Accepted Manuscript

Flavanonol glucosides from the aerial parts of *Agrimonia pilosa* Ledeb. and their acetylcholinesterase inhibitory effects

U. Min Seo, Duc Hung Nguyen, Bing Tian Zhao, Byung Sun Min, Mi Hee Woo

PII: S0008-6215(17)30045-9

DOI: 10.1016/j.carres.2017.04.014

Reference: CAR 7364

To appear in: *Carbohydrate Research*

Received Date: 20 January 2017

Revised Date: 10 April 2017

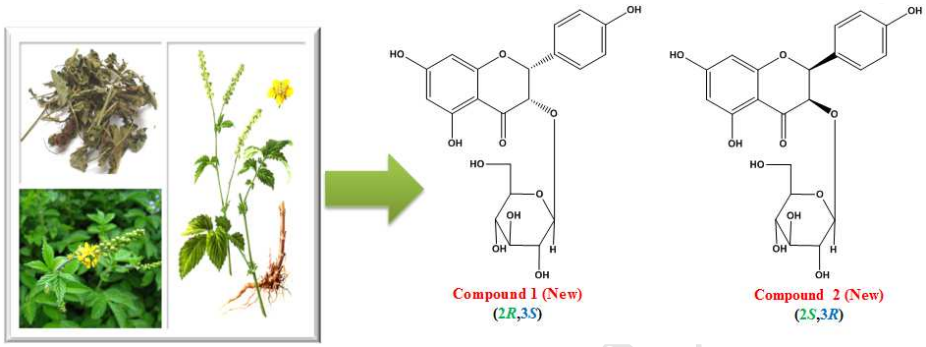
Accepted Date: 13 April 2017

Please cite this article as: U.M. Seo, D.H. Nguyen, B.T. Zhao, B.S. Min, M.H. Woo, Flavanonol glucosides from the aerial parts of *Agrimonia pilosa* Ledeb. and their acetylcholinesterase inhibitory effects, *Carbohydrate Research* (2017), doi: 10.1016/j.carres.2017.04.014.

Graphical abstract

Flavanonol glucosides from the aerial parts of *Agrimonia pilosa* Ledeb. and their acetylcholinesterase inhibitory effects

U Min Seo, Duc Hung Nguyen, Bing Tian Zhao, Su Hui Seong, Jae Sue Choi, Jeong Ah Kim, Byung Sun Min, and Mi Hee Woo



Flavanonol glucosides from the aerial parts of *Agrimonia pilosa* Ledeb. and their acetylcholinesterase inhibitory effects

U Min Seo[a], Duc Hung Nguyen[a,b], Bing Tian Zhao[a], Byung Sun Min[a], and Mi Hee Woo[a,*]

[a]*College of Pharmacy, Catholic University of Daegu, Gyeongsan 38430, Republic of Korea*

[b]*Phutho College of Pharmacy, Viettri City, Phutho Province 290000, Vietnam*

*Corresponding author.

E-mail address: woomh@cu.ac.kr (M.H. Woo).

ABSTRACT

Two new flavanonol glucoside isomers, (2*R*,3*S*)-dihydrokaempferol 3-*O*-*β*-D-glucoside (**1**) and (2*S*,3*R*)-dihydrokaempferol 3-*O*-*β*-D-glucoside (**2**), were isolated from the aerial parts of *Agrimonia pilosa* Ledeb., along with eight known flavanonol glucosides (**3**–**10**). Their structures were determined on the basis of spectroscopic analysis. In addition, these compounds were evaluated to determine their acetylcholinesterase inhibitory activities. The results indicated that these compounds have moderate inhibitory effects, with IC_{50} values ranging from 76.59 ± 1.16 to 97.53 ± 1.64 μM, except compounds **1** and **4** were inactive.

Keywords: Agrimonia pilosa, Flavanonol glucoside, Acetylcholinesterase activity

1. Introduction

In Alzheimer's disease (AD), cholinergic neurons are lost and the endogenous level of acetylcholine is decreased. Drugs that penetrate the blood brain barrier and partially inhibit AChE can increase the levels of acetylcholine, potentiate its physiological effects, and provide symptomatic relief [1]. The most frequently prescribed anti-AD drugs are cholinesterase inhibitors, which act by increasing ACh levels in the brain *via* the inhibition of AChE. Several flavonoid compounds have been reported to be AChE inhibitors [2].

Agrimonia pilosa Ledeb. (AP) is a perennial herb in the Rosaceae family. It is mainly distributed in Northern Asia and Eastern Europe. The roots and the aerial parts of AP also have different uses in Traditional Chinese Medicine. Its roots have been used for hemostatic, antimalarial, and anti-dysenteric treatment in Chinese herbal medicine for a long time [3]. Additionally, some flavonoids from AP plants showed significant acetylcholinesterase (AChE) inhibitory activity [4].

