From yeast to alkaloids

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Alkaloids, which include caffeine and morphine, are a large class of pharmacologically active plant compounds that are often difficult to chemically synthesize. Incorporation of benzylisoquinoline alkaloid pathways in yeast will facilitate the production of natural and non-natural alkaloids.

One of the dreams of synthetic biology is that we will one day be able to produce any chemical in a microorganism by introducing the appropriate biosynthetic and tailoring enzymes and, in the process, replace environ-mentally unfriendly and/or arduous chemical synthesis routes. That dream has come closer to reality with an article from Hawkins and Smolke on the production of benzylisoqui-noline alkaloids in yeast.

Of the many types of natural products[2], alkaloids—nitrogen-containing, low-molecular-weight compounds found in a vari-ety of plants—are some of the most pharma-cologically interesting. Approximately 12,000 alkaloid structures have been described, many of which serve as defense agents in plants against herbivores and pathogens. Alkaloids are also known for their medicinal uses: mor-phine and codeine as analgesics; vinblastine, vincristine and camptothecin as anticancer agents; (+)-tubocurarine as a muscle relax-ant; and ajmaline as an anti-arrhythmic, to name a few. As evidence of their importance, the worldwide sales of some of the most widely used alkaloids, including caffeine, nicotine, cocaine and heroin, exceeded $US 4 billion in 2002.

One of the largest and most structurally diverse families of alkaloids are the benzyliso-quinoline alkaloids (BIAs), which comprise approximately 2,500 member compounds.

Examples of BIAs are codeine and morphine, the microtubule-disrupting colchicines, the antibiotic berberine, the muscle relaxant (+)-tubocurarine, and sanguinarine, which is currently added to toothpastes and oral rinses owing to its antiplaque properties. Several analgesics (buprenorphine and oxycodone) are produced synthetically from thebaine, an intermediate in the BIA pathway. Another intermediate in the BIA pathway, reticuline, is used as a starting material for the synthesis of several molecules that have shown poten-tial as antimalarial and anticancer drugs, and reticuline itself has been shown to have hair growth activity (of major interest to the author).

Until recently, BIAs have only been avail-able by extraction from plant sources. Two recently published articles make it clear that this large group of molecules will be produc-ible in microorganisms. In a previous article, Minami *et al.* engineered *Escherichia coli* to produce 55 mg l^{-1} of (*S*)-reticuline and a coculture of *E. coli* and *Saccharomyces cerevi-siae* to produce magnoflorine and scoulerine from the *E. coli*–produced (*S*)-reticuline[3]. In this issue of *Nature Chemical Biology*, Hawkins and Smolke describe their work to engi-neer *S. cerevisiae* with enzymes from plants (*Thalictrum flavum* and *Papaver somniferum*) and humans to produce a racemic mixture of the BIA metabolite (*R*,*S*)-reticuline, in titers of 10 to 150 mg l^{-1}, from the commercially available intermediate norlaudanosoline. By expressing three additional enzymes from these two plants and a reductase partner from *Arabidopsis thaliana*, they were able to engineer yeast to produce (*S*)-scoulerine, (*S*)-tetrahydrocolumbamine and (*S*)-tetra-hydroberberine from (*S*)-reticuline. The authors also demonstrated that it may be possible to mix and match enzymes more broadly, as the expression of a human P450 and its reductase partner endowed yeast with the ability to produce (*S*)-salutaridine from (*R*)-reticuline (**Fig. 1**).

