel 60, Merck, Darmstadt, Germany), octadecyl silica gel (ODS) (LiChroprep RP-18, 40-60 μm, Merck), and Sephadex LH-20 (Amersham Pharmacia Biotech, Uppsala, Sweden). Thin layer chromatography (TLC) analysis was carried out using a Kiesel gel $60F_{254}$ and a RP-18 F_{254}s plates (Merck), and the spots on the TLC plates were detected using a UV lamp Spectroline Model ENF-240 C/F (Spectronics Corporation, Westbury, NY, USA) and spraying a 10% H_2SO_4 solution followed by heating. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Varian Unity Inova AS-400 FT-NMR spectrometer (Palo Alto, CA, USA). ^{1}H NMR (600 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Bruker Avance 600 MHz spectrometer (Bruker, Billerica, MA, USA). Melting points were determined using a Fisher-John's Melting Point apparatus (Miami, FL, USA) with a microscope and the values obtained were uncorrected. Optical rotation was measured using a JASCO P-1010 digital polarimeter (Jasco, Tokyo, Japan). IR spectra were obtained using a Perkin Elmer

Spectrum One FT-IR spectrometer (Buckinghamshire, England). HRFAB-MS and HREI-MS were conducted on a JEOL JMSAX-700 mass spectrometer (Tokyo, Japan). Alloxan monohydrate and sea salts were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 2-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG) was obtained from Invitrogen (Eugene, OR, USA). Fluorescence microscopy was used by an Olympus 1X70 microscope (Olympus, Tokyo, Japan). To gain the image analysis of pancreatic islet in zebrafish, Focus Lite (Focus Co, Daejeon, Korea) and Image J (National Institutes of Health, Bethesda, MD, USA) were used.

4.2. Plant materials

The fruits of *Magnolia obovata* were collected at Kyung Hee University, Yongin, Republic of Korea in September 2010 and identified by Prof. Seung-Woo Lee, Department of Horticultural Biotechnology, Kyung Hee University. A voucher specimen (KHU-NPCL-201009) has been deposited at the Laboratory of Natural Products Chemistry, Kyung Hee University.

4.3. Extraction and isolation

The dried fruits (11 kg) were chopped and extracted with 80% MeOH (40 L × 4) at room temperature for 24 hr, filtered and concentrated *in vacuo*. The MeOH extracts (740 g) were successively partitioned with water (3.5 L), EtOAc (3.5 L × 4), and *n*-BuOH (3 L × 3). Each solvent layer was concentrated to yield EtOAc (MOE, 238 g), *n*-BuOH (MOB, 97 g) and water (405 g) fractions, respectively. The *n*-BuOH fraction (MOB, 97 g) was applied to a SiO_2 c.c. (Φ 8.0 × 12 cm) and eluted with $CHCl_3$-MeOH-water (12:3:1 → 10:3:1 → 7:3:1 → 6:4:1, 3 L of each) with monitoring by TLC to provide 21 fractions (MOB-1 to MOB-21). MOB-5 [1.01 g, elution volume/total volume (Ve/Vt) 0.014-0.025] was subjected to the SiO_2 c.c. (Φ 4.5 × 15 cm) and eluted with $CHCl_3$-MeOH-water (20:3:1, 3.5 L), yielding 15 fractions (MOB-5-1 to MOB-5-15). Fraction MOB-5-6 (21 mg, Ve/Vt 0.180-0.020) was subjected to the ODS c.c. (Φ 1 × 8 cm) and eluted with EtOH-water (1:2, 45 mL), yielding nine fractions (MOB-5-6-1 to MOB-5-6-9) and along with a purified compound **3** [MOB-5-6-5, 2.5 mg, Ve/Vt 0.410-0.500, TLC (RP-18 F_{254}s) R_f 0.50, MeOH-water (2:1)]. Fraction MOB-10 (5.16 g, Ve/Vt 0.151-0.216) was subjected to the SiO_2 c.c. (Φ 6.5 × 14 cm) and eluted with $CHCl_3$-MeOH-water (16:3:1 → 14:3:1 → 12:3:1 → 10:3:1 → 8:3:1 → 6:4:1, 3.8

