Carbohydrate Research 416 (2015) 70–74

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

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

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ARTICLE INFO

Article history:
Received 8 June 2015
Received in revised form 13 July 2015
Accepted 16 July 2015
Available online 23 July 2015

Keywords:
Alloxan
Anti-diabetic
Magnolia obovata
Obovatoside
Pancreatic islet
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.

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

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http://dx.doi.org/10.1016/j.carres.2015.07.010
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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 (Fig. 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 comparing 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**).

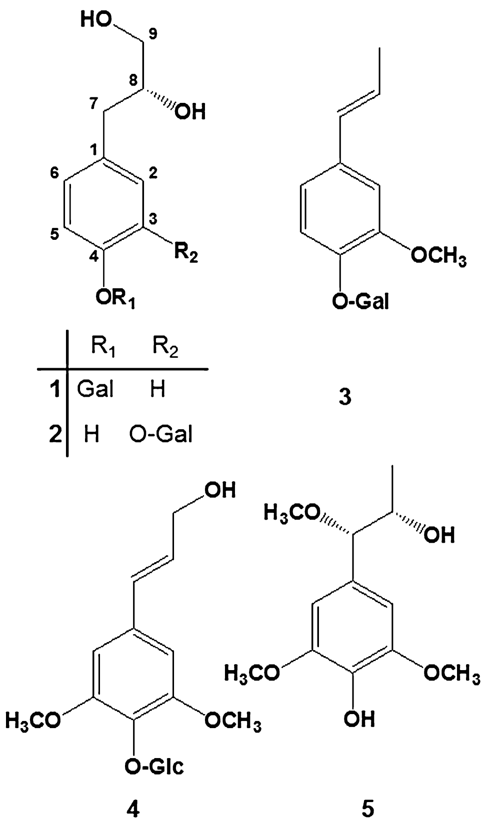

Fig. 1. Structures of phenylpropanoids from *Magnolia obovata* fruit. Gal: β-D-galactopyranosyl, Glc: β-D-glucopyranosyl.

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 a 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)-