Previous phytochemical studies have shown that the aerial parts of AP contain polyphenols, phenolic glucosides, flavonoids [5-7], triterpenoids [8], and coumarins [3]. Flavanonol glucosides possess two chiral centers at C-2 and C-3 in their skeleton and give four diastereomeric forms [(2*S*,3*S*), (2*R*,3*R*), (2*R*,3*S*), (2*S*,3*R*)]. Meanwhile, their two diastereomeric forms [(2*S*,3*S*)-dihydrokaempferol 3-*O*-*β*-D-glucoside and (*2R*,3*R*)-dihydrokaempferol 3-*O*-*β*-D-glucoside] were found from acai (*Euterpe oleracea* Mart.) pulp [9]. Kato et al. also reported (2*S*,3*S*)-dihydrokaempferol 3-*O*-*β*-D-glucoside in the aerial parts of AP [5].

In this study, we isolated flavanonol glucoside compounds from the ethyl acetate fraction of the aerial parts of AP and elucidated their structures by spectroscopic data

analyses (IR, UV, HRMS, and 1D and 2D NMR). Furthermore, these isolated compounds were evaluated for AChE inhibitory effects.

2. Results and discussion

2.1. Structural elucidation

The isolation was performed *via* multiple chromatographic steps over silica gel, YMC RP-18 column chromatography and a semi-preparative HPLC. In this paper, two new flavanonol glucoside isomers (**1** and **2**) were isolated from the EtOAc fraction of the aerial parts of AP, along with eight known flavanonol glucosides.

The structures of known compounds were identified as (2*R*,3*R*)-dihydrokaempferol 3-*O*-*β*-D-glucoside (**3**) [9], (2*S*,3*S*)-dihydrokaempferol 3-*O*-*β*-D-glucoside (**4**) [5,9], (2*R*,3*S*)-taxifolin 3-*O*-*β*-D-glucoside (**5**) [10], (2*R*,3*R*)-taxifolin 4'-*O*-*β*-D-glucoside (**6**) [10], (2*R*,3*R*)-taxifolin 3-*O*-*β*-D-glucoside (**7**) [11], (2*R*,3*R*)-taxifolin 7-*O*-*β*-D-glucoside (**8**) [12], (2*S*,3*R*)-taxifolin 3-*O*-*β*-D-glucoside (**9**) [11], and (2*S*,3*S*)-taxifolin 3-*O*-*β*-D-glucoside (**10**) [11], by comparing their NMR data with those in the literature (see Figure 1).

Compound **1** was obtained as a yellow amorphous powder. The molecular formula of **1** was determined as $C_{21}H_{22}O_{11}$ by the positive mode HR-FAB-MS data at *m/z* 473.1057 $[M+Na]^+$ (cald for $C_{21}H_{22}O_{11}Na$, 473.1054). The IR spectrum of **1** showed absorptions for hydroxyl and carbonyl groups at 3324 and 1635 cm^{-1}. The ^1H and ^{13}C NMR spectra of **1** displayed characteristic signals for a flavanonol moiety due to two *meta*-coupled aromatic protons at δ_H 6.41 (2H, m, H-6, 8), four AA'BB'-type aromatic ring protons at δ_H 7.99 (2H, d, J = 8.4 Hz, H-2', 6') and 7.20 (2H, d, J = 8.4 Hz, H-3', 5'), and two coupled oxymethine protons at δ_H 5.84 (1H, br s, H-2) and 5.13 (1H, br s, H-3), and 15 carbon signals at δ_C 82.0 (C-2), 76.3 (C-3), 194.6 (C-4), 165.9 (C-5), 97.9 (C-6), 169.4 (C-7), 96.9 (C-8), 163.8 (C-9), 102.3 (C-10), 127.3 (C-1'), 130.8 (C-2', 6'), 116.3 (C-3', 5') and 159.7 (C-4') (Tables 1 and Table 2).

These data suggested that compound **1** contain a dihydrokaempferol moiety [5]. In addition, the ^{1}H and ^{13}C NMR signals of **1** also displayed a glucose group at δ_H 5.09 (1H, d, J = 7.7 Hz, H-1"), 4.03 (1H, br t, J = 7.6 Hz, H-2"), 3.90 (1H, br s, H-3"), 4.15 (2H, m, H-4", 5"), 4.50 (1H, dd, J = 2.2, 11.6 Hz, H-6"a), and 4.30 (1H, dd, J = 5.8, 11.6, H-6"b), and δ_C 104.0 (C-1"), 75.6 (C-2"), 78.9 (C-3"), 72.1 (C-4"), 79.0 (C-5"), and 63.3 (C-6") (Tables 1 and Table 2). Acid hydrolysis of **1** yielded D-glucose which was identified by HPLC analysis after conversion of sugar to thiocarbamoyl-thiazolidine derivative [13].

