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To cite this article: Zhao Lu *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **657** 012044

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Advances in research on metabolic pathways of podophyllotoxin in plants of Berberaceae

Zhao Lu^{1,2}, Meifang Hou^{1*}, Rongyan Xu^{2*}, Xiaoheng Zhang

¹ School of Ecological Technology and Engineering, Shanghai Institute of Technology, Shanghai 201499; ² Shanghai Chen Shan Plant Science Research Center Chinese Academy of Sciences, Shanghai 201600

About the author: Lu Zhao, E-mail: LuZhao1366@163.com. Tel:18830635819.

* Corresponding author: Hou Meifang, E-mail: mfhou@sit.edu.cn;

Xu Rongyan, E-mail: ryxu822@fudan.edu.cn.

Abstract: Podophyllotoxin is a natural aryl naphthalene lignan derived from the plant of the Berberaceae family, and has attracted much attention due to its remarkable antitumor and antiviral activity. This article reviews recent research progress on podophyllotoxin synthesis and metabolic pathways at home and abroad, analyzes key enzyme genes in its synthesis pathway, and summarizes the current status of the design and synthesis of podophyllotoxin derivatives. And the further development and utilization of its derivatives provide a feasible basis.

1. Introduction

Berberidaceae is a plant group with strong physiological activity, belonging to the magnolia subclass of angiosperms and dicotyledons. Berberis are usually herbaceous or shrub plants with single or compound leaves and rhizomes or tubers. According to literature statistics, berberis contains 17 genera and about 650 species, among which 11 genera and about 320 species are in China, mostly distributed in Sichuan, Xizang, Yunnan and other western regions^[1-3].

Podophyllotoxin belongs to natural aryl naphthalene lignans and is a natural product with certain anti-tumor and antiviral activity. It mainly exists in *Dioscorea*, *Sinopodophyllum* and *Diphyllia* of Podophylloideae, a perennial herbaceous group of Berberidaceae. Its molecular formula for C₂₂H₂₂O₈, molecular weight of 414.41, It is white crystalline powder at room temperature^[4,5]. As a secondary metabolite of plant cells, podophyllotoxin is similar to Colchicine in inhibiting cell growth. During the mitotic activity of cancer cells, podophyllotoxin can effectively inhibit tubulin to prevent vascular bundle aggregation and formation of mitotic spindles to play a role, which ultimately leads to the failure of normal cell division of cancer cells, thus inhibiting the proliferation of cancer cells^[6]. Due to the high toxicity of podophyllotoxin itself, in recent years, many scholars have modified and changed the basic skeleton of podophyllotoxin, and selected several highly active anti-tumor drugs such as Etoposide (VP-16) and teniposide (VM-26). The chemical structure is shown in Figure 1. It is mainly used to treat diseases such as testicular lymphopathy, leukocytosis, uterine cancer, gastric cancer and breast cancer^[7,8].



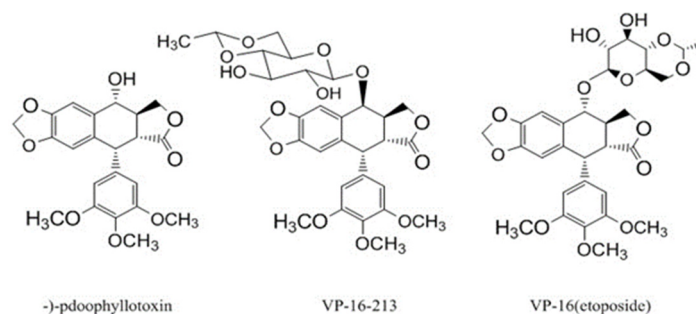


Fig1. The chemical structure of podophyllotoxin and its derivatives

2. Synthesis of podophyllotoxin

2.1. Biosynthetic pathway

As early as 1984-1990, Biosynthetic pathways for the synthesis of podophyllotoxin are beginning to be discovered (Dewick et al.). Initially, the scientists made podophyllotoxin by reducing styrene or styrene dimers, and then converted it to podophyllotoxin by changing the carbon atoms in PPA(Phenylpropanolamine) and other methods. Later, it was proposed to realize the synthesis of podophyllotoxin by adding organic somposide. However, due to the high cost of somposide and considering economic problems, large-scale production was not realized^[9-11].

2.2. Cell synthesis pathway

In the 19th century, more scientists found that the content of 4'-demethyl-podophyllotoxin of podophyllotoxin was significantly increased after a short period of time when the Gamborg-based B5 medium supplemented with 6-BA, 2,4-D and GA3 was used to culture the callus cells of Sinopodophyllum. People found that podophyllotoxin and its derivatives can also be obtained from flax cell culture^[12,13]. The contents of 5-deoxypodophyllin in cell cultures of flax plants and in regenerated plants were compared. Demonstrated that cell cultures can produce concentrations of 5-deoxypodophyllotoxin comparable to those found in fully differentiated plants. The results showed that the use of 2, 4-dichlorophenoxyacetic acid as a growth regulator was beneficial to the production of 5-deoxypodophyllotoxin compared with naphthoic acid. Subsequently, inducers such as NAA were added to MS culture medium to suspend the cells of Sinopodophyllum, a wild plant, and then the contents of podophyllotoxin and its derivatives were detected to increase relatively^[14].

