Two new isoflavone 7-O-α-4′'-anhydro-4''',5'''-didehydroglucuronides from Streptomyces sp. LZ35ΔgdmAI

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Two isoflavone 7-O-α-4′'-anhydro-4''',5'''-didehydroglucuronides, namely daidzein 7-O-α-4′'-anhydro-4''',5'''-didehydroglucuronide (1) and genistein 7-O-α-4′'-anhydro-4''',5'''-didehydroglucuronide (2), were isolated and identified from the mutant strain of Streptomyces sp. LZ35ΔgdmAI. Their structures were elucidated by the analysis of their high resolution mass spectrometry (HR–MS) and 1D, 2D Nuclear magnetic Resonance (NMR) spectroscopic data. They are new natural products and maybe the transformed products of the soybean meal by Streptomyces sp. LZ35ΔgdmAI.

Keywords: Streptomyces sp. LZ35ΔgdmAI; isoflavone 7-O-α-4′'-anhydro-4''',5'''-didehydroglucuronide; biotransformation

1. Introduction

Streptomyces sp. LZ35 is a geldanamycin high-yield strain which was isolated from the intertidal soil collected at Jimei, Xiamen, P.R. China. So far, five different classes of natural products have been identified from strain LZ35, including geldanamycins (Shi et al. 2011), cuevaenes (Deng, Lu, Li, Li, et al. 2014), hygrocin (Lu et al. 2013), 16,17-dihydroxycyclooctatin (Zhao et al. 2013) and echosides (Deng, Lu, Li, Hao, et al. 2014). The analysis of genome sequence indicated that Streptomyces sp. LZ35 has the potential of producing different types of secondary metabolites (data not shown). In the course of our ongoing research activities towards the isolation of new natural products, the mutant strain Streptomyces sp. LZ35ΔgdmAI, which was constructed by disrupting the first PKS module of geldanamycin gene cluster (gdm), was fermented in soybean meal media.

Herein, we report the isolation and structural characterisation of two new isoflavone 7-O-α-4′'-anhydro-4''',5'''-didehydroglucuronides (1-2) (Figure 1) from the metabolites of the strain LZ35ΔgdmAI through column chromatography over Sephadex LH-20, medium pressure liquid...
chromatography (MPLC) and high performance liquid chromatography (HPLC), and elucidated based on their HR–MS and 1D, 2D NMR spectroscopic data.

2. Results and discussion

The MeOH extract from a 6-L agar plate fermentation of the strain LZ35ΔgdmAI was fractionated by a combination of various column chromatographic methods, resulting in the isolation of two new isoflavone 7-O-α-4″-anhydro-4″,5″-didehydroglucuronides (1 and 2).

The molecular formula of compound 1 was determined to be C_{21}H_{16}O_{9} by HR-ESI-MS (m/z 413.3543 [M+H]^+, calcd for 413.3537). The UV absorptions at λ_{max} 275 and 320 nm attributable to the bands I and II of the benzoyl and cinnamoyl chromophores of an isoflavone (Farag et al. 2001).

The 1H NMR spectrum displayed 12 resonances for 9 unsaturated protons (δ_H 6.17–8.21), 3 oxymethine protons (δ_H 5.82, 4.26, 3.98). A characteristic singlet resonance for H-2 of isoflavone was observed at δ_H 8.15 (Horie et al. 1998). This assignment was confirmed by its long-range connectivity to the quaternary carbons at δ_C 178.1 (C-4), 159.1 (C-9), 126.2 (C-3) and 124.0 (C-10) in the HMBC spectrum. The presence of a tri-substituted benzene ring was indicated by two doublets and a singlet with the equal J values of 8.8 Hz at δ_H 7.29, 8.15 and 7.40 in the 1H NMR, and HMBC correlations from H-8 to C-6, C-7 and C-10, and H-6 to C-7, C-9 (w) and C-10, and H-5 to C-7, C-4 and C-9. The remaining aromatic proton resonances comprised two doublets at δ_H 7.39 and 6.85, representing a p-substituted ring B, which was confirmed by the HMBC correlations from the proton at δ_H 7.39 (H-2′, H-6′) to C-3, and C-4′ (δ_C 158.8), and the proton at δ_H 6.85 (H-3′, H-5′) to C-1′, C-4′ and C-5′. According to the down field shifts of C-4′ (δ_C 158.8) and C-7 (δ 162.7), two oxy carbons were deduced at C-4′ and C-7. Therefore, the aglycone of compound 1 was determined as daidzein (Li et al. 2009; Yang et al. 2013).

