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Biotransformation of artemisinin by *Catharanthus roseus* and *Ginkgo biloba* cell suspension cultures

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Abstract **Object** To investigate the biotransformation of the antimalarial compound artemisinin (Ⅰ) by *Catharanthus roseus* and *Ginkgo biloba* cell suspension cultures. **Methods** Plant tissue culture technology was employed. The product was isolated on silica gel column chromatography and its structure was elucidated by spectroscopic evidence. **Results** One product was obtained and its structure was characterized as 3α-hydroxydeoxyartemisinin (Ⅱ). **Conclusion** Both of *C. roseus* and *G. biloba* cell suspension cultures can bioconvert artemisinin.

Key words *Catharanthus roseus* (L.) G. Don; *Ginkgo biloba* L.; biotransformation; artemisinin; cell suspension culture

长春花及银杏植物细胞悬浮培养对青蒿素的生物转化研究

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摘 要: 目的 对抗疟药物青蒿素(Ⅰ)进行了生物转化研究。方法 利用长春花及银杏植物细胞悬浮培养细胞进行生物转化。用硅胶柱色谱进行产物的分离,波谱方法鉴定产物的结构。结果 此两种植物悬浮细胞体系均能将青蒿素转化成 3α-羟基去氧青蒿素(Ⅱ)。结论 此两种植物悬浮细胞体系均能有效转化青蒿素。

关键词: 长春花;银杏;生物转化;青蒿素;悬浮细胞
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Artemisinin (Qinghaosu), a sesquiterpene lactone, is an antimalarial agent isolated from the Chinese herbal medicine *Artemisia annua* L.[1]. Thereafter artemisinin and its derivatives have received considerable attention because of their activity against resistant strains of *Plasmodium falciparum* and efficacy against cerebral malaria. As an antimalarial drug, the high reversion rate and poor solubility of artemisinin in water, was limited its use in clinics. A number of derivatives of artemisinin have been synthesized from dihydroartemisinin, and out of these, artemether, arteether, artesunic and artelinic acid are either currently in use or being evaluated for use[2]. At the same time some biotransformation of artemisinin and its analogues, such as arteannuin B, artemether and arteether *etc*., has been carried out during the past years[3-6]. Moreover, the cytotoxic effect of some artemisinin derivatives have been studied[7,8]. It can be concluded that artemisinin and its derivatives are getting to be the focus of many investigators. To date, there is no report on the biotransformation of artemisinin by plant cell suspension cultures. In the present paper, The biotransformation of I by *Catharanthus roseus* (L.) G. Don and *Ginkgo biliba* L. cell suspension cultures was reported, respectively.

1 Results and discussion

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Compound Ⅰ (Fig. 1) was administered to the ten-day-old cell cultrues, and incubated for additional seven days. The cells and medium were harvested and extracted as described in the experimental section. One product was isolated from the medium by chromatographic methods, and its structure was identified as 3α-hydroxydeoxyartemisinin (Fig. 1) on the basis of its chemical and spectral data. Mass spectral data of product Ⅱ indicated that there was no change in the molecular weight (*m* /*z* [M]$^+$ 282). The IR spectrum showed a broad peak at 3 490 cm^{-1}, which suggested the presence of a hydroxyl group, while no characteristic signal of a peroxide bridge (831, 881, 1 115 cm^{-1}) was observed. The ^{13}CNMR spectrum showed a new signal at 69.4. All of these data indicated that Ⅱ was a hydroxylated deoxyartemisinin. The ^{13}CNMR and ^{1}HNMR data of Ⅱ were in good agreement with those reported in literature[3]. The yields of Ⅱ by *C. roseus* and *G. biloba* cultured cells were 10% and 13%, respectively. Additional test showed that compound Ⅰ added to the same medium without cell cultures and incubated in the same condition yielded no products, suggesting that Ⅱ be an enzymatic product. The fact that Ⅰ was converted to Ⅱ by both *C. roseus* and *G. biloba* cultured cells suggested that some plant cell suspension cultures might possess similar enzyme systems to result in the loss of one of the peroxide oxygen atoms to give the epoxide and have the ability of hydroxylation though the precise order of the pathway is still unknown. The bioactivity studies on the antitumor and antimalarial activities of the transformed product Ⅱ is under way.

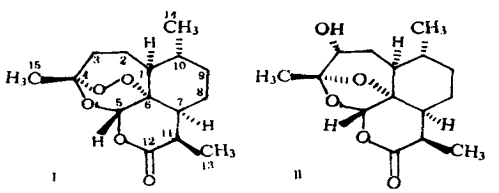

Fig. 1 Structures of compound Ⅰ and Ⅱ

This result should be of some value for the future biotransformation studies of artemisinin. Although there has been some chemical methods to synthesize artemisinin and its derivatives, the toxic risk and high cost of these methods limit their uses. Until now plants are still the main source for the production of artemisinin. But the content of artemisinin in natural resources or cultured cells of *A. annua* is low, which made it urgent to develop some effective methods with biotechnology and biochemical engineering to improve the production of artemisinin.