L of each) to obtain 27 fractions (MOB-10-1 to MOB-10-27) and a pure compound **4** [MOB-10-14, 55 mg, Ve/Vt 0.147-0.155, TLC (Kiesel gel 60 F_{254}) R_f 0.47, $CHCl_3$-MeOH-water (65:35:10)]. Fraction MOB-10-22 (659 mg, Ve/Vt 0.612-0.742) was subjected to an ODS c.c. (Φ 4.5 × 5 cm) and eluted with MeOH-water (1:3, 1.1 L) to obtain 14 fractions (MOB-10-22-1 to MOB-10-22-14) and a pure compound **1** [MOB-10-22-2, 188 mg, Ve/Vt 0.038-0.058, TLC (Kiesel gel 60 F_{254}) R_f 0.51, $CHCl_3$-MeOH-water (6:4:1)]. Fraction MOB-15 (7.38 g, Ve/Vt 0.593-0.633) was subjected to the ODS c.c. (Φ 6 × 8 cm) and eluted with MeOH-water (1:2 → 1:1, 3.4 L of each) to obtain 18 fractions (MOB-15-1 to MOB-15-18). Fraction 15-22 (556 mg, Ve/Vt 0.042-0.051) was subjected to a Sephadex LH-20 c.c. (Φ 3 × 30 cm) and eluted with MeOH-water (4:1, 0.7 L) to obtain 10 fractions (MOB-15-2-1 to MOB-15-2-10). Fraction MOB-15-2-2 (155 mg, Ve/Vt 0.245-0.296) was subjected to the SiO_2 c.c. (Φ 3 × 19 cm) and eluted with $CHCl_3$-MeOH-water (10:3:1, 7.5 L) to obtain 11 fractions (MOB-15-2-2-1 to MOB-15-2-2-10) and a pure compound **2** [MOB-15-2-2-4, 21 mg, Ve/Vt 0.382-0.485, TLC (Kiesel gel 60 F_{254}) R_f 0.35, $CHCl_3$-MeOH-water (65:35:10)]. The EtOAc fraction (MOE, 238 g) was applied to the SiO_2 c.c. (Φ 12 × 15 cm) and eluted with *n*-hexane-EtOAc (5:1 → 2:1 → 1:2, 2.8 L of each) to provide 15

fractions (MOE-1 to MOE-15). MOE-7 (1.56 g, Ve/Vt 0.451-0.574) was subjected to the SiO_2 c.c. (Φ 4.5 × 19 cm) and eluted with $CHCl_3$-MeOH-water (20:3:1, 1.3 L), yielding six fractions (MOE-7-1 to MOE-7-6). Fraction MOE-7-2 (607 mg, Ve/Vt 0.101-0.183) was subjected to the ODS c.c. (Φ 3 × 8.5 cm) and eluted with MeOH-water (3:2 → 2:1, 1.8 L of each), yielding 12 fractions (MOE-7-2-1 to MOE-7-2-12) and along with a pure compound **5** [MOE-7-2-1, 7.1 mg, Ve/Vt 0.000-0.032, TLC (RP-18 F_{254}s) R_f 0.70, MeOH-water (2:1)].

4.4. Compound characterization

4.4.1. Obovatoside A (1)

Obovatoside A (**1**) orange needles; m.p. 98-100 °C; $[\alpha]_D$ -62.0° (*c* 0.11, MeOH); IR_v (KBr, cm^{-1}) 3357, 2918, 1610; HREI-MS *m/z* 330.1328 (calcd. for $C_{15}H_{22}O_8$, 330.1315) $[M]^+$; ^1H NMR (CD_3OD, δ_H) 7.15 (2H, d, *J* = 8.8 Hz, H-2, 6), 7.01 (2H, d, *J* = 8.8 Hz, H-3, 5), 5.22 (1H, d, *J*=8.0 Hz, H-1'), 4.14 (1H, dd, *J*=2.8, 2.8 Hz, H-4'), δ 3.87 (1H, dd, *J*=12.4, 2.4 Hz, H-6'a), 3.83 (1H, dddd, *J*=7.2, 6.0, 5.6, 4.4 Hz, H-8), 3.77 (1H, m, H-5'), 3.69 (1H, dd, *J*=12.4, 5.6 Hz, H-6'b), 3.61 (1H, dd, *J*=8.8, 8.0 Hz, H-2'), 3.58 (1H, dd, *J*=8.8, 2.8 Hz, H-3'), 3.50 (1H, dd, *J*=11.2, 4.4 Hz, H-9a), 3.43 (1H, dd, *J*=11.2, 6.0 Hz, H-9b), 2.78 (1H, dd, *J*=14.0, 5.6 Hz, H-

7a), 2.65 (1H, dd, *J*=14.0, 7.2 Hz, H-7b); ^{13}C NMR (CD_3OD, δ_C) 157.7 (C-4), 133.8 (C-1), 131.2 (C-2, 6), 117.6 (C-3, 5), 100.2 (C-1'), 75.6 (C-5'), 74.5 (C-8), 72.9 (C-4'), 72.0 (C-2'), 68.6 (C-3'), 66.4 (C-9), 62.8 (C-6'), 40.1 (C-7).

