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Plasma-induced dimerization of phloridzin as a new class of anti-adipogenic agents

Gyeong Han Jeong [a,c], Jae-Hyeon Cho [b,c], Seong-Ho Kim [a], Tae Hoon Kim [a,*]

[a] Department of Food Science and Biotechnology, Daegu University, Gyeongsan 38453, Republic of Korea
[b] Institute of Animal Medicine, College of Veterinary Medicine, Gyeongsang National University, Jinju 52828, Republic of Korea

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ABSTRACT

Phloridzin is a natural phloretin glucoside found in several parts of apple trees and is an attractive target for structural modification as novel pharmaceutical agent. Nonthermal dielectric barrier discharge (DBD) plasma-induced structural changes in dihydrochalcone phloridzin (**1**) resulted in the isolation of three new methylene-bridged dihydrochalcone dimers, methylenebisphloridzin (**2**), deglucosylmethylenebisphloridzin (**3**), and methylenebisphloretin (**4**), along with phloretin (**5**). The chemical structures of these newly generated compounds were elucidated by interpretation of their spectroscopic data. The new phloretin dimer **4** connected by a methylene linkage exhibited significantly improved anti-adipogenic properties against pancreatic lipase as well as differentiation of 3T3-L1 preadipocytes compared to the parent compound phloridzin.

Obesity is associated with an imbalance between energy intake and expenditure and is widely recognized as a serious health risk. Obesity is closely correlated to several metabolic syndromes, including hyperlipidemia, hypertension, osteoarthritis, diabetes mellitus, arteriosclerosis, and coronary heart disease.[1] Substantial efforts to develop treatments for obesity have been conducted in recent decades, and their distinct mechanisms of action such as lipase inhibition, inhibition of adipocyte differentiation, and control of lipid metabolism have been clearly elucidated.[2] Currently, one of the most advanced strategies for the treatment of obesity involves inhibition of triglyceride cleavage into bioavailable glycerol and fatty acids in the gastrointestinal tract by suppression of pancreatic lipase using effective antiobesity agents.[3] Pancreatic lipase is known an important enzyme in the hydrolysis and absorption of dietary triglycerides.[4] Thus, inhibition of pancreatic lipase is considered to be a valuable therapeutic strategy for treating diet-induced obesity in humans. Orlistat™ is a commercially available representative agent of this anti-obesity therapeutic mechanism.[5] Physiologically, obesity is also known to be correlated with excess adipocyte tissue mass caused by increases in the number and size of adipocytes differentiated from preadipocytes.[6] Consequently, control of lipid absorption and accumulation via inhibition of pancreatic lipase activity and adipocyte differentiation is considered to be an important targets in the development of novel anti-obesity agent.

Phloridzin is described as a bitter phenolic constituent with anti-pyretic effects and was firstly isolated from the bark of apple trees.[7] This secondary metabolite belongs to the chemical class of dihydrochalcones, which have structures closely similar to that of the immediate in flavonoid biosynthesis and is easily found in apple fruits, root barks, shoots, leaves, and seeds. Phloridzin and its analogues have been widely used in alternative medicine and possess a diverse spectrum of potential pharmacological efficacies such as antioxidant, antidiabetic, and melanogenic stimulatory properties.[8–11] Due to its potential applicability as a pharmacological agent, phloridzin has recently received great attention from the research community. Biotransformation phloridzin using polyphenol oxidase and human intestinal flora was previously demonstrated in limited studies to be able to create new valuable derivatives.[12,13] Recent research has shown that nonthermal plasma treatment is an effective environmental-friendly technology for providing of structurally modified natural products with enhanced bioactivity.[14] Among the advanced cold plasma instruments, DBD plasma is known to cause several chemical changes associated with abundant generation of reactive oxygen and nitrogen species. On the other hand, systematic research on the construction of drug-like molecules induced by plasma treatment of naturally occurring secondary metabolites with different scaffolds is still very limited. As part of our continuing investigation into creating potential bioactive constituents from ubiquitous natural

* Corresponding author.
E-mail address: skyey7@daegu.ac.kr (T.H. Kim).
[c] These authors contributed equally to this work.

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products, we herein report the efficient structural modification of phloridzin using a DBD plasma, which resulted in the formation of new phlorizin derivatives with significantly enhanced biological efficacies based on *in vitro* assay systems against pancreatic lipase and preadipocyte differentiation compared to the of parent compound phloridzin.

The sample preparation procedure was undertaken according to the previously reported method.[15] A sample solution containing phloridzin (1.0 g) in methanol (7.0 L) was directly treated for 20, 40, and 60 min using a DBD plasma, and the converted patterns were monitored by reversed-phase HPLC. The dried treated phloridzin for 60 min showed most enhanced inhibitory activity with an IC_{50} value of 29.8 ± 1.3 μg/mL in the pancreatic lipase assay. Successive chromatographic separation[16] resulted in the isolation of three new methylene-bridged compounds **2**–**4**, along with previously known phloretin (**5**).[17] (Fig. 1). The known compound was identified by comparison of the spectroscopic data with the literature value.