The J value of the anomeric proton [δ_H 5.09 (1H, d, J = 7.7 Hz)] suggested β-configuration of D-glucose [14] which was supported by NOESY experiment (Figure 3). Additionally, the ^{1}H-^{13}C HMBC spectrum of **1** indicated the correlation of the oxymethine proton (δ_H 5.13) to C-1' (δ_C 104.0) (Figure 2). With all of the above data, compound **1** was determined as dihydrokaempferol 3-*O*-β-D-glucoside. The diastereomeric forms of dihydrokaempferol 3-*O*-β-D-glucoside include four forms [(2*R*,3*S*), (2*S*,3*R*), (2*R*,3*R*), and (2*S*, 3*S*)]. Meanwhile, compounds **3** and **4** were determined as (2*R*,3*R*) dihydrokaempferol 3-*O*-β-D-glucoside and (2*S*,3*S*) dihydrokaempferol 3-*O*-β-D-glucoside by comparing spectroscopic data with literature values, respectively [9]. The mixture of **1**, **2**, **3**, and **4** was analyzed by high performance liquid chromatography (HPLC) on a C18 column. The result displayed four peaks at different retention times at 27.4, 33.1, 41.3, and 43.0 min for **3**, **4**, **1**, and **2**, respectively. These above data suggested that compounds **1** and **2** were (2*R*,3*S*) or (2*S*,3*R*) form (see Supplementary Material). In CD spectrum, compound **1** displayed the negative Cotton effect at 294 nm and positive one at 341 nm. The CD spectrum of **1** was similar to those of (2*R*,3*S*)-*cis*-dihydrokaempferol reported (Figure 4) [15], suggesting that the stereochemistry of **1** was (2*R*,3*S*). Therefore, compound **1** was determined as (2*R*,3*S*)-dihydrokaempferol 3-*O*-β-D-glucoside, a new natural product.

Compound **2** was obtained as a yellow amorphous powder. The molecular formula of **2** was determined as $C_{21}H_{22}O_{11}$ by the positive mode HR-FAB-MS data at *m/z* 473.1058 $[M+Na]^+$ (cald for $C_{21}H_{22}O_{11}Na$, 473.1054). The ^1H and ^{13}C NMR spectra of **2** were similar to those of **1** except for *J* values of H-2 and H-3 [(br s) in **1** and (d, *J* = 2.8 Hz) in **2**]. In ^1H-^{13}C HMBC spectrum, compound **2** displayed the correlation between H-3 [δ_H 5.29 (d, *J* = 2.8 Hz)] and C-1" (δ_C 104.8) (Figure 2). These data suggested that **2** also was a dihydrokaempferol 3-*O*-glucoside. The sugar moiety was identified as *β*-D-glucoside by methods mentioned above and *J* value of the anomeric proton [δ_H 5.39 (d, *J* = 7.7 Hz)]. The correlation of H-2 to H-3 was also observed in NOESY experiment. In CD experiment, compound **2** displayed the positive Cotton effect at 296 nm and the negative one at 333 nm that was opposite to those of **1**, suggesting that the stereochemistry of **2** was (2*S*,3*R*). The CD spectrum of **3** was positive at 291 nm and the negative Cotton effect at 328 nm, indicating that the stereochemistry of **3** was (2*R*,3*R*) [15]. Similarly, CD spectrum of **4** showed the negative Cotton effect at 294 nm and the positive one at 323 nm and, suggesting that the stereochemistry of **4** was (2*S*,3*S*) (Figure 4) [15]. Finally, compound **2** was determined to be (2*S*,3*R*)-dihydrokaempferol 3-*O*-*β*-D-glucoside, a new natural product.

2.2. Acetylcholinesterase activity

AChE is targeted by cholinesterase inhibitors employed for the treatment of senile dementia, myasthenia, gravis, Parkinson's disease, and ataxia. The AChE inhibitory effects of isolated compounds were measured using the spectrophotometer method developed by Ellman [16]. Dehydroevodiamine was used as the positive control [4]. Compounds **1**–**10** were evaluated for inhibitory AChE effects at the applied concentrations (4, 20, and 100 μM). The results indicated that these compounds have moderate inhibitory activity, with IC_{50} values ranging

from 76.59 ± 1.16 to 97.53 ± 1.64 μM. Compounds **1** and **4** were very weak or inactive (IC_{50} values > 100 μM). Some conclusions were deduced from investigating the structure-activity relationships of these flavanonol glucosides. Compound **2**, a (2*S*,3*R*) form of dihydrokaempferol 3-*O*-*β*-D-glucoside, displayed better inhibitory effect than the other forms. Compounds **6**, **7**, and **8**, the diastereomeric (2*R*,3*R*) forms of taxifolin-glucoside, displayed similar inhibitory effects on the AChE enzyme. This result indicates that the position of *β*-D-glucose at C-4', C-3, and C-7 in the structure of taxifolin glucoside did not affect the inhibitory effects of the compounds. AChE inhibitor is used as a drug for the symptomatic treatment of Alzheimer's disease [17]. A survey of medicinal plants and their extracts to evaluate their medicinal and therapeutic potential revealed that plants rich in polyphenols and flavonoids have the potential to act as AChE inhibitors [17,18]. The flavanonol glucoside compounds in this study may have partial benefits for the treatment of Alzheimer's disease.

