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Microbial transformations of artemisinin by *Cunninghamella echinulata* and *Aspergillus niger*

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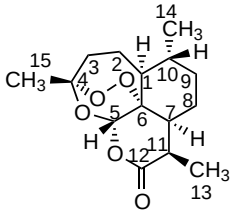
Abstract—Microbial transformations of artemisinin **1** by *Cunninghamella echinulata* (AS 3.3400) and *Aspergillus niger* (AS 3.795) were carried out. Two products, 10β-hydroxyartemisinin **2** and 3α-hydroxydeoxyartemisinin **3**, were obtained. Their structures were identified on the basis of chemical and spectroscopic data. 10β-Hydroxyartemisinin is a new compound.

Artemisinin **1**, qinghaosu, a sesquiterpene lactone endoperoxide was isolated from the Chinese herbal plant, *Artemisia annua* L. in 1972.[1] Its structure was determined by X-ray analysis in 1979.[2] Artemisinin and its derivatives are important therapeutic agents because they can be used for combating multidrug-resistant *Plasmodium falciparum* strains of malaria that cannot be eliminated by classical quinoline and antifolate antibiotics. It has been proposed that its highly reactive endoperoxide is converted into free radicals by the iron in the free heme molecules concentrated in the food vacuoles of malarial parasites. The resulting free radicals lead to cellular destruction or alkylation damage of some target proteins in the parasite cells.[3–6] In recent studies some derivatives of artemisinin showed antiarrhythmic[7] and antitumor activities.[8–10]

As an antimalarial drug, artemisinin has the disadvantages of a high recrudescence rate and poor solubility in water. To find more potent antimalarial drugs, structural modification of artemisinin and its analogues by chemical and biological methods has been studied in recent years,[11–14] and more effective antimalarial derivatives, such as artemether **4**, have been obtained.[15] Microbial transformation of these substrates has resulted in isomeric, rearranged, hydrolyzed and reduced products. In addition, microbial transformations may be used to predict metabolic pathways in mammals, and it has been reported that microorganisms can be used successfully as in vitro models for the prediction of mammalian drug metabolites (Scheme 1).[16]

Some 1-, 2-, 3-, 9-, 13- and 14-hydroxylated derivatives have previously been isolated from transformation processes of artemisinin and its analogues by microorganisms. In the present study, artemisinin was biotransformed in cultures of *Cunninghamella echinulata* (AS 3.3400) and *Aspergillus niger* (AS 3.795). Two biotransformed products **2** and **3** were isolated from the fermentation broth of *C. echinulata* and *A. niger*, respectively. These two compounds were structurally identified as 10β-hydroxyartemisinin **2** and 3α-hydroxydeoxyartemisinin **3** on the basis of their IR, ^{1}H, ^{13}C NMR, ^{1}H–^{1}H COSY, HMQC, ^{1}H–^{1}H NOESY and TOFMS spectroscopic data.



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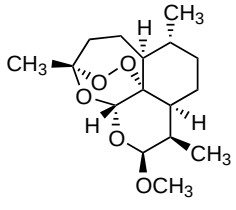

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Scheme 1.

Keywords: microbial transformations; artemisinin; *Cunninghamella echinulata*; *Aspergillus niger*.

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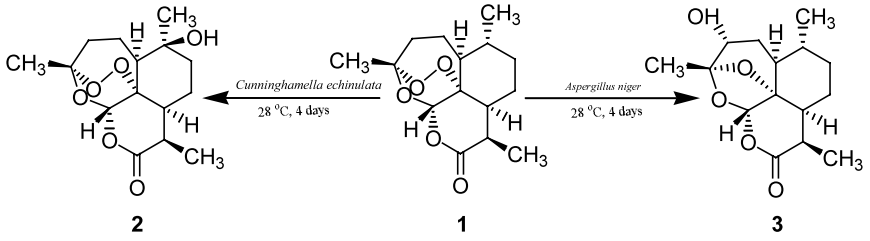

Scheme 2.

The screening scale biotransformations were performed in 250 mL Erlenmeyer flasks containing 60 mL potato media. The cultures were cultivated on rotary shakers at 160 rpm at 28°C. After 48 hours of cultivation of the microorganisms, an acetone solution of the substrate was added into each flask at a concentration of 100 mg/L and the transformation systems were maintained under the same conditions as described above. Culture controls were composed of fermentation blanks in organisms grown under identical conditions with the same volume of acetone. Substrate controls consisted of sterile media containing the same amount of substrate and incubated under the same conditions.

