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Three new fusidic acid derivatives and their antibacterial activity

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ABSTRACT

Two steroid acids, cephalosporin P_1 and isocephalosporin P_1, were isolated from *Hapsidospora irregularis* FERM BP-2511. These compounds are structurally related to fusidic acid. Their NMR data were completely assigned on the basis of the 2D NMR spectra. Incubation of these two compounds with *Microbacterium oxydans* CGMCC 1788 in Luria–Bertani broth yielded the same set of three new 3-dehydrogenated products, 3-keto-isocephalosporin P_1, 3-keto-cephalosporin P_1 and 6-deacetyl-3-keto-cephalosporin P_1. The final pH of the bacterial culture was 9.0. Incubation of 3-keto-isocephalosporin P_1 or 3-keto-cephalosporin P_1 in Tris–HCl buffer (pH 9.0) revealed that these two compounds can convert to each other by shifting the acetyl group between C-6 and C-7. The acetyl group at C-6 or C-7 can also be removed by hydrolysis to yield the minor product 6-deacetyl-3-keto-cephalosporin P_1. These fusidic acid derivatives were tested for the antibacterial activity against the Gram-positive pathogen *Staphylococcus aureus*. 3-Keto-cephalosporin P_1 showed the highest activity among the five compounds, with a minimal inhibition concentration (MIC) of 4 μg/mL, which is more potent than the substrate cephalosporin P_1. Both cephalosporin P_1 and 3-keto-cephalosporin P_1 were active against methicillin-resistant *S. aureus*, with the same MIC of 8 μg/mL.

Infections are one of the top causes of death. Antibiotic drugs have been effective in treating bacterial infections and saving lives. However, extensive use of antibiotics may also induce the occurrence of resistant bacteria. For example, *Staphylococcus aureus* (SA) is recognized as one of the most common pathogenic bacteria that cause diseases in humans, such as pneumonia, surgical wound and bloodstream infections. Its variant methicillin-resistant *Staphylococcus aureus* (MRSA) is resistant to all beta-lactam antibiotics such as penicillin and methicillin. This strain is called 'super bug' because of its drug-resistance and serious threat to human lives. The spread of resistant bacteria has led to untreatable infection, which has become one of the world's most pressing public health threats.[1] Thus, new antibacterial agents are needed.

Most antibiotics were discovered from microorganisms such as soil-derived actinomycetes. Many of these antibiotics were isolated between 1940s and 1960s. After that, discovery of new antibacterial natural products slowed down. This creates a crisis of lacking effective anti-infectious drugs to combat infections, especially considering that antibiotic resistance is rising. Our group has been interested in discovering antibacterial natural products and creating new derivatives using biocatalytic tools for bioactivity screening. In the course of screening antibacterial microbial extracts, we found that *Hapsidospora irregularis* FERM BP-2511 is active against SA and MRSA, with the minimal inhibition concentrations (MICs) of 500 and 1000 μg/mL, respectively. *H. irregularis* belongs to the family of Pseudeurotiaceae.[2] It is known to produce a cyclodepsipeptide, leualacin, which can inhibit the binding of ^{3}H-nitrendipine to cardiac Ca^{2+} channel in a competitive manner.[3,4] No antibacterial compounds have ever been reported from this fungus. We thus grew *H. irregularis* FERM BP-2511 in K2 broth and isolated two metabolites **1** and **2** (Fig. 1) from the culture using open column chromatography and HPLC.[5] Their structures were elucidated on the basis of the spectral data.

Both **1** and **2** have a molecular weight of 574 according to the $[M-H]^-$ ion peak at *m/z* 573.3 in their ESI-MS spectra. The ^{13}C NMR spectra of these two compounds revealed that they have thirty three carbons including 3 carbonyls, 4 oxygenated methines, 4 olefinic carbons, and 22 sp^3 carbons. Accordingly, the molecular formula of **1** and **2** was deduced to be $C_{33}H_{50}O_8$, suggesting that they have nine degrees of unsaturation. Extensive analysis of the 2D NMR spectra including ^{1}H–^{1}H COSY, HSQC, and HMBC spectra (Fig. 2) allowed **1** and **2** to be identified as cephalosporin P_1 (Fig. 1) and isocephalosporin P_1 (Fig. 1), respectively. Their NMR data were assigned and are shown in Tables S1 and S2.

