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BIOSYNTHESIS

Engineering etoposide

Etoposide is an important anticancer drug — included on the WHO (World Health Organization) list of essential medicines. Despite its importance, there have been several recent shortages of the drug, because it is usually produced by semisynthesis using an extract from the Himalayan mayapple (*Sinopodophyllum hexandrum*), a species that is considered endangered. Now, Elizabeth Sattely and co-workers from Stanford University, California, have successfully engineered a complete biosynthetic route to (–)-deoxypodophyllotoxin (DPT) in a type of tobacco plant.

(–)-Podophyllotoxin has been extracted from the mayapple, and attempts to use plant tissue culture systems have been made but have not proved scalable. In previous work, Sattely's team has elucidated the complete biosynthetic pathway of atoposide aglycone and have now decided to engineer its production into a plant chassis.

Engineered routes to plant natural products have been described before, most notably for artemisinic acid — the precursor to artemisinin, an important anti-malaria drug. In this case, the biosynthesis has been engineered into yeast, which is an appealing strategy as it makes production highly scalable. However, the work to produce artemisinic acid in yeast is estimated to have taken 150 person-years.

Instead, Sattely and co-workers chose to engineer their synthesis in *Nicotiana benthamiana*, a wild relative of tobacco. There are several advantages of using a plant platform to produce a plant-derived natural product. First, it has proved challenging to express some plant enzymes — particularly the cytochromes P450, essential for the final steps of the synthesis — in microbial hosts. Second, transferring a pathway plant to plant is simplified because the same cofactors and metabolic precursors are already available.

The team had previously shown that co-expression of the eight enzymes that produce DPT from coniferyl alcohol (CA) in their tobacco strain led to a yield of 11.4 ± 3.8 μg g^{-1} dry weight of the plant. The yield was improved eightfold by direct addition of (+)-pinoresinol, indicating that it is production of this intermediate that is yield limiting. Pinoresinol itself is produced by dimerization of CA and the addition of the latter to a tobacco already infiltrated by the DPT pathway enzymes also increased the yield. Reasoning that it is both the production and dimerization of CA that limit the production of DPT, Sattely and co-workers investigated the addition of genes to produce CA to their plant chassis.

The biosynthetic pathway to CA — which is ultimately derived from phenylalanine — has been studied in detail, and the team was able to select the best genes encoding the eight enzymes that catalyse these nine steps and incorporate them into the same plant. They went on to study each of the eight enzymes individually (and subsets of them), but the most consistent increase in yield resulted when all eight enzymes were included.

The optimized result of the biosynthesis is a system that yields 0.71 mg of high purity DPT per gram of dry weight of the plant. Some 10% of WHO essential medicines are derived from plant natural products, a majority of which rely on the native plant for production. "We are interested in expanding the idea of metabolic engineering in a plant chassis with other high-value plant natural products with (at least partially) known biosynthetic pathways," says Sattely.

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ORIGINAL ARTICLE Schultz, B. J. et al. Total biosynthesis for milligram-scale production of etoposide intermediates in a plant chassis. *J. Am. Chem. Soc.* **141**, 19231–19235 (2019)
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