3. Conclusion

Flavonoids are a huge class in nature and their biological activities are known as antiviral, antitumor, antiplatelet, anti-inflammatory, and antibacterial [19]. Previous studies showed that flavonoid constituents in AP possess potential inhibitory PTP1B and AChE activities [4,19]. In our study, two new flavanonol glucosides (**1** and **2**) were isolated from aerial parts of AP, along with eight known flavanonol glucosides (**3**–**10**). These compounds were investigated for AChE inhibitory activity. Most of the isolates displayed moderate inhibitory activity, except for inactive compounds **1** and **4**.

4. Experimental

4.1. General experimental procedures

The specific rotations were determined on a JASCO DIP-370 digital polarimeter. The circular dichroism (CD) spectra were recorded in methanol on the JASCO J–715 spectropolarimeter (Jasco, Easton, MD, USA). The infrared (IR) spectra were obtained on a Mattson Polaris FT/IR-300E spectrophotometer. UV spectra were measured in MeOH using a Shimadzu spectrophotometer. The nuclear magnetic resonance (NMR) spectra were recorded in pyridine-d_5 or methanol-d_4 on an Oxford AS 400 MHz instrument (Varian, Palo Alto, CA, USA) or a Bruker 500 MHz instrument (Bruker, Billerica, MA, USA). Mass spectra were recorded using a Quattro II mass spectrometer. Column chromatography was performed using silica gel (Merck, 63–200 μm particle size), RP-18 (Merck, 150 μm particle size), and Sephadex LH-20 (Pharmacia Co. Ltd.). Thin Layer Chromatography (TLC) tests were performed on silica gel 60 F254 plates (Merck, Darmstadt, Germany). Fractions were monitored by TLC and spots were visualized by spraying with 10% H_2SO_4 in ethanol, followed by heating. The semi-preparative HPLC runs were carried out using a Gilson system with an UV detector and an Optima Pak C-18 column (10 × 250 mm, 10 μm particle size, RS Tech. Corp., Korea). The analyses were conducted on an HPLC chromatography (Waters, Texas, USA) and a Kinetex C-18 column (4.6 × 250 mm, 5 μm particle size, Phenomenex., USA). All other chemicals and solvents were of analytical grade and used without further purification.

4.2. Plant material

The aerial parts of AP were collected from the herbal garden at the Catholic University of Daegu, Korea, in August 2012. The plant material was authenticated by one of the authors (B. S. Min). A voucher specimen (AP-2012143) has been deposited at the College of Pharmacy, Catholic University of Daegu, Republic of Korea.

164 *4.3. Extraction and isolation*

165 The aerial parts of *Agrimonia pilosa* Ledeb. (32 Kg) were initially dried (5.8 Kg). Then it
166 was extracted with 80% EtOH (3 × 4 L) at room temperature for 7 days. The ethanol extract
167 was then concentrated under reduced pressure (2.0 Kg). The ethanol extract was further
168 suspended in H_2O (1.5 L) and successfully partitioned with CH_2Cl_2 (1 L × 7, 800 g), EtOAc
169 (1 L × 7, 163.9 g), *n*-BuOH (1 L × 5, 500.5 g) and H_2O-soluble fraction. The EtOAc fraction
170 (163.9 g) was subjected to open flash column chromatography over silica gel, eluted with
171 CH_2Cl_2/MeOH (100:0, 50:1, 25:1, 20:1, 15:1, 12:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1,
172 and 1:1) to give 19 fractions (A–S). Fraction O (4.1 g) was then subjected to RP-C18 column
173 chromatography, eluted with MeOH/H_2O (1:2, 2:1, 4:1, 8:1, and 10:1) to afford six
174 subfractions (OA–OF). Sub-fraction EA-O-A (820 mg) was chromatographed on a Sephadex
175 LH-20 column and eluted with MeOH/H_2O (1:1) to yield compounds **6** (8.3 mg) and
176 compound **8** (5.2 mg), respectively. Sub-fraction OA1 (133 mg) was subjected to semi-
177 preparative Gilson HPLC [YMC C18 column (10 × 250 mm, 5 μm particle size)] eluted with
178 ACN/H_2O (18:82, flow rate 2 mL/min, UV 254 nm) to yield compounds **3** (t_R 27.4 min, 6.2
179 mg), **4** (t_R 33.1, min 5.1 mg), **1** (t_R 41.3 min, 3.4 mg), and **2** (t_R 43.0 min, 4.1 mg). Sub-
180 fraction OB (1.1 g) was further purified by semi-preparative HPLC [YMC C18 column (10 ×
181 250 mm, 5 μm particle size)] eluted with MeOH/H_2O (33:67, flow rate 2 ml/min, UV 254 nm)
182 to yield compounds **7** (t_R 26.5 min, 18.9 mg), **10** (t_R 28.1 min, 8.7 mg), **5** (t_R 53.0 min, 4.3
183 mg), and **9** (t_R 55.2 min, 2.9 mg), respectively.