Cephalosporin P_1 (**1**) and isocephalosporin P_1 (**2**) are triterpenoid antibiotics that belong to the fusidic acid family of natural products. Fusidic acid is a bacteriostatic antibiotic from the fungus

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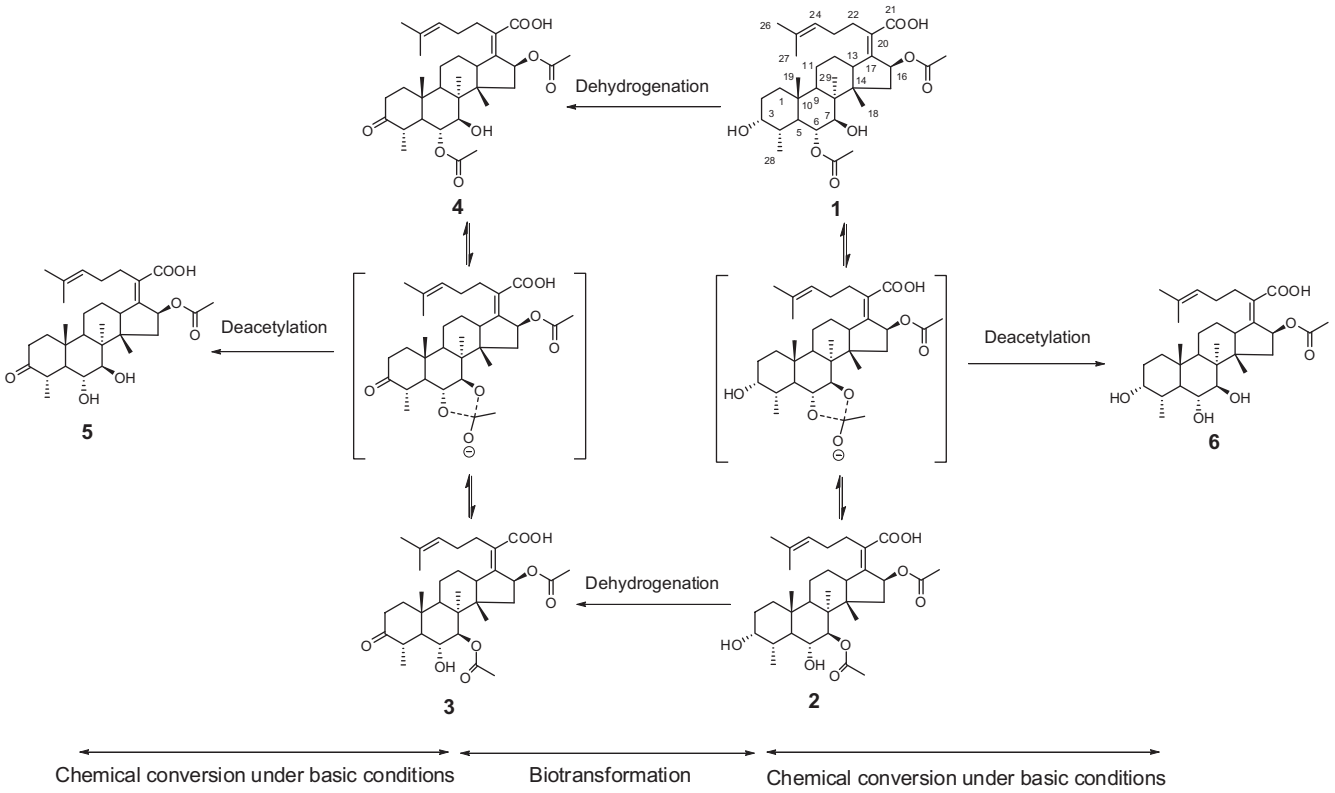

Figure 1. Synthesis of three new fusidic acid derivatives **3**–**5** from cephalosporin P_1 (**1**) and isocephalosporin P_1 (**2**).