Compound **2** was obtained as a new compound in the form of a yellow amorphous powder, $[\alpha]_D^{20}$ −18.1° (MeOH). A pseudomolecular ion peak at *m*/*z* 833.2657 $[M-H]^-$ observed in the negative HRFABMS in combination with ^{13}C NMR spectroscopic data corresponded to a molecular formula of $C_{43}H_{48}O_{20}$. The characteristic absorption maxima at 288 nm in the UV spectrum were attributed to the presence of the dihydrochalcone skeleton.[18] The ^1H NMR spectrum of **2** suggested the occurrence of one A_2B_2-type aromatic signals at δ_H 7.04 (2H, d, *J* = 8.4 Hz, H-2, 6) and 6.67 (2H, d, *J* = 8.4 Hz, H-3, 5), as well as one isolated aromatic signal at δ_H 6.26 (1H, s, H-3′), indicating the presence of a *para*-substituted and 1,2,3,4-tetra-substitued aromatic ring systems. In addition to aromatic signals, the spectrum also exhibited two diagnostic methylene triplets at δ_H 3.50 (2H, t, *J* = 7.8 Hz, H-8) and 2.87 (2H, t, *J* = 7.8 Hz, H-7), which further suggests the existence of the dihydrochalcone backbone. The ^1H NMR spectrum also displayed resonances for one characteristic anomeric protons at δ_H 5.03 (1H, d, *J* = 7.8 Hz, Glc-I-1″) and six oxygen-containing signals at 3.88–3.36. Consistent with these interpretations, the ^{13}C NMR and HSQC spectra of **2** closely resembled those for the parent phloridzin (**1**),[19] except for the presence of a quaternary carbon at C-5′ (δ_C 109.1) and an isolated methylene signal at CH_2-moiety (δ_H 3.79) in **1**. Careful comparison of the above mentioned NMR features and mass spectra between compounds **1** and **2** readily indicated that compound **2** consists of two equivalent dihydrochalcone moieties connected symmetrically through a methylene bridge between C-5′ and C-5a′. The splitting pattern for H-3′ was simplified due to the absence of H-5′, which further allowed the dimerization of phloridzin through C-5′ to C-5a′. The linkage positions of the methylene bridge and glucosyl residue in **2** were unambiguously elucidated by key HMBC correlations as shown in Fig. 2. Thus, the symmetrical structure of compound **2** was elucidated as shown, and this compound was assigned the trivial name methylenebisphloridzin.[20]

Compound **3**, $[\alpha]_D^{20}$ −11.2° (MeOH), was obtained as a white amorphous powder and displayed a dark blue color in the $FeCl_3$ test. The negative-ion mode HRFABMS showed a pseudomolecular ion peak at *m*/*z* 721.2141 $[M-H]^-$, corresponding to the molecular formula of $C_{37}H_{38}O_{15}$. Extensive comparison of the NMR data between compounds **3** and **2** demonstrated that these compounds have closely similar NMR resonances for the aglycone unit but different signals for the sugar moiety. Briefly, the ^1H NMR spectrum of **3** observed diagnostic signals for only one anomeric signal at δ_H 5.04 (1H, d, *J* = 7.8 Hz, Glc-I-1″), one upfield shift of the H-3a′ signal[17,19] at δ_H 5.92 (1H, s), and one additional H-3′ signal at δ_H 6.29 (1H, s), indicating the presence of a β-glucose and two phloretin moieties. Combinational analysis of FABMS as well as ^1H and ^{13}C NMR spectra indicated that compound **3** is a derivative of **2**, which is missing one glucosyl group at the C-2a′ position of the phloretin moiety. The locations of a methylene bridge and a glucose moiety in the molecule were confirmed unambiguously from the key HMBC interactions (Fig. 2). Consequently, the structure of compound **3** was assigned the name deglucosylmethylenebisphloridzin,[21] as shown in Fig. 1.

The molecular formula of compound **4** was determined to be $C_{31}H_{28}O_{10}$ based on by negative HRFABMS (*m*/*z* 559.1599), and **4** was shown to contain one less glucose residue than methylenebisphloridzin (**2**). The ^1H and ^{13}C NMR spectra of **4** were also closely

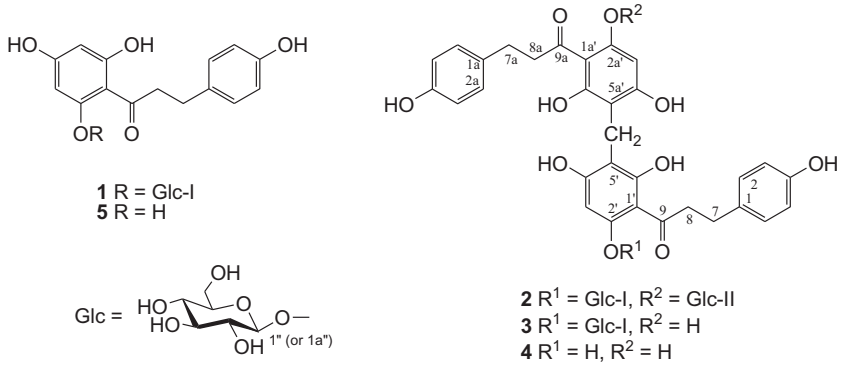

Fig. 1. Structures of new compounds **2**–**4** isolated from phloridzin induced by plasma treatment.

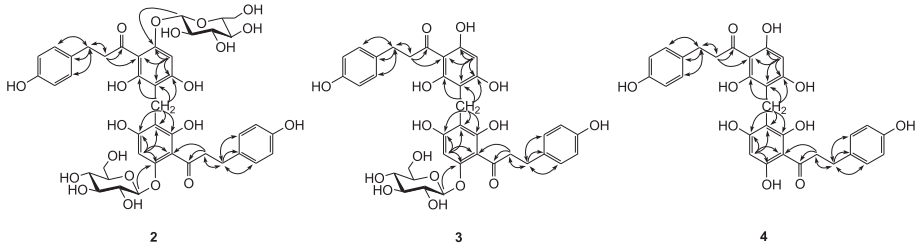

Fig. 2. Selected HMBC correlations of **2**–**4**.