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New antibacterial isocoumarin glycosides from a wetland soil derived fungal strain *Metarhizium anisopliae*

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ABSTRACT

Eight new isocoumarin glycosides (**1**–**8**) were obtained from the solid culture of the wetland soil-derived fungus *Metarhizium anisopliae* (No. DTH12-10). Their chemical structures were elucidated by analyses of HR ESI-TOF MS, ^{1}H, ^{13}C NMR, ^{1}H–^{1}H COSY, HSQC, and HMBC spectra. The absolute configurations were determined by single crystal X-ray diffraction, circular dichroism (CD) spectrum, and chemical derivatization methods. In addition, inhibition of the biofilm formation and the secretion of virulence factor of the new isocoumarin glycosides against *Pseudomonas aeruginosa* strain PAOA (clinical isolates) were evaluated. The result revealed that compound **1** showed antibacterial activity comparable with (*Z*)-4-bromo-5-(bromomethylene)-2(5*H*)-furanone (BF).

Pseudomonas aeruginosa is a major nosocomial pathogen that can cause a wide variety of acute and chronic infection, because of its extensive arsenal of virulence factors. What's more, it usually colonizes in the specialized surface, living as biofilm style with the help of quorum-sensing communication, which makes *P. aeruginosa* become more resistant to antibiotics.[1–3] An estimate by the National Institutes of Health (NIH) stated that 80% of microbial infections were biofilm-based.[4] So, development of QS antagonists, which can block the QS system of *P. aeruginosa* with mechanism of reducing biofilm formation and virulence factor secretion, is a new strategy to treat bacterial infection.[5]

Although studies of bioactive secondary metabolites from wetland soil derived fungus were rare, it is a potential resource for discovery of new bioactive constituents.[6–8] In our search for bioactive components from microorganisms,[9–11] eight new isocoumarin glycosides (**1**–**8**) were obtained from the solid culture of the wetland soil-derived fungus *M. anisopliae* (No. DTH12-10). Herein, we described the isolation and structure elucidation of these compounds. In addition, inhibition of the biofilm formation and the secretion of virulence factor of the new isocoumarin glycosides against *P. aeruginosa* strain PAOA (clinical isolates) were evaluated.

A solid culture of *M. anisopliae*[12] on cooked rice was extracted with EtOAc thoroughly. The EtOAc-soluble portion was subjected to repeated silica gel, ODS column chromatographies and RP HPLC to yield eight new isocoumarin glycosides (**1**–**8**) (Fig. 1).[13]

Compound **1**[14] was obtained as yellow needle crystal (MeOH/H_2O). The HR ESI-TOF MS showed quasimolecular ion at *m*/*z* 519.1855 $[M+Na]^+$ (calcd. for 519.1842), indicating the molecular formula of $C_{24}H_{32}O_{11}$ and accounting for 9° of unsaturation. Comparison of the ^{13}C NMR data with that of 3-ethyl-6-*O*-(4′-*O*-methyl-β-D-glucopyranoside)-8-hydroxyl-7-methyl-3,4-dihydroisocoumarin revealed that **1** had a 3,4-dihydroisocoumarin skeleton and a 4′-*O*-methyl-glucopyranose moiety, which were confirmed by ^{1}H–^{1}H COSY, HSQC, and HMBC spectra of **1** (Fig. 2).[15] The HMBC correlation from H-6′$_a$ (δ 4.20, 1H, dd, 11.8, 5.6)/H-6′$_b$ (δ 4.36, 1H, dd, 11.8, 2.1) and H-8′ (δ 2.03, 3H, s) to C-7′ (δ 172.4) suggested that an acetyl group was adjacent to C-6′ of the 4′-*O*-methyl-glucopyranose moiety in **1**. The linkage position of 4′-*O*-methyl-6′-acetyl-glucopyranose moiety in **1** was defined to C-6 by the HMBC correlation from δ_H 5.01 (1H, d, 7.5, H-1′) to δ_C 157.3