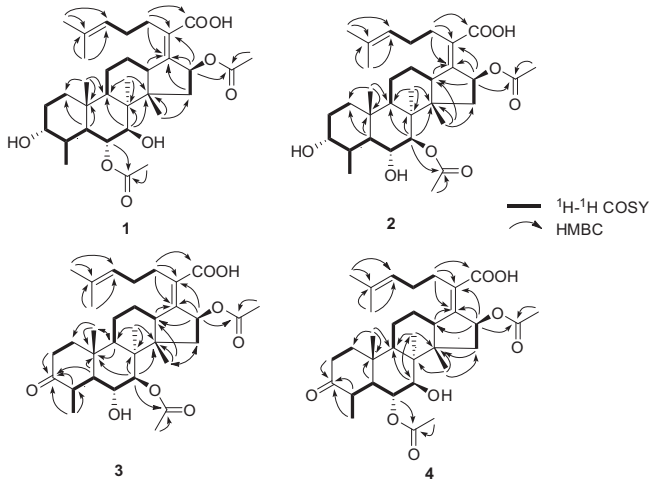

Figure 2. Selected ^{1}H–^{1}H COSY and HMBC correlations for **1**–**4**.

Fusidium coccineum and was developed by Leo Laboratories (Ballerup, Denmark) and released for clinical use in the 1960s. Its sodium salt has been used in many countries. Fusidic acid is primarily effective against Gram-positive bacteria. **1** was first previously isolated from a species of *Cephalosporium*,[6] and **2** was produced by *Cephalosporium acremonium*.[7] Early work demonstrated that **1** has an antibacterial spectrum that predominantly encompasses Gram-positive organisms, with particularly potent activity against SA.[8,9] This is the first time that **1** and **2** were isolated from *H. irregularis*. The NMR data of **2** were not completely assigned in the previous work. In this study, we conducted complete NMR analysis for these two molecules and assigned all the NMR signals for both **1** and **2**.

Biotransformation has shown its promise in creating chemical diversity in natural products.[10,11] Because **1** has been reported to inhibit Gram-positive bacteria,[12] we chose a strain of Gram-negative bacterium *Microbacterium oxydans* CGMCC 1788 for the biotransformation experiment to prepare new analogs from **1** and **2**. This strain was previously used for glycosylation of the plant natural product puerarin.[13] HPLC analysis revealed that compared to the culture control (trace iii, Fig. 3A), incubation of *M. oxydans* CGMCC 1788 with **1** in LB medium at 30 °C for 4 days yielded three products **3**–**5** (trace i, Fig. 3A). The UV spectra of these products are similar to that of the substrate (Fig. S1), suggesting that they are derivatives of **1**. Interestingly, incubation of the same strain with **2** gave rise to the same set of three products (trace ii, Fig. 3A). These compounds were then isolated from the extract of the biotransformation broth of **1** using open column chromatography and HPLC.[14] The purified products were subjected to MS and NMR analyses.

Compounds **3** and **4** showed the same molecular weight of 572 according to the $[M-H]^-$ ion peak at m/z 571.3 and $[M+Na]^+$ peak at m/z 595.3 in the ESI-MS spectra. Both compounds showed a $[M-H]^-$ ion peak at m/z 571.3258 ($C_{33}H_{47}O_8$, calcd 571.3276) in the HR-MS spectrum. Accordingly, the molecular formula was determined to be $C_{33}H_{48}O_8$, suggesting that these two compounds are isomers and they are two protons less than the substrates **1** and **2**.

The ^{13}C NMR spectra of **3** showed a low field carbon signal at δ_C 216.4, suggesting that a ketone group was generated during the biotransformation process. In the meantime, an oxygenated methine signal was missing compared to the substrates **1** and **2**. This observation suggested that this oxygenated methine might have been converted to a ketone group through dehydrogenation. To find out which oxygenated methine was oxidized, 2D NMR including ^{1}H–^{1}H COSY, HSQC, and HMBC were recorded. ^{1}H–^{1}H COSY correlations revealed a spin system of CH_3(28)–CH(4)–