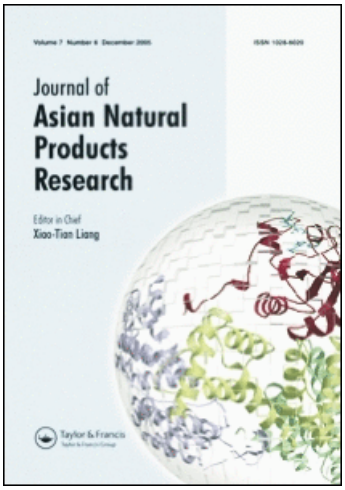

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Microbial transformation of fraxinellone by *Aspergillus niger*

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Note

Microbial transformation of fraxinellone by *Aspergillus niger*

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Microbial transformation of fraxinellone (**1**) by *Aspergillus niger* (AS 3.421) has been carried out. Two converted products, dasycarpol (**2**) and a new compound fraxinigerllone (**3**) were obtained. Their structures have been identified on the basis of spectroscopic methods. Dasycarpol shows moderate inhibitory activity on lung cancer cell line A549.

Keywords: Microbial transformation; Fraxinellone; Dasycarpol; Fraxinigerllone; *Aspergillus niger*

1. Introduction

Fraxinellone is one of the most abundant active constituents isolated from the root bark of *Dictamnus dasycarpus* Turcz (Bai-Xian-Pi), a traditional Chinese medicine used to treat jaundice, cough, rheumatism and some skin diseases [1]. *Aspergillus niger* has been widely used to biotransform various compounds because of its broad substrate specificity and high regioselectivity [2]. To obtain more active compounds, *A. niger* was used for the biotransformation of fraxinellone and two converted products were obtained (Scheme 1), one of which was identified as dasycarpol (**2**) by comparison of its NMR data with that of the known compound [3] and the other turned to be a new compound which was named as fraxinigerllone (**3**).

2. Results and discussion

Fraxinigerllone (**3**) was obtained as a colorless oil. Its molecular formula was determined to be $C_{14}H_{16}O_4$ by HRMS (*m/z* 248.1061, M^+). Comparison of its NMR data with that of fraxinellone indicates that rings C and D are the same as those in fraxinellone. At the same

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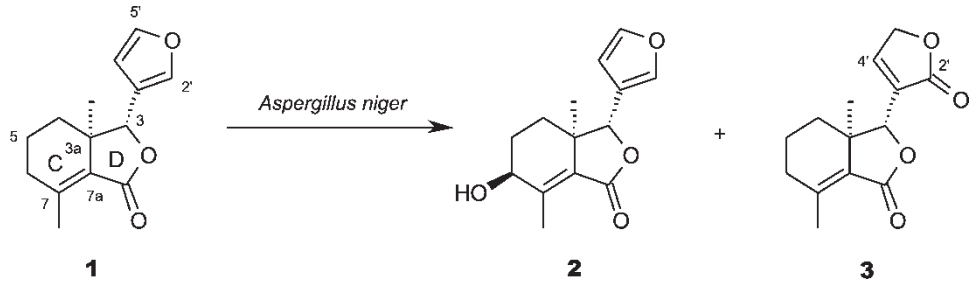

Scheme 1.

time, signals due to another ester carbonyl group (δ_C 171.7 ppm), an oxymethylene (δ_C 71.1 ppm) and an olefinic proton (δ_H 7.59 ppm) suggest the oxidation of the furan ring to an unsaturated lactone ring. There then remains the question of where the ester carbonyl group is located. Comparison of the NMR data of **3** with related structures indicates that the chemical shift of the olefinic proton is less than 6.0 ppm when the carbonyl group is at C-5′, but when it is at C-2′ the shift is higher than 7.10 ppm [4,5]. Therefore the ester carbonyl group should be at C-2′ in compound **3**. As no changes were observed in the chemical shift of C-3, the stereochemistry of **3** should be the same as fraxinellone. Thus the structure of compound **3** is confirmed as shown in Scheme 1.

The cytotoxic activities of **1**–**3** were tested *in vitro* against EC9706 and A549 cell lines. A preliminary biological study revealed that **2** and **3** show moderate cytotoxicity against the A549 cell line. Compound **2** has an IC_{50} of 20 $\mu g\,ml^{-1}$, and **3** has an inhibitory rate of 32% at a concentration of 87 $\mu g\,ml^{-1}$.

3. Experimental

3.1 General experimental procedures

The melting point was recorded on a X-4 micromelting point apparatus and is uncorrected. ^1H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, using a Bruker ARX 400 MHz spectrometer. MS spectra were obtained on a Micromass ZAB-HS spectrometer.