After 4 days of incubation, the mixtures were pooled and filtered. The culture filtrates were extracted three times with the equivalent volume of ethyl acetate. TLC chromatography showed that *C. echinulata* and *A. niger* had the ability to biotransform artemisinin. Both transformed products were more polar than the substrate artemisinin. No transformation product was found in the controls.

A preparative scale fermentation was performed with *C. echinulata*, and the product **2** was isolated and purified by chromatographic methods. 300 mg of substrate was added to the fermentation broth and 150 mg of **2** (50% yield) was obtained. A TOFMS analysis showed a molecular weight of 298, suggesting that a hydroxyl group had been introduced into the substrate molecule. The ^{13}C NMR spectrum showed a new peak at δ 72.3 and the IR spectrum showed a strong absorption at 3396 cm^{-1}, suggesting **2** to be a hydroxylated product of **1**. A DEPT analysis showed that the number of tertiary carbons had decreased from five to four and the number of quaternary carbons increased from three to four, which indicated that one methine group must have been hydroxylated. The 14-Me proton had shifted downfield to 0.37 ppm and became a singlet instead of a doublet as compared with that of artemisinin. From the above result, the hydroxyl group was assigned to the C-10 position. This result was confirmed by ^1H–^1H COSY and HMQC data, and all the remaining proton and carbon signals were assigned.[17] In the 2D ^1H–^1H NOESY spectrum, the correlation of the 10-OH to 5-H and 1-H to 14-CH_3 strongly supported the orientation of the 10-OH as having the β configuration. Finally, **2** was identified as 10β-hydroxyartemisinin, which is a new compound.

A preparative scale biotransformation of artemisinin (300 mg) by *A. niger* gave 45 mg of product **3** (15% yield). TOFMS analysis suggested its molecular weight to be 282. A comparison of the ^1H and ^{13}C NMR with those of the known compound, 3α-hydroxydeoxyartemisinin, indicated that product **3** indeed was 3α-hydroxydeoxyartemisinin. This compound, which has previously been reported to be one of the biotransformation products of artemisinin by *Penicillium chrysogenum* (ATCC 9480),[18] has been obtained for the first time from the biotransformation process of artemisinin by *Aspergillus niger*. Product **3** could also be produced in 67% yield when artemisinin is treated with ferrous sulfate in acetonitrile/water (Scheme 2).[19]

In this study, one new compound has been obtained by a microbial method and its biological activity is under investigation. It has been reported that a hydroxy group can be introduced at the C-10 position of artemisinin with the endoperoxide remaining intact.

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17. 10β-Hydroxyartemisinin **2**: colorless needles; mp 156–158°C, $[\alpha]_D^{20}$ +60.3 (*c* 0.55 MeOH); IR ν_{max} (KBr): 3396, 2937, 1714, 1462, 1385, 1221, 1179, 1124, 1091, 1033, 995, 958, 888, 832, 793 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.49 (1H, s, H-5), 3.34–3.39 (1H, m, H-11), 2.42–2.48 (1H, m, H-3), 2.10–2.14 (1H, m, H-3), 1.99–2.05 (1H, m, H-2), 1.72–1.86 (4H, m, H-2, H-7, H-8, H-9), 1.66 (1H, dd, $J=12.0$, 5.0 Hz, H-1), 1.58 (1H, s, OH-10), 1.47–1.49 (2H, m, H-8, H-9), 1.46 (3H, s, Me-15), 1.31 (3H, s, Me-14), 1.23 (3H, d, $J=7.5$ Hz, Me-13); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 172.4 (C-12), 105.2 (C-4), 94.3 (C-5), 79.9 (C-6), 72.2 (C-10), 51.6 (C-1), 45.3 (C-7), 40.0 (C-9), 35.6 (C-3), 32.9 (C-11), 29.6 (C-14), 25.2 (C-15), 19.6 (C-8), 19.1 (C-2), 12.6 (C-13); TOFMS (*m*/*z*) 337 (*M*+K), 321 (*M*+Na).
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