4.4.2. Obovatoside B (2)

Obovatoside B (**2**) a pale yellow viscous oil; $[\alpha]_D$ -51.8° (*c* 0.12, MeOH); IR_ν (KBr, cm^{-1}) 3352, 2923, 1601; positive HRFAB-MS *m/z* 369.1251 (calcd. for $C_{15}H_{22}O_9Na$, 369.1264) $[M+Na]^+$; ^{1}H NMR (CD_3OD, δ_H) 7.09 (1H, d, *J*=2.0 Hz, H-2), 6.79 (1H, dd, *J*=8.0, 2.0 Hz, H-6), 6.75 (1H, d, *J*=8.0 Hz, H-5), 5.12 (1H, d, *J*=8.0 Hz, H-1'), 4.16 (1H, dd, *J*=2.8, 2.8 Hz, H-4'), 3.89 (1H, dd, *J*=12.0, 2.4 Hz, H-6'a), 3.84 (1H, m, H-8), 3.77 (1H, m, H-5'), 3.71 (1H, dd, *J*=12.0, 5.6 Hz, H-6'b), 3.63 (1H, dd, *J*=8.8, 8.0 Hz, H-2'), 3.61 (1H, dd, *J*=8.8, 2.8 Hz, H-3'), 3.50 (1H, dd, *J*=11.2, 4.4 Hz, H-9a), 3.44 (1H, dd, *J*=11.2, 6.0 Hz, H-9b), 2.73 (1H, dd, *J*=13.6, 5.6 Hz, H-7a), 2.61 (1H, dd, *J*=13.6, 6.8 Hz, H-7b); ^{13}C NMR (CD_3OD, δ_C) 146.7 (C-4), 146.7 (C-3), 131.7 (C-1), 125.4 (C-6), 119.8 (C-2), 116.7 (C-5), 102.1 (C-1'), 75.9 (C-5'), 74.5 (C-2'), 72.8 (C-4'), 72.2 (C-8), 68.5 (C-3'), 66.5 (C-9), 62.8 (C-6'), 40.2 (C-7).

4.4.3. Obovatoside C (3)

Obovatoside C (**3**) a pale brown viscous oil; IR_v (KBr, cm^{-1}) 3383, 2919, 1568; $[\alpha]_D$ -22.8° (*c* 0.20, MeOH); HREI-MS *m/z* 326.1299 (calcd. for $C_{16}H_{22}O_7$, 326.1365) $[M]^+$; ^1H NMR (600 MHz, CD_3OD, δ_H) 7.07 (1H, d, *J*=8.4 Hz, H-5), 6.97 (1H, d, *J*=1.8 Hz, H-2), 6.86 (1H, dd, *J*=8.4, 1.8 Hz, H-6), 6.34 (1H, d, *J*=15.6 Hz, H-7), 6.20 (1H, dq, *J*=15.6, 6.6 Hz, H-8), 5.21 (1H, d, *J*=8.4 Hz, H-1'), 4.14 (1H, dd, *J*=3.0, 3.0 Hz, H-4'), 3.85 (3H, s, $-OCH_3$), 3.82 (1H, m, H-5'), 3.68 (1H, dd, *J*=12.0, 6.0 Hz, H-6'a), 3.61 (1H, dd, *J*=9.6, 8.4 Hz, H-2'), 3.61 (1H, dd, *J*=12.0, 3.6 Hz, H-6'b), 3.59 (1H, dd, *J*=9.6, 3.0 Hz, H-3'), 1.84 (3H, d, *J*=6.6 Hz, H-9); ^{13}C NMR (125 MHZ, CD_3OD, δ_C) 151.0 (C-3), 147.4 (C-4), 134.7 (C-1), 131.9 (C-7), 125.3 (C-8), 120.1 (C-6), 118.0 (C-5), 111.1 (C-2), 100.9 (C-1'), 76.0 (C-5'), 73.0 (C-4'), 72.3 (C-2'), 68.8 (C-3'), 63.0 (C-6'), 56.8 ($-OCH_3$), 18.6 (C-9).