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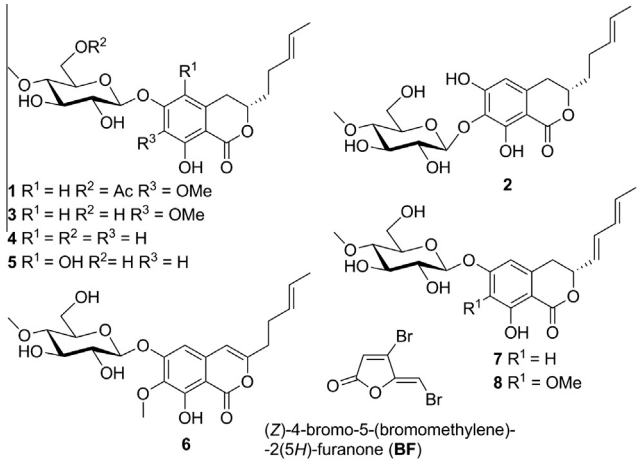

Figure 1. Chemical structures of **1**–**8** and BF.

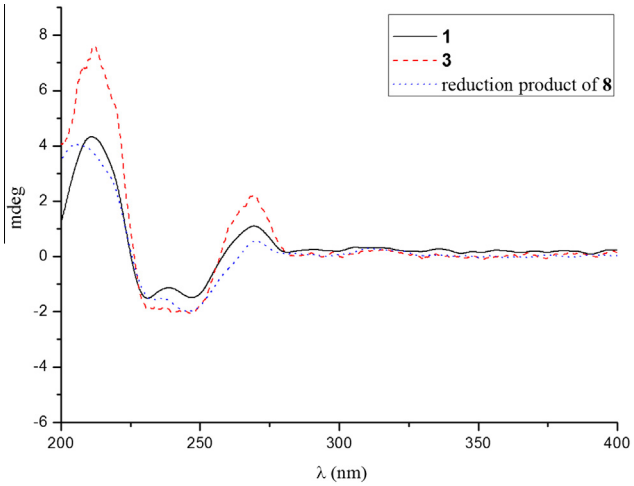

Figure 3. CD spectra of **1**, **3** and reduction product of **8** in MeOH.

(C-6). The HMBC correlation from δ_H 3.83 (3H, s, 7-OCH_3) to δ_C 137.0 (C-7) attached the methoxyl group to C-7 position of the 3,4-dihydroisocoumarin moiety. In the ^{1}H–^{1}H COSY spectrum of **1**, successive correlations of H-4 (δ 2.89, 2H, m), H-3 (δ 4.54,1H, m), H-11 (δ 1.74, 1H, m; δ 1.86, 1H, m), H-12 (δ 2.17, 2H, m), H-13 (δ 5.47, 1H, overlap), H-14 (δ 5.47, 1H, overlap), and H-15 (δ 1.63, 3H, d, 5.1) revealed the partial structure of C4–C3–C11–C12–C13–C14–C15 (Fig. 2). The HMBC correlation from H-11 (δ 1.74, 1H, m; 1.86, 1H, m) to C-4 (δ 33.6) and C-3 (δ 80.6) suggested that the aliphatic chain C11–C12–C13–C14–C15 was adjacent to C-3 of the 3,4-dihydroisocoumarin skeleton in **1**. According to the molecular formula, the substitution in C-8 was a hydroxyl group. Therefore, the gross structure of **1** was determined. The double bond of C-13 and C-14 was determined to be *E* configuration by the ^{13}C chemical shift of methyl group connected to the double bond.[16] The positive cotton effect at 268 nm in the circular dichroism (CD) spectrum of **1** suggested the (3*S*)-configuration (Fig. 3),[17] which was confirmed by the single-crystal X-ray diffraction. The absolute configuration of the sugar moiety was assigned by the single-crystal X-ray diffraction experiment conducted with Cu Kα.[18] Thus, compound **1** was determined as (3*S*)-6-*O*-(4′-*O*-methyl-6′-acetyl-β-D-glucopyranoside)-7-*O*-methyl-8-hydroxyl-3-[(3*E*)-penta-3-enyl]-3,4-dihydroisocoumarin.