2 Experiment

2.1 General experimental conditions. IR spectra were obtained on a Perkin-Elmer 983B spectrophotometer (KBr). NMR spectra (^{1}HNMR and ^{13}CNMR) were recorded in $CDCl_3$ on INOVA-500 spectrometer, and chemical shifts were recorded in δ using TMS as internal standard. TOF mass spectra was measured on a MALDI TOF mass spectrometer. All chemicals were obtained from Beijing Chemical Factory.

2.2 Tissue and cell culture of *C. roseus*. The seedlings of *C. roseus* (identified by Prof. GUO De-an) were obtained from the Medicinal Plant Garden of Peking University Health Science Center. Young leaves were used to initiate calli. The plant materials were disinfected by immersing in 70% ethanol for 30 seconds, followed by 0.1% $HgCl_2$ for ten minutes, washed five times with sterilized water, then cut into small pieces (about 0.5 cm × 0.5 cm) and aseptically transferred to Murashige and Skoog's medium (MS) supplemented with 1.0 mg/L of 2, 4-dichlorophenoxyacetic acid (2, 4-D) and 7.0 g/L of agar. The pH of the medium was adjusted to 5.8 before being autoclaved at 121 ℃ for 20 minutes. The calli were initiated from all of explants within four weeks of culture in the darkness at (25± 2)℃. The calli cultures were maintained on the medium of the same composition and the same culture condition by subculture every four weeks. Three-week-old friable calli were used for initiation of suspension cultures. MS medium supplemented with 0.5 mg/L of 6-benzyl-aminopurine (6-BA), 0.5 mg/L of NAA and 0.2 mg/L of 2, 4-dichlorophenoxyacetic acid (2, 4-D) which was proved to be the best for good growth of the cul-

tures in liquid medium. Cell cultures were subcultured every three weeks at the inoculation of 5 g/L of dry weight in 500 mL of Erlenmeyer flasks with 150 mL of fresh medium and incubated on a rotary shaker at 110 r/min in the darkness at (25± 2) ℃. The procedure of the cultivation of *G. biloba* cells was performed as described previously[9].

2.3 Biotransformation of Ⅰ. The suspension cells were cultured in 500 mL of flask with 150 mL liquid medium. One milliliter of the stock substrate solution(10 mg/mL) was added to one flask with suspension cell cultures, and one additional flask without substrate as the control. After additional seven days of incubation, the cell cultrues were filtered under vacuum and washed three times with distilled water. The filtrate was collected and extracted three times by equivalent volume of EtOAc, and all the extracted solutions were concentrated under vacuum at 40 ℃. The residues were dissolved in acetone and analyzed by TLC developed with acetone-petroleum ether(1∶2.5), and detected by spraying with 10% EtOH(in H_2SO_4) followed by heating at 105 ℃. The TLC results showed that one new spot appeared in the chromatogram of the medium extract. For preparative biotransformation experiment, 1 mL of substrate solution was added to each flask on the 10th cultural day, and the total amount of Ⅰ administered in *C. roseus* and *G. biloba* was 300 mg, 150 mg, respectively. After additional seven days of incubation, all the media were collected, extracted and concentrated as described above. The obtained residue was separated by silica gel chromatography (silica gel G, 200~300 meshes), eluting with acetone-petroleum ether(60 ℃~90 ℃)(1∶5~1∶1) to yield 30 mg and 20 mg of Ⅱ by *C. roseus* and *G. biloba* cell suspension cultures, respectively.

2.4 Identification. 3α-Hydroxydeoxyartemisinin Ⅱ: white needles; ^{1}HNMR (CDCl$_3$, 500 MHz) δ 0.93 (3H, d, *J*= 6 Hz, Me-14), 1.17 (3H, d *J*= 7 Hz, Me-13), 1.56 (3H, s, Me-15), 1.81 (1H, m, H-8), 1.92 (1H, m, H-8), 1.97 (1H, m, H-2), 2.07 (1H, dt, *J*= 12.9, 4.5 Hz, H-7), 3.18 (1H, dq, *J*= 4.5, 7.2 Hz, H-11), 3.60 (1H, s, H-3), 5.62 (1H, s, H-5). ^{13}CNMR (CDCl$_3$, 500 Hz): δ12.6 (13-C), 18.4 (14-C), 20.5 (15-C), 23.5 (8-C), 30.3 (2-C), 32.7 (11-C), 33.4 (9-C), 35.1 (10-C), 40.6 (1-C), 42.0 (7-C), 69.4 (3-C), 82.9 (6-C), 98.9 (5-C), 108.9 (4-C), 171.3 (12-C).

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