2.3. Transgenic synthesis pathway

The hairy roots of plants have the characteristics of stable genetic performance, strong growth ability and high content of medicinal ingredients. In 1991-1992, The high yield of hairy roots of yellow flax was promoted by the removal of naphthoic acid from culture medium (Uden et al.)^[15,16]. In addition, phenylpropane, L-tyrosine and L-phenylalanine were added to increase the content of 5-deoxypodophyllin significantly. Vitamin and plant hormones were then adjusted, resulting in a sixfold increase in levels of 5-deoxypodophyllin. At the beginning of the 20th century, different agrobacterium strains were used to transform paeoniflorin embryos to determine the transformation characteristics of callus and to maintain monoclonal culture(Giri et al). HPLC analysis showed that the content of podophyllotoxin increased by three times compared with the blank control^[17].

3. Metabolic pathways of podophyllotoxin

Podophyllotoxin mainly comes from the rhizome of Sinopodophyllum, a plant of the genus podophyllotoxin in Berberidaceae. The production means is coniferyl alcohol as the starting substrate, after derived protein (DIR), rosin rapeseed resin alcohol reductase (PLR) and cocoanut isomaltol alcohol dehydrogenase (SDH) and a series of enzyme catalytic role generated intermediates (-)-pluviatolide, then to (-)-pluviatolide for intermediate compound synthesis of yatein go back into

deoxypodophyllotoxin, The final process of obtaining podophyllotoxin is shown in FIG. 2. In 2015, Six enzymes involved in completing the etoposide Aglycone biosynthesis pathway have been found, such as O-methyltransferase. Several key genes that may catalyse podophyllotoxin synthesis have also been identified. (Warren Lau et al.)^[18-20].

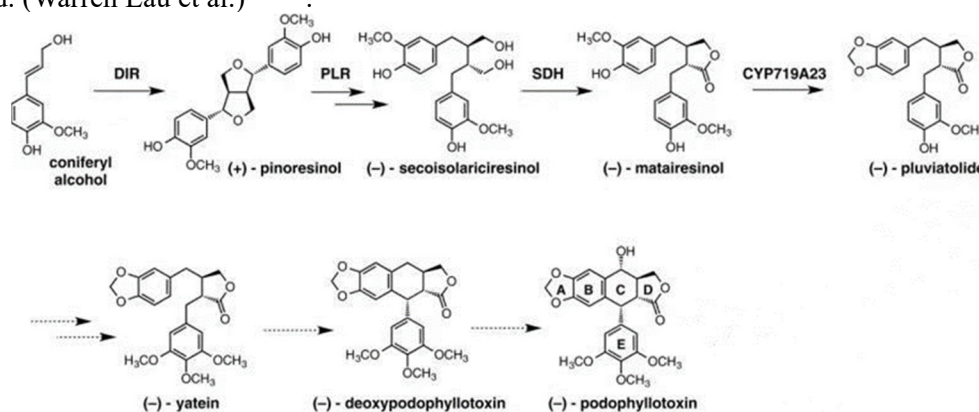


Fig 2 .The metabolic process of podophyllotoxin

4. Expression of key enzyme genes

O-methyltransferase (OMT) is the most widely studied methyltransferase and has been systematically classified and studied and applied in catalytic mechanism. Caffeic acid OMT and CCoAOMT have been studied the most. In 2014, The transfer of the *CCoAOMT* gene from jute was found to increase the lignin content in *Arabidopsis thaliana* by about 20.14 percent (Zhang Gaoyang et al.). Next, the *OMT1* and *OMT3* genes may be involved in the podophyllotoxin synthesis pathway, and it was discovered that the oxymethyltransferase encoded by the *OMT* gene plays a key role in the transformation of the intermediate compound (-)-pluviatolide into yatein (Warren Lau et al.)^[21,22].

5. Design and synthesis of podophyllotoxin derivatives

In order to reduce the toxicity of podophyllotoxin, nine new derivatives of podophyllotoxin were synthesized by introducing five-element azacyclic compounds based on podophyllotoxin. These compounds were screened by ¹H and ¹³C nuclear magnetic resonance spectroscopy (HMR), high resolution electrospray mass spectrometry (ESMS) and Single X-ray, and proved to have good cytotoxic activity and certain anti-tumor effect (Pei-fang Zhu et al.). Subsequently, a variety of novel podophyllotoxin derivatives, such as 4a~4f and 6a~6i, were synthesized and their growth inhibition activities on leukemia cells, hela cell lines and human cervical cancer cells were investigated using etoposide as a positive control. The results showed that the properties of the 9 synthetic derivatives were superior to those of the control drug etoposide (dan-li Tian et al.). The method of introducing compounds with high drug activity, such as amino acids, with podophyllotoxin as the mother nucleus was established, thus screening out the dominant compound P-02, which has strong selectivity to lung cancer cell AS49 and low toxicity to human liver cell L-02 (gao-rong Wu et al.)^[23-25].

6. summary and outlook

As podophyllotoxin plays an irreplaceable role in anti-cancer, anti-tumor and other medical treatment, the analysis of metabolic pathways of podophyllotoxin has become a hot topic at home and abroad, and the whole metabolic process has been further improved with the efforts of scientists.

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