Compound **2**[19] was obtained as light yellow needle (MeOH/H_2O). The HR ESI-TOF MS showed quasimolecular ion at *m*/*z* 441.1758 $[M+H]^+$ (calcd. for 441.1761), indicating the molecular formula of $C_{21}H_{28}O_{10}$ and accounting for 8° of unsaturation. Comparison of the ^{13}C NMR data with that of **1** and 3-ethyl-6-*O*-(4′-*O*-methyl-glucopyranoside)-8-hydroxyl-7-methyl-3,4-dihydroisocoumarin[15] revealed that **2** had a 3-[(3*E*)-pent-3-enyl]-3,4-dihydroisocoumarin moiety and a 4′-*O*-methyl-glucopyranose unit. The difference between them lay in the substitutions in C-6, C-7, and C-8 position (Table 1). The HMBC correlation from δ_H 5.42 (1H, overlap) to δ_C 133.0 (C-7) attached the 4-*O*-methyl-glucopyranose to C-7 position of the 3-[(3*E*)-pent-3-enyl]-3,4-dihydroisocoumarin moiety. The other two substitutions in C-6 and C-8 positions were hydroxyl groups by molecular formula given by HR ESI-TOF MS. Thus, the gross structure of **2** was determined, which was verified by ^{1}H–^{1}H COSY and HMBC spectra. Similarly, **2** had 3*S* configuration according to the positive cotton effect at 268 nm in the circular dichroism (CD) spectrum (Fig. 4).[17] The absolute configuration of the sugar moiety was assigned by the single-crystal X-ray diffraction experiment conducted with Cu Kα.[20] Therefore, **2** was assigned as (3*S*)-7-*O*-(4′-*O*-methyl-β-D-glucopyranoside)-6,8-dihydroxyl-3-[(3*E*)-pent-3-enyl]-3,4-dihydroisocoumarin.

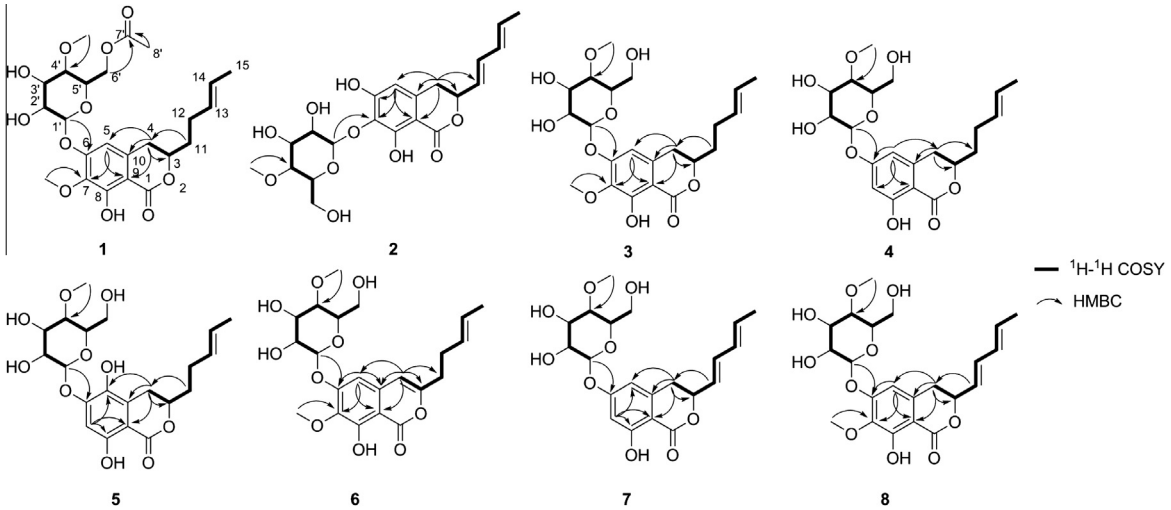

Figure 2. Key HMBC and ^{1}H–^{1}H COSY correlations of **1**–**8**.