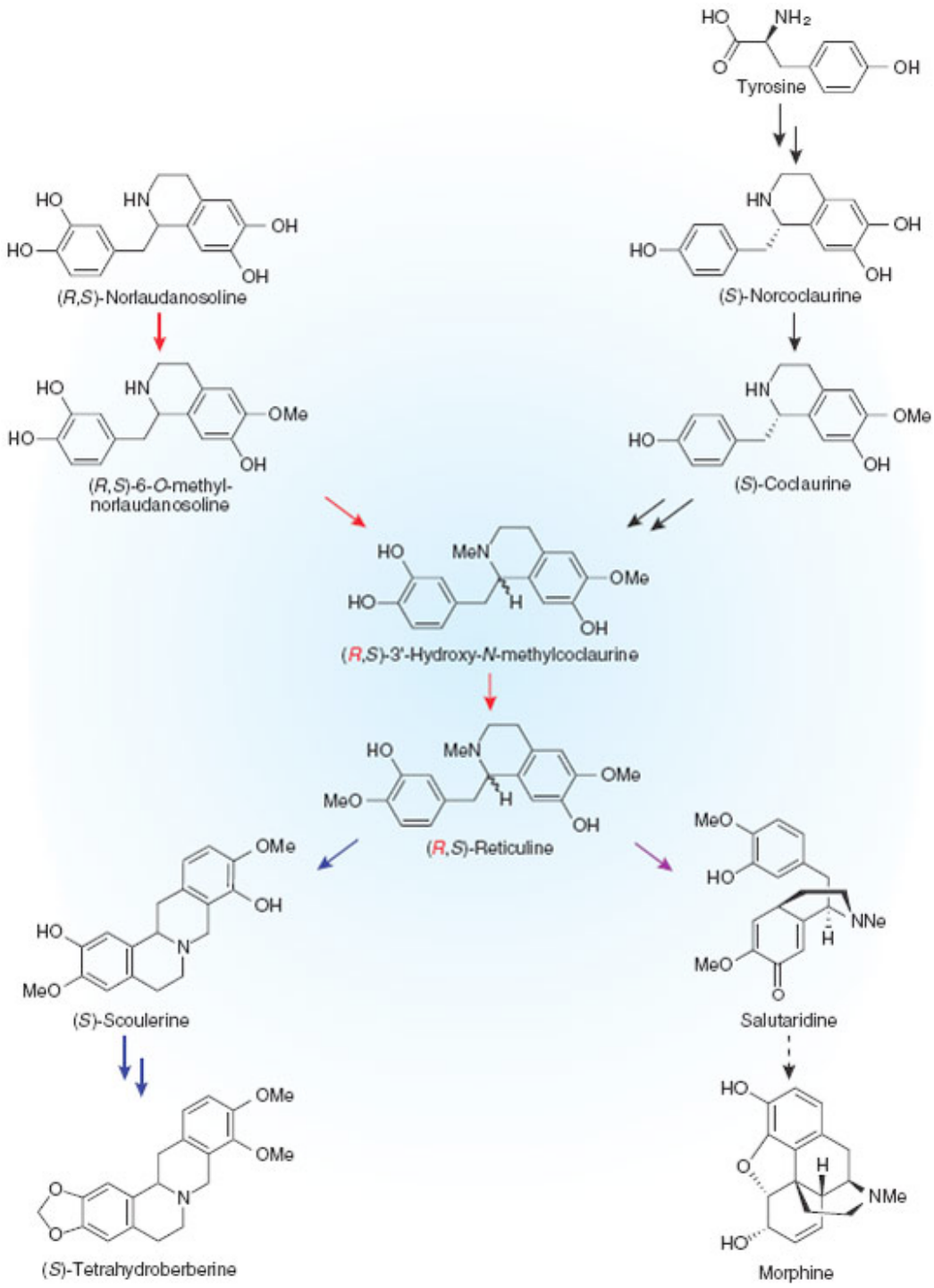

Figure 1. Pathways for the production of BIAs. Native pathway for the production of (*S*)-reticuline (black arrows). Hawkins and Smolke[1] report a pathway for the production of (*R*,*S*)-reticuline (red arrows) and products produced from (*S*)-reticuline using plant enzymes (blue arrows), and for the conversion of (*R*)-reticuline to salutaridine using a human P450 enzyme (purple arrow). Red '*R*'s signify a racemic mixture is produced by the pathway introduced by Hawkins and Smolke, rather than the single stereoisomer that is produced by the native pathway.

Synthetic biology has made significant inroads into synthetic chemistry, and the Hawkins and Smolke and Minami *et al.* papers add another group of natural products onto the ever-growing list of

compounds that can now be produced microbially[2,4,5]. However, as these results also demonstrate, the road is not always smooth. The enzymes that convert tyrosine to BIAs are either not available or are not expressible in the microbial hosts used for these studies. As one of the key advantages of microbial synthesis is the ability to produce complicated molecules from an inexpensive and readily available starting material, one important future challenge will be finding a way to express these genes in yeast or *E. coli* production platforms to make microbial alkaloid production economically viable.

Though a number of natural products have found use as anticancer, antibacterial and antifungal drugs, natural products evolved for purposes other than the treatment of human disease, and hence their pharma-cological properties may not be optimal. One important advance in the Hawkins and Smolke paper is the demonstration that the plant enzymes involved in BIA synthesis are somewhat flexible in their use of substrates, which suggests that any number of BIA derivatives might be producible in a micro-bial host. This new platform may therefore offer an important advantage in the search for new alkaloid drugs.

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