The presence of α-4-anhydro-4,5-didehydroglucuronate moiety was revealed by the 1H NMR signals at δ_H 5.82 (d, J = 4.4 Hz), 4.26 (br s), 3.28 (br s) and 6.17 (br s), and 13C NMR signals at δ_C 99.6d, 68.0d, 71.7d, 113.2d, 142.5d and 165.5s. These assignments were further confirmed by 1H–1H COSY and HMBC correlations (Figures S5 and S4). The 1H–1H COSY cross-peaks H-1″/H-2″, H-2″/H-3″ and H-3″/H-4″ and HMBCs from H-2″ to C-1″, C(3), and C (4), and from H-2″ to C-3″ and C-1″, and from H-3″ to C-2″, C-4″ and C-5″, and from H-1″ to C-2″, C-5″, C-6″ and C-7. The key HMBC correlation from the anomeric proton H-1″ (δ_H 5.82) to C-7 revealed that the α-4-anhydro-4,5-didehydroglucuronate moiety was located at C-7. The structure of 1 was further confirmed by comparison of the corresponding data with those reported previously (Agrawal 1992; Al-Maharik & Botting 2006). Thus, the structure of compound 1 was established to be daidzein 7-O-α-4″-anhydro-4″,5″-didehydroglucuronide.

Figure 1. The structures of compounds 1 and 2.
protons at \( \delta_H 7.39 \) and \( \delta_H 6.85 \) with the \( J \) values of 7.5 and 8.8 Hz. The down field shifts of C-4' (\( \delta_C 158.9 \)), C-5 (\( \delta_C 163.9 \)) and C-7 (\( \delta_C 163.6 \)) revealed the presence of oxy carbons. Therefore, the aglycone moiety of 2 was determined as genistein (Wang et al. 1999; Kozerski et al. 2003). Additionally, the \( \alpha \)-4-anhydro-4,5-didehydroglucuronate moiety was determined by NMR comparison with compound 1. Therefore, the structure of 2 was determined as genistein 7-O-\( \alpha \)-4\(^{00}\)-anhydro-4\(^{00}\),5\(^{00}\)-didehydroglucuronide.

It has been demonstrated that isoflavones isolated from Streptomyces cultivated in media containing plant-derived nutrients such as soybean are not of microbial biosynthetic origin (Anyanwutaku et al. 1992). Moreover, Streptomyces is well-known for its ability of biotransformation of isoflavones, such as converting genistein (5,7,4'-trihydroxyisoflavone), the major isoflavone of soybeans, into 8-methyl genistein (Hosny & Rosazza 1999), and daidzein into 1\(^{\prime}\)-O-methyl-8-hydroxymethyl daidzein (Yang et al. 2013). Therefore, the two new isoflavone glucuronides (1 and 2) might be transformed from daidzein and genistein of the culture media by glucuronidation (Maatooq & Rosazza 2005). Indeed, glucuronidation is broadly involved in xenobiotic metabolism of substances such as drugs (Guillemette 2003; Sugatani 2013), and elimination of endobiotics such as biosynthetic intermediates (Li et al. 2014). Previously, five \( p \)-terphenyl \( O \)-\( \beta \)-glucuronides, namely echosides A–E, were isolated from the strain Streptomyces sp. LZ35\( \Delta \)gdmAI (Deng, Lu, Li, Hao, et al. 2014), indicating the presence of UDP-glucuronosyltransferases in this strain and further supporting the biotransformation origin of 1 and 2. Accordingly, we proposed that the production of 1 and 2 was derived from daidzein and genistein by the glucuronidation at 7-hydroxyl group and the dehydration of 5-hydroxyl group in the glucuronate moiety, respectively (Figure 2).

The isolation of compounds 1 and 2 in this study indicated the readiness of glucuronidation of daidzein and genistein by Streptomyces species, which was consistent to the results of previous research. However, the dehydration of 5-hydroxyl group in the glucuronate moiety was novel among isoflavone glucuronides. Moreover, soybean isoflavones have been reported to possess numerous physiological properties, such as antitumour, anti-menopausal (female) osteoporosis and antiageing, antioxidant, anti-inflammation. They have also been reported to improve learning and memory skills in menopausal women and to aid in the prevention and treatment of heart disease, diabetes and Kawasaki disease (KD) (Wang et al. 2013). Therefore, the bioactivities and biosynthesis of compounds 1 and 2 are worth further investigation (Al-Maharik & Botting 2006; Kgomoitso et al. 2008).

3. Experimental
3.1 General experimental procedures

NMR spectra were measured on Bruker DRX-600 MHz NMR spectrometer (Bruker, Switzerland) with TMS as an internal standard. HR-ESI-MS were carried out on an LTQ-
Strain LZ35\textit{gdmAl} 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 L 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 H\textsubscript{2}O and EtOAc. The EtOAc extract was partitioned between petroleum ether and 95\% MeOH until the petroleum ether layer was colourless. The MeOH extract (3.5 g) was subjected to CC over Sephadex LH-20 (40–70 \textmu m, column ID: 9.4 x 250 mm, flow rate: 4 mL/min, elution: CH\textsubscript{2}Cl\textsubscript{2}/MeOH (10–21), 50\% MeOH (22–34), 70\% MeOH (35–40) and 100\% MeOH (41–43) to afford 43 subfractions with 16 mL each. These fractions were pooled to Fr. 7a–7e according to TLC results. Fr. 7c (35 mg) was purified by HPLC (ZORBAX Eclipse XDB-C\textsubscript{18}, 5 \textmu m, column ID: 9.4 x 250 mm, flow rate: 4 mL/min, elution: CH\textsubscript{2}Cl\textsubscript{2}/MeOH/AcOH 80: 15: 5 (v/v/v) at room temperature and partitioned between H\textsubscript{2}O and EtOAc) equipped with a ZORBAX XDB-C\textsubscript{18} column (9.4 x 250 mm, 5 \textmu m). All solvents used were of analytical grade. Compounds were visualised under UV light and/or by spraying with H\textsubscript{2}SO\textsubscript{4}/EtOH (1:9, v/v) followed by heating.

