SUPPLEMENTARY MATERIAL

Two new isoflavone 7-*O*- α -4"-anhydro-4",5"- didehydroglucuronides from *Streptomyces* sp. LZ35 Δ gdmAI

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Abstract

Two isoflavone 7-*O*- α -4"-anhydro-4",5"-didehydroglucuronides, namely daidzein7-*O*- α -4"-anhydro-4",5"-didehydroglucuronide (**1**) and genistein7-*O*- α -4"-anhydro-4",5"-didehydroglucuronide (**2**), were isolated and identified from the mutant strain of *Streptomyces* sp. LZ35 Δ gdmAI. Their structures were elucidated by the analysis of their high resolution mass spectrometry and 1D, 2D NMR spectroscopic data. They are new natural products, and maybe the transformation products of the soybean meal by *Streptomyces* sp. LZ35 Δ gdmAI.

Keywords

Streptomyces sp. LZ35 Δ gdmAI; isoflavone7-*O*- α -4"-anhydro-4",5"-
didehydroglucuronide; biotransformation.

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Figure S6. The ^1H NMR spectrum for compound **2**

Figure S7. The ^{13}C NMR spectrum for compound **2**

Experimental Section

General experimental procedures

NMR spectra were measured on Bruker DRX-600 MHz NMR spectrometer (Bruker) with (tetramethyl silane) TMS as an internal standard. HRMS were carried out on an LTQ-Orbitrap XL. Reversed-phase (RP) C₁₈ silica gel for column chromatography was obtained from Merck (Darmstadt, Germany) and Sephadex LH-20 from GE Amersham Biosciences (Piscataway, New Jersey). Silica gel (200 – 300 mesh) for column chromatography and silica gel GF₂₅₄ for TLC were purchased from Qingdao Marine Chemical Ltd. (Qingdao, China). Semipreparative high performance liquid chromatography (HPLC) were performed on an Agilent 1260 equipped with a ZORBAX XDB-C₁₈ 5 μ m column (9.4 $\times$ 250 mm). All solvents used were of analytical grade. Compounds were visualized under UV light and by spraying with H₂SO₄/EtOH (1 : 9, v/v) followed by heating.

Fermentation, extraction and isolation:

Strain LZ35 Δ *GdmAI* was cultured in petri dishes laid with ca. 20 mL medium (soybean meal 1.5%, 2.5% Soluble starch, 0.2% yeast extract and 1.5% agar, pH 7.2) with a total volume of 6 litres for 11 days at 28 °C. The culture was diced and extracted three times overnight with AcOEt/MeOH/AcOH 80:15:5 (v/v/v) at room temperature and partitioned between H₂O and EtOAc. The EtOAc extract was partitioned with petroleum ether and MeOH until the petroleum ether layer was colorless. The MeOH extract (3.5 g) was subjected to Sephadex LH-20 eluted with CH₂Cl₂/MeOH (1:2) to obtain 10 fractions, i.e., Fr. 1–10. Fr. 7 (235 mg) was further subjected to MPLC over RP-18 silica gel 30 g. 43 subfractions with 16 mL for each gradient were obtained from the elution with 15% MeOH (1–9), 30% MeOH (10–21), 50% MeOH (22–34), 70% MeOH (35–40), and 100% MeOH (41–43) in water, respectively. According to TLC results, Fr. 7a–7e were obtained. Fr. 7c (35 mg) was purified by HPLC (ZORBAX Eclipse XDB-C₁₈ 5 μ m, column ID: 9.4 $\times$ 250 mm, flow rate: 4 mL/min, elution: CH₃CN/H₂O (1 : 4, v/v), UV : at 274 nm) to afford **1** (tr 7.8, 7mg). Fr. 7d (10 mg) was purified by HPLC eluted with CH₃CN/H₂O (25 : 75, v/v) to yield **2** (tr 8.2 min, 2 mg).

Figure S1. The ^1H NMR spectrum for compound 1

Figure S2. The ¹³C NMR spectrum for compound 1

Figure S3. The HMQC spectrum for compound 1

Figure S4. The ^1H MBC spectrum for compound 1

Figure S5. The COSY spectrum for compound 1

Figure S6. The ^1H NMR spectrum for compound 2

Figure S7. The ^{13}C NMR spectrum for compound 2