3.2 Materials

Fraxinellone was isolated from Cortex Dictamni (*Dictamnus dasycarpus* Turcz) purchased from Peking Tongrentang Drugstore and was identified on the basis of spectroscopic data [6].

3.3 Extraction and isolation

The fungi used were purchased from the Institute of Microbiology, Chinese Academy of Sciences (AS). The fermentation experiment was carried out in a medium composed of sucrose (30 g), $NaNO_3$ (3 g), $MgSO_4{\cdot}7H_2O$ (0.5 g), KCl (0.5 g), $K_2HPO_4{\cdot}3H_2O$ (1.3 g), $FeSO_4{\cdot}7H_2O$ (0.06 g) and distilled water (1000 ml). Cells of *Aspergillus niger* were transferred from two-week old slants into a sterile culture media and kept on a rotary shaker

(180 rpm) at 28°C for 48 h to give a stage I culture. Stage I cultures (5 ml) were used as inocula for stage II cultures (50 ml in a 250 ml flask, 80 flasks in total). After 24 h incubation of stage II cultures, the solution of fraxinellone (1.0 g) was added (12.5 mg/400 μl Me_2CO). After 4 days of incubation the mixture was filtered and the culture filtrates extracted with ethyl acetate (3 ×) to give a brown gum. Purification of the transformed mixture using column chromatography and HPLC afforded compounds **2** and **3**.

Compound **1**: colourless needles; mp 118°C. ^{1}H NMR ($CDCl_3$) δ (ppm): 7.47 (1H, brs), 7.44 (1H, brs), 6.35 (1H, brs, H-4′), 4.88 (1H, s, H-3), 2.28 (1H, dd, J = 19.5, 6.9 Hz), 2.19 (1H, dd, J = 10.0, 6.9 Hz), 2.13 (3H, s, 7-Me), 1.71–1.87 (3H, m), 1.45 (1H, dt, J = 12.4, 3.1 Hz), 0.86 (3H, s, 3a-Me); ^{13}C NMR ($CDCl_3$) δ (ppm): 169.8 (C-1), 148.4 (C-7a), 143.3 (C-5′), 139.7 (C-2′), 127.3 (C-7), 120.6 (C-3′), 108.5 (C-4′), 83.3 (C-3), 42.9 (C-3a), 32.0 (C-4), 31.6 (C-6), 20.3 (3a-Me), 18.4 (7-Me), 18.2 (C-5).

Compound **2**: yellow oil; EI-MS (*m*/*z*): 248 (M^+); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.48 (1H, m), 7.45 (1H, m), 6.35 (1H, m, H-4′), 4.94 (1H, s, H-3), 4.13 (1H, brs, H-6), 2.26 (3H, s, 7-Me), 0.85 (3H, s, 3a-Me); ^{13}C NMR ($CDCl_3$) δ (ppm): 169.7 (C-1), 145.9 (C-7), 143.6 (C-5′), 139.9 (C-2′), 130.5 (C-7a), 120.1 (C-3′), 108.5 (C-4′), 83.2 (C-3), 67.4 (C-6), 43.6 (C-3a), 27.8 (C-5), 26.8 (C-4), 18.9 (3a-Me), 15.7 (7-Me).

Compound **3**: colorless oil; HRMS (*m*/*z*): 248.1061 (M^+) (calcd. for $C_{14}H_{16}O_4$, 248.1048); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.59 (1H, brd, J = 1.6 Hz, H-4′), 4.94 (2H, t, J = 1.9 Hz, H-5′), 4.75 (1H, brd, J = 1.9 Hz, H-3), 2.28 (1H, dd, J = 19.6, 6.6 Hz), 2.13 (3H, s, 7-Me), 2.19 (1H, dd, J = 10.4, 7.0 Hz), 1.81–1.88 (1H, m, H-5), 1.66–1.78 (1H, m, H-5), 1.57 (1H, dt, J = 13.0, 3.4 Hz), 0.90 (3H, s, 3a-Me); ^{13}C NMR ($CDCl_3$) δ (ppm): 171.7 (C-2′), 169.1 (C-1), 150.5 (C-7a), 148.0 (C-4′), 130.5 (C-3′), 126.5 (C-7), 82.4 (C-3), 71.1 (C-5′), 42.7 (C-3a), 32.1 (C-4), 32.1 (C-6), 20.8 (3a-Me), 18.6 (7-Me), 18.3 (C-5).

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