4.4.4. Syringin (4)

Syringin (**4**) white crystals; m.p. 193-194 °C; IR_v (KBr, cm^{-1}) 3390, 2989, 1588; $[\alpha]_D$ -15.8° (*c* 0.21, MeOH); EI-MS *m/z* 372 $[M]^+$.

4.4.5. Pavonisol (5)

Pavonisol (**5**) colorless crystals; m.p. 140 °C; IR_v (KBr, cm^{-1}) 3450, 3310, 1605, 1520; $[\alpha]_D$ +89.0° (*c* 0.15, $CHCl_3$); EI-MS *m/z* 242 $[M]^+$.

4.5. Acid hydrolysis of 1-2 and isolation of aglycones

A solution of compound **1** (10 mg) in 1M HCl (3 ml) was heated at 80 °C on a heating block for 3 h. The reaction mixture was added to 2 ml MeOH and extracted with EtOAc (5 ml). The EtOAc layer was concentrated and purified by Supeleclean LC-Si SPE tube (*n*-hexane-EtOAc = 2:1) to obtain the aglycone [**1a**, 1.8 mg, R_f 0.45 TLC (RP-18 F_{254}s), MeOH-water (2:3)].

A solution of compound **2** (10.8 mg) in 1M HCl (3 ml) was treated in the same manner as compound **1** to obtain the aglycone (**2a**, 2.2 mg, R_f 0.50 TLC (RP-18 F_{254}s), MeOH-water (2:3)].

4.6. Zebrafish maintenance

Adult zebrafish were maintained in a zebrafish system S type [1500(W) × 400(D) × 2050(H) mm] (Daejeon, Korea) on a cycle of 14 h light and 10 h dark at 28.5 °C. To obtain zebrafish larvae, two pairs of adult zebrafish were put in a spawning box overnight and induced to spawn by providing light for 30 minutes. Zebrafish embryos were collected at 3 h post-fertilization (hpf) for experiments and incubated in a petridish in 0.03% sea salt solution. Embryos were maintained under a 14 h light and 10 h dark cycle in an incubator at 28.5 °C.

4.7. Recovery effect of compounds 1-5 on alloxan-induced pancreatic islet damage in zebrafish larvae

Zebrafish larvae were divided into seven groups: normal, alloxan-induced (control), and alloxan-induced with treatment with compounds **1**-**5**. Wild-type zebrafish larvae at 5 days post-fertilization (dpf) were placed into 96-well plates. Larvae were exposed to 25 μM 2-NBDG for 12h and rinsed for 10 min. To induce pancreatic islet damage, alloxan was added at 100 μM for 15 min. At 4 h and 45 min after alloxan treatment, larvae were stained for 1 h with 25 μM 2-NBDG and

mounted in 96-well plates and observed using a fluorescence microscope. To determine compound effects, alloxan-induced larvae were treated with 10 μM compounds for 1 h and restained for 1 h with 25 μM 2-NBDG and observed under a fluorescence microscope. All images were analyzed for pancreatic islet size and histograms of fluorescence intensity were generated using Focus Lite and Image J software.

4.8. Statistical analysis

Statistical analysis was performed using Graphpad Prism (version.5). Data were expressed as mean ± standard error of the mean (SEM). Significance was determined using repeated one-way ANOVA followed by Tukey's test. Probability levels for statistical significance were $p<0.05$, 0.01 and 0.001.

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Figure Legends

Figure 1. Structures of phenylpropanoids from *Magnolia obovata* fruit. Gal: *β*-D-galactopyranosyl, Glc: *β*-D-glucopyranosyl.

Figure 2. Effect of compounds **1**-**5** on recovery from alloxan-induced pancreatic islet damage in zebrafish. (A) Pancreatic islet size change by group. (B) Mean value as green color histogram analysis of zebrafish pancreatic islets. (C)

Pancreatic islet image of (a) normal (NOR); (b) alloxan-induced (CON) or treatment of (c) alloxan-induced + obovatoside A (**1**); (d) alloxan-induced + obovatoside B (**2**); (e) alloxan-induced + obovatoside C (**3**); (f) alloxan-induced + syringin (**4**); and (g) alloxan-induced + pavonisol (**5**). $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$; compared to NOR; $*p<0.05$, $**p<0.01$, $***p<0.001$; compared to CON.