184 *4.3.1. (2R,3S)-dihydrokaempferol 3-O-β-D-glucoside (**1**)*

185 Yellow amorphous powder; $[\alpha]_D^{25}$ –71 (*c*, 0.2, MeOH); UV (MeOH) λ_{max} nm 214, 296;
186 IR (KBr) ν_{max} cm^{-1} : 3324, 2943, 2831, 1635, 1448, 1115, 1024; For ^1H and ^{13}C NMR data
187 see Tables 1 and 2; HR-FAB-MS *m/z* 473.1057 $[M+Na]^+$ (calcd for $C_{21}H_{22}O_{11}Na$, 473.1054).

188 *4.3.2. (2S,3R)-dihydrokaempferol 3-O-β-D-glucoside (**2**)*

189 Yellow amorphous powder; $[\alpha]_D^{25}$ +20 (*c*, 0.2, MeOH); UV (MeOH) λ_{max} nm 214, 296 ;
190 IR (KBr) ν_{max} cm^{-1} : 3329, 2943, 2831, 1634, 1449, 1112, 1024. For ^1H and ^{13}C NMR data
191 see Tables 1 and 2; HR-FAB-MS *m/z* 473.1058 $[M+Na]^+$ (calcd for $C_{21}H_{22}O_{11}Na$, 473.1054).

192 *4.3.3. Acid hydrolysis and HPLC analysis for **1** and **2***

193 Each compound (2 mg) was dissolved in 2 mol/L HCl (10 mL) and then heated at 80 ºC
194 for 6 h. The mixtures were concentrated *in vacuo* to dryness, and the residue was partitioned
195 between CH_2Cl_2 and H_2O. The aqueous layer was evaporated to dryness. The dried product
196 was then dissolved in anhydrous pyridine (1.0 mL) and D-cysteine methyl ester hydrochloride
197 (4.0 mg) and the mixture was further heated at 60 ºC for 1 h. After the reaction mixture was
198 concentrated to dryness, *o*-tolyl isothiocyanate (10 μL) was added, and the mixture was
199 heated at 60 ºC for 1 h. The product of the reaction mixture was dissolved in 1 mL MeOH
200 and analyzed using HPLC chromatography (Waters, Houston, TX, USA) with a Kinetex C18
201 column (4.6 × 250 mm, 5 μm particle size; Phonomenex, Torrance, CA, USA).
202 Chromatographic analytical methods utilized MeOH containing 0.05% H_3PO_4 in H_2O (20%
203 MeOH to 35% MeOH) for 40 min at a flow rate of 0.8 mL/min, with ultraviolet (UV)
204 detection at 227 nm. The reaction conditions for authentic samples were the same as
205 described above. Comparison of the retention times for *β*-D-glucose using **1** and **2** in aqueous

206 solution was carried out (t_R 27.66 and 27.45 min of **1** and **2**, respectively) using authentic
207 samples (t_R 27.45 min of *β*-D-glucose) (Supplementary material).

208 *4.3.4. Acetylcholinesterase inhibitory assay*

209 Ellman's method was applied for evaluating AChE inhibitory activity of isolated
210 compounds (Ellman et al., 1961). Acetylthiocholine iodide (ATCI) was used as substrates.
211 Briefly, the mixtures of 140 μL of sodium phosphate buffer (pH 8.0), 20 μL of each tested
212 compound with different concentrations (4, 20, and 100 μM), and 20 μL enzyme solution were
213 incubated at room temperature for 15 min. The reactions were initiated by the addition of 10
214 μL of 0.01 M 5,5-bisdithionitrobenzoic acid (DTNB) and 10 μL of 0.075 ATCI. The reaction
215 solutions were then incubated at 37 ºC for 20 min. A yellow compound (5-thio-nitrobenzoate)
216 production was produced by the reaction of the product thiocholine with DTNB. It was
217 detected at 412 nm. All tested samples and the positive control (dehydroevodiamine) were
218 dissolved in 10% analytical grade dimethyl sulfoxide (DMSO). The reaction was performed in
219 triplicate and recorded in 96-well microplates using a microplate reader [VERSA max
220 (Molecular Devices, Sunnyvale, CA, USA)]. Percent inhibition was calculated using the
221 formula: % AChE inhibition = (1-*S*/*E*) × 100, where *E* and *S* were the enzyme activities with
222 and without the tested sample, respectively. The 50% inhibition concentration of each tested
223 compound was calculated from the log dose-inhibition the log dose-inhibition curve.

224 **Acknowledgements**

225 This work was supported by research grants from Catholic University of Daegu in 2017.

226 **Supplementary data**

227 Supplementary data associated with this article can be found, in the online version.

228 **References**

229 [1] C. Chen, Y. Zhang, C. Huang, Biochem. Biophys, Res. Commun. 397 (2010) 543–547.

230 [2] I. Uriarte-pueyo, M.I. Calvo, Curr. Med. Chem. 18 (2011) 5289–5302.

231 [3], E.J. Park, H. Oh, T.H. Kang, D.S. Sohn, Y.C Kim, Arch. Pharm. Res. 27 (2004) 944–946.

232 [4] M. Jung, M. Park, Molecules 12 (2007) 2130–2139.

