Hot Topics in Pharmacognosy: Opiates from Modified Microbes

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As known by all pharmacognosists, throughout the ages humans and other animals relied on nature for their basic needs. Plants, in particular, formed the basis of sophisticated traditional medicine systems, with the earliest records dating from around 2900-2600 BCE,[1] documenting the uses of approximately 1,000 plant-derived substances in Mesopotamia[2] and the active transportation of medicinal plants and oils around what is now known as Southwest Asia. These included oils of *Cedrus* species (cedar) and *Cupressus sempervirens* (cypress), *Glycyrrhiza glabra* (licorice), *Commiphora* species (myrrh), and the star of this story, *Papaver somniferum* (poppy juice). It should be noted that all are still used today for the treatment of ailments ranging from coughs, colds, and analgesia to parasitic infections and inflammation.

Although it is not often realised, the initial discoveries that may be considered to have revolutionized drug discovery and development were made by European chemists in the 1803-1805 time frame, building upon the physico-chemical principles evolving in the recent past from the work of experimental and theoretical chemists such as Proust, Davy, Gay-Lussac, Berzelius, and Dalton. This body of theory and experiment which moved "healers" away from "polypharmacy" towards "pharmacology of single (pure) agents" was probably first enunciated by Cadet de Gassicourt[3] in 1809.

ALKALOIDS

This brings us to the star of this discourse, the story of morphine (1). The initial report of isolation of fractions from the opium poppy was reputedly made by Derosne[4] in 1803 at the Institute of France and then published in 1814.[5] There was one flaw, however; this preparation had no narcotic properties whatsoever and was probably noscapine with a little meconic acid extracted by the ethanol-water system. A controversy arose. The German pharmacist Seturner then published his work in 1805[6] claiming that he had commenced work before Derosne. Inspection of this title implies investigation of the acidic and not the basic fractions of opium, probably meconic acid, as demonstrated in a paper published the next year.[7]

In 1817 however, using hot water extraction followed by precipitation with ammonia, led to colorless crystals that had the narcotic properties of opium.[8] What surprised scientists at the time reading this publication was that the material obtained was alkaline, not acidic; thus, this was the first non-acidic compound with biological properties purified from a plant.

Subsequent conversion into heroin (2) was first reported in 1874 by Wright in the United Kingdom as a result of boiling morphine acetate. It was commercialized by Bayer AG in 1898 and sold as a "tonic" by then Smith Kline and French laboratories (precursor of GlaxoSmithKline) in the United States around the turn of the 20th Century. The use and abuse of these compounds is much too complex to discuss here, but in 1973, Pert and Synder reported the identification of opioid receptors in brain tissue,[9] and this report was closely followed in 1975 by Kosterlitz and Hughes.[10] This identification of "endogenous morphine-like substances" over the next few years led to the discovery of enkephalins, endorphins, and dynorphins, all of which had the common N-terminal sequence of Tyr-Gly-Gly-Phe-(Met/Leu), leading to the concept that morphine actually mimics this sequence.[11]

In the years between the "use" of morphine and heroin (both legal and illegal), derivatives were made by either direct synthesis, or by modification of the purified natural products (semisynthesis), leading to 15 plus agents that have been approved to date that are either opioids or direct opioid antagonists. This number includes both morphine and heroin but not thebaine.[12]

Why is this of import in the scientific community and of some concern to law enforcement? The reasons are as follows. In 2006, the Keasling group in California reported the production of artemisinic acid in genetically engineered yeast following the addition of the necessary gene clusters from the producing plant. This demonstrated that to obtain reasonable yields, they had to make certain that the necessary precursors for both the normal cell growth and production of the "parasite pathway" (my comment) were available for production.[13] This is a well-known problem for anyone who used to "persuade microbes to produce antibiotics on an industrial scale" but has often been forgotten by investigators in academia who modify microbes.

In 2008 Hawkins and Smolke reported that they had been successful in producing benzyl isoquinoline alkaloids in a modified yeast strain by adding selected gene clusters from *Thalictrum flavum* and *Papaver somniferum* to the yeast.[14] The "choke point" in the biological production was the provision of reticuline as the correct enantiomer. Hawkins and Smolke overcame this by using an enzymatic conversion of exogenous (*R,S*)-norlaudanosoline to (*R,S*)-reticuline as feedstock for the production of the morphinan pathway as well as sanguinarine and berberine. They also showed that a human P450 converts (*R*)-reticuline to salu-