3.2 Fermentation, extraction and isolation

3.3 Structure and identification

\textit{7-O-\alpha-4\textsuperscript{\prime}-anhydro-4\textsuperscript{\prime},5\textsuperscript{\prime}-didehydroglucuronide (1)}. Yellowish powder, UV (EtOH) \(\lambda_{\text{max}}\) 275, 320 nm. \(^1\text{H} NMR\) data (in CD\textsubscript{3}OD, \(\delta\) in ppm, \(J\) in Hz): 8.21 (s) (H-2), 8.15 (d, 8.8) (H-5), 7.29 (d, 8.8) (H-6), 7.40 (s) (H-8), 7.39 (d, 7.9) (H-2\textprimed), 6.85 (d, 7.6) (H-3\textprimed), 6.85 (d, 7.6) (H-5\textprimed), 7.39 (d, 7.9) (H-6\textprimed), 5.82 (d, 4.4) (H-1\textprimed), 3.98 (br s) (H-2\textprimed\textprimed), 4.26 (br s) (H-3\textprimed\textprimed), 6.17 (br s) (H-4\textprimed\textprimed). \(^{13}\text{C} NMR\) data (in CD\textsubscript{3}OD, \(\delta\) in ppm): 155.2 (C-2), 126.2 (C-3), 178.1 (C-4), 128.4 (C-5), 117.2 (C-6), 162.7 (C-7), 159.1 (C-9), 120.5 (C-10), 124.0 (C-1\textprimed), 131.4 (C-2\textprimed), 116.2 (C-3\textprimed), 158.8 (C-4\textprimed), 116.2 (C-5\textprimed), 131.4 (C-6\textprimed), 99.6 (C-1\textprimed\textprimed), 71.7 (C-2\textprimed\textprimed), 68.0 (C-3\textprimed\textprimed), 113.2 (C-4\textprimed\textprimed), 142.5 (C-5\textprimed\textprimed), 165.5 (C-6\textprimed\textprimed). HR-ESI-MS: \textit{m/z} 413.3543 [M + H]\textsuperscript{+} (calcd for C\textsubscript{21}H\textsubscript{16}O\textsubscript{9}, 413.3537).

\textit{7-O-\alpha-4\textsuperscript{\prime}-anhydro-4\textsuperscript{\prime},5\textsuperscript{\prime}-didehydroglucuronide (2)}. \(^1\text{H} NMR\) data (in CD\textsubscript{3}OD, \(\delta\) in ppm, \(J\) in Hz): 8.15 (s) (H-2), 6.60 (s) (H-6), 6.83 (s) (H-8), 7.40 (d, 7.5) (H-2\textprimed), 6.85 (d, 8.8) (H-3\textprimed), 6.85 (d, 8.8) (H-5\textprimed), 7.40 (d, 7.5) (H-6\textprimed), 5.74 (d, 3.6) (H-1\textprimed\textprimed), 3.93 (br s) (H-2\textprimed\textprimed\textprimed), 4.24 (br s) (H-3\textprimed\textprimed\textprimed), 6.16 (br s) (H-4\textprimed\textprimed\textprimed). \(^{13}\text{C} NMR\) data (in CD\textsubscript{3}OD, \(\delta\) in ppm): 155.4 (C-2), 125.0 (C-3), 182.5 (C-4), 163.9 (C-5), 96.1 (C-6), 163.6 (C-7), 101.3 (C-8), 159.2 (C-9), 108.3 (C-10), 123.0 (C-1\textprimed), 131.4 (C-2\textprimed), 116.3 (C-3\textprimed), 158.9 (C-4\textprimed), 116.3 (C-5\textprimed), 131.4 (C-6\textprimed), 99.5 (C-1\textprimed\textprimed\textprimed), 71.1 (C-2\textprimed\textprimed\textprimed), 68.0 (C-3\textprimed\textprimed\textprimed), 113.2 (C-4\textprimed\textprimed\textprimed), 142.4 (C-5\textprimed\textprimed\textprimed), 165.5 (C-6\textprimed\textprimed\textprimed). HR-ESI-MS: \textit{m/z} 429.3537 [M + H]\textsuperscript{+} (calcd for C\textsubscript{21}H\textsubscript{16}O\textsubscript{10}, 429.3531).

Supplementary material

Supplementary material including NMR spectra of compounds 1 and 2 are available online.
Disclosure statement
No potential conflict of interest was reported by the authors.

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