233 [5] H. Kato, W. Li, M. Koike, Y. Wang, K. Koike, Phytochemistry 71 (2010) 1925–1929.

234 [6] J.Taira, H. Nanbu, K. Ueda, Food chem. 115 (2009) 1221–1227.

235 [7] S. Kasai, S. Watanabe, J. Kawabata, S.Tahara, J. Mizutani, Phytochemistry 31 (1992)
236 787–789.

237 [8] I. Kouno, N. Baba, Y. Ohni, N. Kawano, Phytochemistry 27 (1988) 297–299.

238 [9] J. Kang, C. Xie, Z. Li, S. Nagarajan, A.G. Schauss, Food chemistry 128 (2011) 152–157.

239 [10] T. Fossen, A. Pedersen, Y.M. Andersen, Phytochemistry 47 (1998) 281–285.

240 [11] A. Sakushima, K. Ohno, M. Coskun, K. Seki, K. Ohkura, Nat. Prod. Lett. 16 (2002)
241 383–387.

242 [12] P.H. Nguyen, V.V. Dung, B.T. Zhao, Y.H. Kim, B.S. Min, M.H. Woo., Arch. Pharm. Res.
243 37 (2014) 1394–1402.

244 [13] J.G. Diaz, A.J. Carmona, P.P.D. Paz, W. Herz, Phytochem. Lett. 1 (2008) 125–129.

245 [14] K.W. Woo, E. Moon, S.Y. Park, S.Y. Kim, K.R. Lee, Bioorg. Med. Chem. Lett. 22 (2012)
246 7465–7470.

247 [15] A.G. Prescott, N.P.J. Stamford, G. Wheeler, J.L. Firmin, Phytochemistry 60 (2002) 589–
248 593.

249 [16] G.L. Ellman, D.K. Courtney, V. Andres, R.M. Featherstone, Biochem. Pharmacol. 7
250 (1961) 88–95.

251 [17] T. Stevanovic, P.N. Diouf, M.E. Garcia-Perez, Curr. Nutr. Food Sci. 5 (2009) 264–295.

252 [18] U. Pueyo, M.I. Calvo, Curr. Med. Chem. 18 (2011) 5289–5302.

253 [19] R. Vinayagam, B. Xu, Nutr. Metab. 12 (2015) 1–20.

254

255 **Figure captions**

256 **Figure 1**. Chemical structures of compounds **1**–**10** from the aerial parts of *A. pilosa* Ledeb.

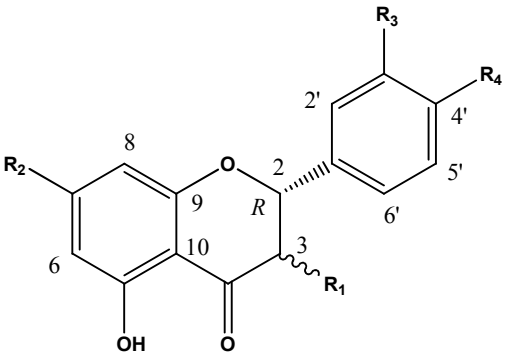
257 **Figure 2**. Key HMBC (⟶) correlations established for compounds **1** and **2**.

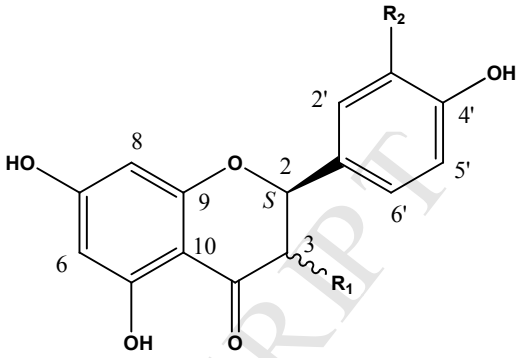
258 **Figure 3**. Key NOESY (⟷) correlations established for compounds **1** and **2**.

259 **Figure 4**. CD spectra of compounds **1**−**4**.

260

261 **Figure 1**. .





262

Compound	R_1	R_2	R_3	R_4
1	*α*-*O*-Glc	OH	H	OH
3	*β*-*O*-Glc	OH	H	OH
5	*α*-*O*-Glc	OH	OH	OH
6	*β*-OH	OH	OH	*O*-Glc
7	*β*-*O*-Glc	OH	OH	OH
8	*β*-OH	*O*-Glc	OH	OH

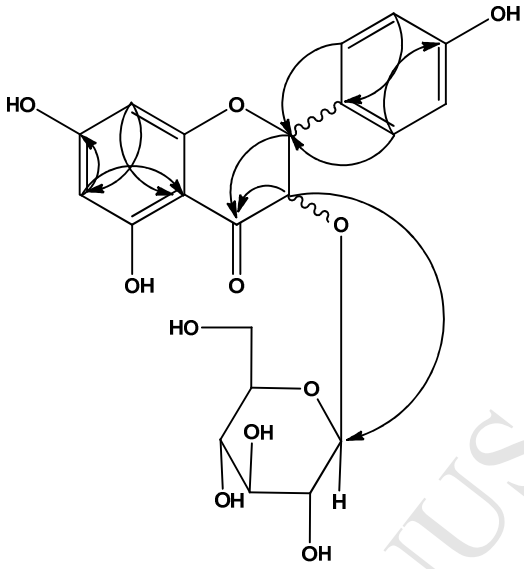
Compound	R_1	R_2
2	*β*-*O*-Glc	H
4	*α*-*O*-Glc	H
9	*β*-*O*-Glc	OH
10	*α*-OH	OH

