Supporting Information to:

Three Major Metabolites of Mulberroside A in Rat Intestinal Contents and Feces

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HPLC analyses of the gastrointestinal contents and feces
For analysis of the metabolites in the gastrointestinal contents, two male Wister rats were administered mulberroside A orally (50 mg/kg) and were killed 2 h following administration,
and then the stomach and small intestine were collected. Both of the contents were extracted with 10 mL of 80% methanol by ultrasonication for 5 min and then centrifuged at 3000 rpm for 10 min. The supernatant of 20 \( \mu \)L was subjected to an HPLC system after being filtered through a 0.45-\( \mu \)m membrane. The preparation of blank sample was the same as the process described above except that the rats of the control group were not administered with drug.

For analysis of the metabolites in the feces, two male Wister rats were administered mulberroside A orally (50 mg/kg), and then 24-h feces were collected using metabolic cages. The feces samples were extracted with 20 mL of 80% methanol by the ultrasonic method for 10 min and then centrifuged at 3000 rpm for 10 min. The supernatant was evaporated to dryness at 40 \(^\circ\)C, the residue was reconstituted with 2 mL of methanol followed by centrifugation at 3000 rpm for 5 min, and the supernatant of 20 \( \mu \)L was subjected to an HPLC system after being filtered through a 0.45-\( \mu \)m membrane. The preparation of blank sample was the same as the process described above except that the rats of the control group were not administered with drug.

The chromatographic analysis was performed on a C\(_{18}\) reversed-phase column (Inertsil ODS-3, 5 \( \mu \)m, 3.9 \( \times \) 150 mm) at a column temperature of 25 \(^\circ\)C. The mobile phase consisted of 0.1% phosphoric acid aqueous solution (A) and 0.1% phosphoric acid methanol solution (B). The gradient program was as follows: linear gradient 0\( \rightarrow \)40% B for 0\( \rightarrow \)40 min, and linear gradient 40\( \rightarrow \)70% B for 40\( \rightarrow \)60 min. The flow rate was 1.0 mL/min. The detection wavelength was set at 335 nm.

**Hydrolysis of metabolites 1 and 2**

A solution of each compound (1.0 mg) in 5% HCl (5 mL) was stirred at 90 \(^\circ\)C in a stoppered vial for 2 h. The reaction solution was extracted with ethyl acetate and concentrated to yield a residue (containing the aglycone) that was detected by HPLC and MS analyses to compare with the standard sample oxyresveratrol. The remnant water solution was evaporated under a stream of N\(_2\). Anhydrous pyridine solutions (0.1 mL) of each residue and L-cysteine methyl ester hydrochloride (0.06 N) was mixed and warmed at 60 \(^\circ\)C for 1 h. Then
trimethylsilylimidazole (0.15 mL) was added and warmed at 60 °C for another 30 min. After
drying the solution, the residue was partitioned between H₂O and cyclohexane. The
cyclohexane layer was concentrated and then dissolved in 200 μL acetone and analyzed by
GC using a DB-1701 column. The temperatures of the injector and detector were 270 °C and
280 °C, respectively. A temperature gradient system was used for the oven, starting at 160 °C
for 1 min and increasing up to 230 °C at a rate of 5 °C/min. The peaks of authentic samples of
D-glucose and L-glucose after treatment in the same manner were detected at 23.28 min and
24.55 min, respectively.

GC was carried out on a Shimadzu GC-2010 series system and performed with a DB-1701
column (30 m × 0.25 mm × 0.25 μm; Agilent).
Fig. 1S HPLC profile of the 2-h stomach contents following oral administration of mulberroside A to rats.

Fig. 2S HPLC profile of the 2-h intestinal contents following oral administration of mulberroside A to rats.
Fig. 3S HPLC profile of the 0–24-h feces after oral administration of mulberroside A to rats.