SHORT REPORT

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Tyrosinase Inhibitory Chemical Constituents from *Cleyera japonica* Thunberg Branches

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Abstract: Bioassay-guided investigation of the branches of *Cleyera japonica* led to the isolation of four phenolic constituents: 3,3'-di-*O*-methylellagic acid (**1**), 3,3'-di-*O*-methylellagic acid 4'-*O*-β-D-xylofuranoside (**2**), 3,5,7-trihydroxychromone 3-*O*-α-L-arabinofuranoside (**3**) and aviculin (**4**). Their structures were elucidated on the basis of spectral studies, as well as by comparison with literature data. Tyrosinase inhibition activities were carried out for the isolated compounds using arbutin as a positive control. Among them, compound **2** was identified as a potent tyrosinase inhibitor. It inhibited mushroom tyrosinase with an IC_{50} value of 0.078 mM, which is about three times more active than arbutin (IC_{50} =0.25 mM). All of the compounds **1**-**4** were isolated for the first time from this plant.

Keywords: *Cleyera japonica*; isolation; phenolic compounds; tyrosinase inhibition. © 2014 ACG Publications. All rights reserved.

1. Plant Source

In the course of phytochemical studies on the plants growing in Jeju Island located at the southernmost part of Korea [1-3], we have observed the tyrosinase inhibitory activity on the ethanol extract of *Cleyera japonica* Thunberg. We herein describe the structural determination of the isolated compounds as well as their biological activities.

The plant *C. japonica* belonging to Theaceae is a flowering evergreen tree native to warm areas of Korea, Japan and China. The branches of *C. japonica* were collected in September of 2009 from the Halla Botanical Garden located in Jeju Island. Identification of the plant was made by a botanist at the garden. A voucher specimen (sample number 124) was prepared and deposited at the Laboratory of Natural Product Chemistry, Department of Chemistry, Jeju National University.

2. Previous Studies

Flavonoids such as taxifolin, taxifolin glycosides and proanthocyanidin A-1 have been isolated from the extract of *C. japonica* stems [4]. Hydrolysable tannins have been recently identified from leaves of the plant [5].

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3. Present Study

Mushroom tyrosinase inhibition activity was observed on the ethanol extract prepared from the branch parts of *C. japonica* (data not shown). In this experiment, the substrate L-tyrosine was oxidized to *ortho*-quinone (DOPA quinone) by tyrosinase enzyme. The generated DOPA quinone was readily auto-oxidized to dopachrome, the formation of which could be monitored by spectrophotometry at 492 nm [6].

In order to identify the active constituents in the *C. japonica* extract, the phytochemical investigation was conducted. Solvent fractionation of the extract led to *n*-hexane, ethyl acetate (EtOAc), *n*-butanol and water-soluble portions. The EtOAc fraction showing relatively good activities (data not shown) was chosen and subjected to repeated column chromatography over silica gel and/or Sephadex LH-20. From these purification procedures, the compounds **1**-**4** were isolated (Figure 1).

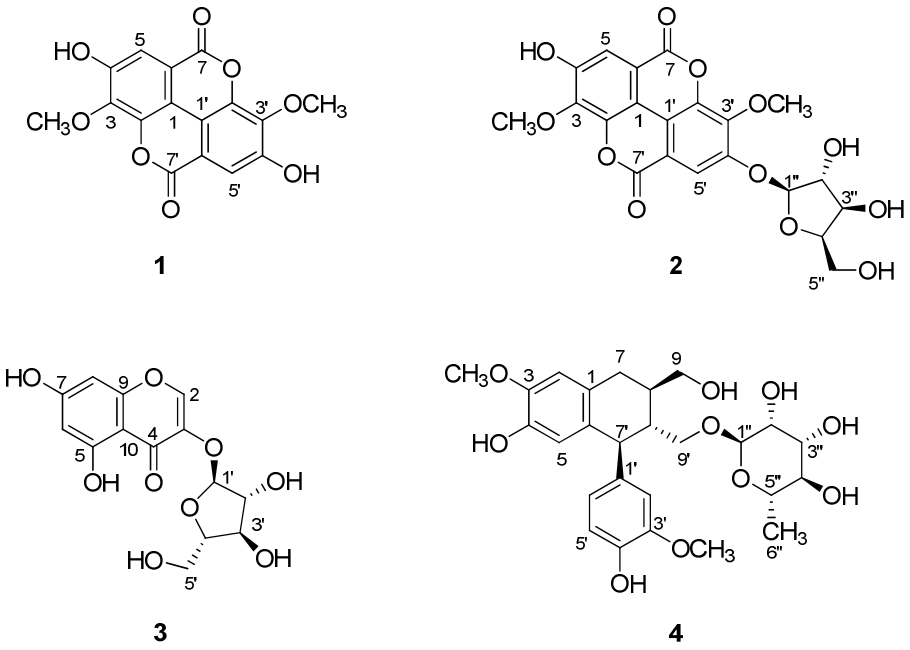

Figure 1. Structures of the isolated compounds **1**-**4** from *C. japonica*.