263

264

265

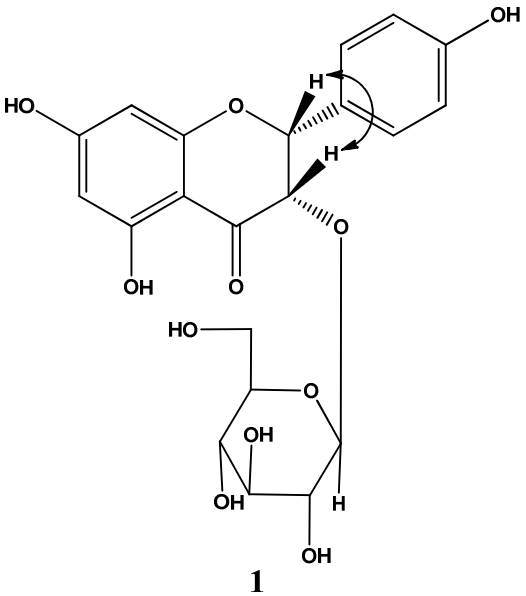
266 **Figure 2**



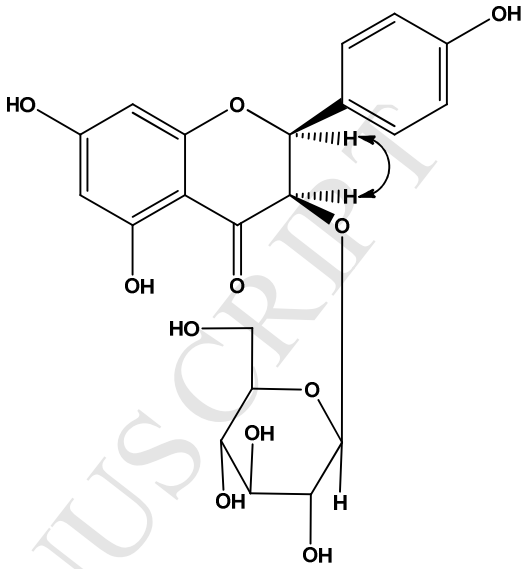
267

268

269 **Figure 3**.



1



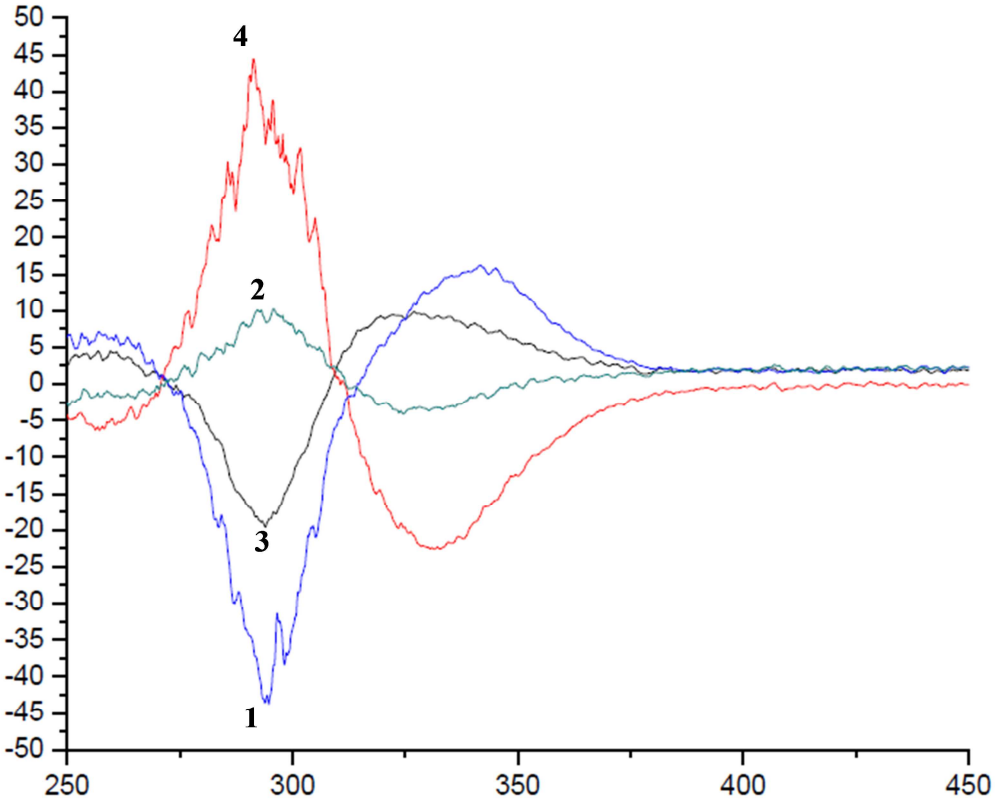
2

270

271

272

273 **Figure 4.**



274

275

276 **Table 1**

277 ^{1}H NMR data of compounds **1**, **2**, **3**, and **4** in pyridine-d_5 (500 MHz)

No	**1**	**2**	**3**	**4**
	δ_H	δ_H	δ_H	δ_H
2	5.84 (br s)	5.82 (d, 2.8)	5.78 (d, 7.6)	5.84 (d, 8.3)
3	5.13 (br, s)	5.29 (d, 2.8)	5.35 (d, 7.6)	5.44 (d, 8.3)
4				
5				
6	6.41 (m)	6.39 (m)	6.37 (d, 1.7)	6.35 (1.9)
7				
8	6.41 (m)	6.39 (m)	6.42 (d, 1.7)	6.44 (d, 1.9)
9				
10				
1'				
2'	7.99 (d, 8.4)	7.79 (d, 8.3)	7.64 (d, 8.4)	7.71 (d, 8.5)
3'	7.20 (d, 8.4)	7.11 (d, 8.3)	7.17 (d, 8.4)	7.21 (d, 8.5)
4'				
5'	7.20 (d, 8.4)	7.11 (d, 8.3)	7.17 (d, 8.4)	7.21 (d, 8.5)
6'	7.99 (d, 8.4)	7.79 (d, 8.3)	7.64 (d, 8.4)	7.71 (d, 8.5)
1"	5.09 (d, 7.7)	5.39 (d, 7.7)	5.49 (d, 7.8)	4.93 (d, 7.4)
2"	4.03 (br t, 7.6)	4.05 (br t, 8.3)	4.01 (m)	4.14 (m)
3"	3.90 (br s)	3.97 (br, s)	4.01 (m)	3.85 (m)
4"	4.15 (m)	4.13 (br t, 9.2)	4.14 (br t, 9.2)	4.14 (m)
5"	4.15 (m)	4.26 (m)	4.31 (m)	4.21 (m)
6"a	4.50 (dd, 2.2, 11.6)	4.47 (br d, 11.4)	4.54 (dd, 2.1, 11.6)	4.53 (dd, 2.1, 11.8)
6"b	4.30 (dd, 5.8, 11.6)	4.26 (m)	4.31 (m)	4.35 (dd, 2.1, 11.8)

278

279

280 **Table 2**

281 ^{13}C NMR data of compounds **1**, **2**, **3**, and **4** in pyridine-d_5 (125 MHz)

No.	**1**	**2**	**3**	**4**
	δ_C	δ_C	δ_C	δ_C
2	82.0	81.7	83.2	83.0
3	76.3	78.4	77.2	76.8
4	194.6	194.7	195.2	194.9
5	165.9	165.8	165.8	165.7
6	97.9	97.9	97.9	97.8
7	169.4	169.2	169.8	169.6
8	96.9	96.8	96.8	96.7
9	163.8	163.9	163.6	163.6
10	102.3	102.6	102.5	102.8
1'	127.3	127.3	127.6	127.9
2'	130.8	130.9	96.9	130.4
3'	116.3	116.6	116.7	116.9
4'	159.7	159.8	160.0	160.1
5'	116.3	116.6	116.7	116.9
6'	130.8	130.9	130.4	130.4
1"	104.0	104.8	105.4	103.3
2"	75.6	75.5	76.0	76.8
3"	78.9	78.9	79.1	79.4
4"	72.1	72.2	72.0	75.5
5"	79.0	78.9	78.8	78.7
6"	63.3	63.5	63.5	63.2

282

283

284 **Table 3**

285 AChE inhibitory activity of compounds **1**–**10**.

Compounds	IC_{50} value[a] (μM)
1	> 100
2	76.59 ± 1.16
3	89.29 ± 3.80
4	> 100
5	97.53 ± 1.64
6	79.00 ± 0.05
7	78.27 ± 1.47
8	78.59 ± 0.93
9	96.29 ± 2.93
10	91.35 ± 2.44
Dehydroevodiamine[b]	34.05 ± 1.11

286 [a]The values indicate 50% AChE inhibitory effect. These data represent the average values of
287 three repeated experiments.

288 Dehydroevodiamine[b] was used as a positive control.

Research highlights

Flavanonol glucosides from the aerial parts of *Agrimonia pilosa* Ledeb. and their acetylcholinesterase inhibitory effects

U Min Seo, Duc Hung Nguyen, Bing Tian Zhao, Su Hui Seong, Jae Sue Choi, Jeong Ah Kim, Byung Sun Min, and Mi Hee Woo

- Two new flavanonol glucoside isomers and eight known compounds were isolated from *Agrimonia pilosa* Ledeb.
- Their structures were determined on the basis of spectroscopic analysis
- All isolated compounds were evaluated for their AChE inhibitory activities
- Most of isolates showed moderate inhibitory effects