Compound **1** showed only two single peaks at δ 4.04 (6H, 3-OCH_3 and 3'-OCH_3) and 7.50 (2H, H-5 and H-5') in ^{1}H NMR spectrum. From the investigation of ^{13}C NMR spectrum, an up-field shifted carbonyl carbon δ 158.4 ppm suggested a structure of an ellagic acid derivative. The dimethoxyl-substitued symmetrical skeleton accounts for the observed NMR spectra. Thus, compound **1** was identified as 3,3'-di-*O*-methylellagic acid, which was further confirmed by literature survey [7]. Compound **2** was inferred to have a chemical structure similar to that of **1**, based on ^{1}H and ^{13}C NMR spectra. The ellagic acid skeleton of **2** was evident due to the similarity of spectral patterns between **1** and **2**. Compound **2**, however, was suggested to bear an additional pentose sugar unit showing ^{13}C NMR peaks at δ 101.8, 76.1, 73.1, 69.3 and 65.8 ppm. The sugar was verified as xylose and was assigned to C-3' position by heteronuclear multiple bond correlation (HMBC) data. Therefore, compound **2** was identified as 3,3'-di-*O*-methylellagic acid 4'-*O*-β-D-xylofuranoside [8]. In the ^{13}C NMR spectrum of compound **3**, a pentose sugar unit was observed at δ 104.6, 73.8, 72.2, 69.4 and 67.3 ppm which correspond to the chemical shifts of arabinose. Besides these peaks, additional 9 carbon peaks appeared for the aglycone unit in **3**. Inspection of the spectral data suggests that a hydroxylchromone skeleton is present as aglycone. Taken together, compound **3** was identified as 3,5,7-trihydroxychromone 3-*O*-α-L-arabinofuranoside [9]. Compound **4** was also observed to be a sugar-containing glycoside. The sugar was inferred to be a hexose and confirmed as rhamnose, based

on its NMR data. Two methoxyl peaks were observed at δ 3.81 (3H, 3-OCH_3) and 3.79 (3H, 3'-OCH_3) in the ^{1}H-NMR spectrum. Besides these peaks, 18 carbon peaks were observed for the skeleton of the non-glyconic unit. It was surmised from the number of carbons, as well as NMR patterns, that the aglycone has a lignan structure. Through inspection of 1D and 2D NMR spectra, the lignin glycoside **4** was elucidated as isolariciresinol 9'-*O*-α-rhamnopyranoside, named as aviculin. This structure was further confirmed by comparing the spectral data to those in literature [10]. As far as we know, all of the compounds **1-4** were identified for the first time from *C. japonica*.

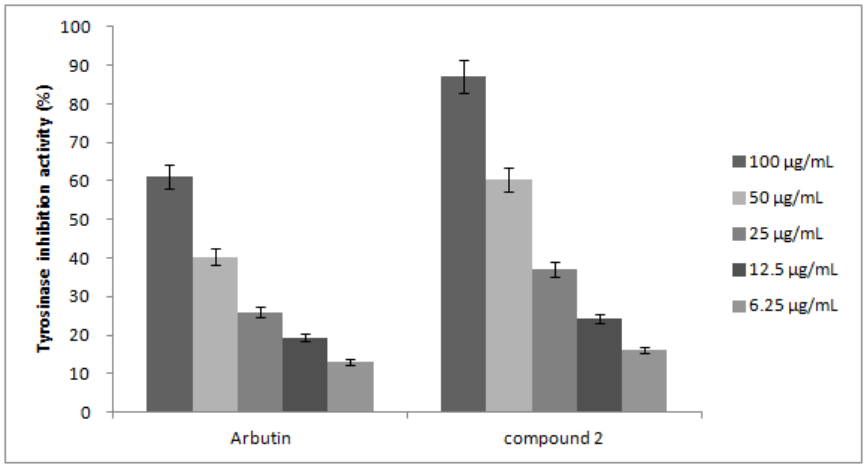

Figure 2. Tyrosinase inhibition activity of the compound **2.**

Tyrosinase (E.C.1.14.18.1) is a metalloenzyme containing copper at its active site. It is distributed widely throughout nature in mammals, fungi and plants. This enzyme plays a crucial role in the production of melanin pigment. Therefore, the inhibition of tyrosinase activity should be an effective measure for down-regulating the pigment formation. The regulation of polymeric melanin is of importance from both medicinal and cosmetic points of view for humans [11].

The anti-tyrosinase properties for the isolates (**1-4**) were examined using L-tyrosine as the substrate. Commercial whitening agent, arbutin (hydroquinone-*O*-α-glucopyranoside), was used as a positive control. While the compounds **1**, **3**, and **4** exhibited very weak tyrosinase inhibition activities, with IC_{50} values of greater than 200 μg/mL, the compound **2** was identified as a potent tyrosinase inhibitor. The ellagic acid derivative **2** reduced the enzymatic activities in a dose dependent manner (Figure 2). The calculated inhibitory activitiy IC_{50} for the compound **2** was 0.078 mM, which exhibits about three times more potency than that of arbutin (IC_{50} = 0.25 mM) . It is interesting to note that compounds **1** and **2** displayed different activities in spite of their identical aglycone skeleton. It is suggested that the sugar unit in **2** results in an enhancement of the tyrosinase inhibitory activity. However, the exact role of the glucose is not clear at this point and should be elucidated in due course.

In conclusion, phytochemical investigation of the stems of *C. japonica* led to the isolation of four compounds. It was demonstrated that the ellagic acid glycoside **2** possessed relatively potent inhibition activity against tyrosinase. Based on these results, the extract of *C. japonica* stems could be applicable as an anti-melanogenic agent.

Acknowledgments

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