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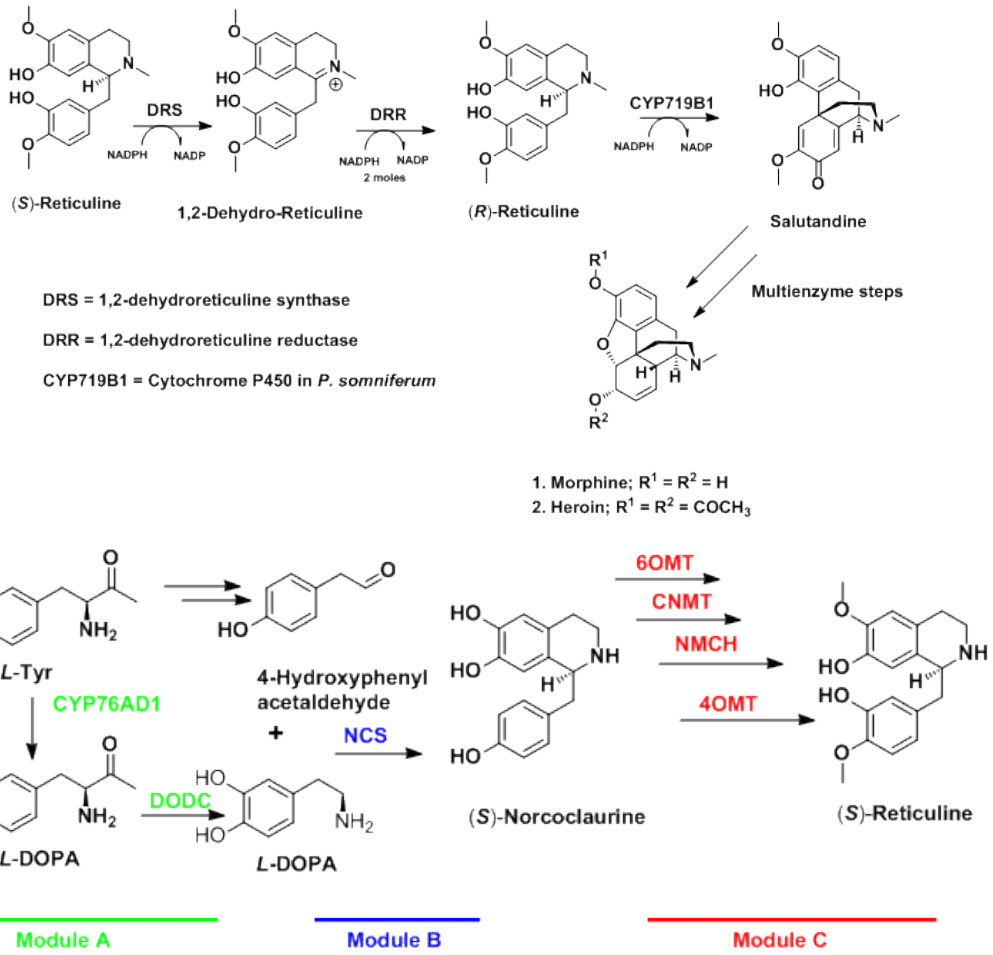
taridine which might explain a report that humans can synthesize small amounts of morphine.[15] Thus, they had demonstrated the later parts of the pathways leading to these agents, but had not yet produced reticuline.

In 2014, the Smolke group reported further work using their modified *Saccharomyces cerevisiae* strains only now adding genes from *Pseudomonas putida* to those from *P. somniferum* that enabled the conversion of thebaine to codeine, morphine, hydromorphone, hydrocodone and oxycodone, thus producing high value added products from the "plant metabolite."[16] In addition, they found a novel pathway to neopine and neomorphine, with total opioid titers of around 130 mg/L.

The last enzymatic step in the "puzzle" came in a very recent paper by Facchini's group at the University of Calgary, where they were able to locate the remaining enzyme in the pathway (Figure 1) and show that it was the result of a fusion between a cytochrome P450 (CYP) and an aldo-keto reductase (AKR) catalyzing the *S*-to-*R* epimerization of reticuline via 1,2-dehydroreticuline, as shown below.[17]



However, what was still missing from the equation was a good producer of the L-DOPA required to produce the necessary intermediates. By some very clever gene manipulations, producing a linked chromophore when the gene required was isolated, the Dueber group identified a tyrosine hydroxylase and then effectively produced the required enantiomer, (*S*)-reticuline from glucose in yeast.[18]

Although it has not yet been reported in the literature, from the work of these investigators the necessary enzymatic processes are now "available" to produce opioids from glucose in genetically modified *S. cerevisiae*. Not only morphine but also the possibilities of producing high-value added compounds such as oxycodone. This latter compound, though extremely valuable as an analgesic, is also a major "player" in drug abuse. To counter this, in 2014 the FDA approved Targiniq(R), which is a mixture of oxycodone and naloxone, and looked at an opioid and an opioid antagonist pharmacologically.

Hot off the press: *Science Express* (August 13, 2015) has a paper from the Smolke group demonstrating the production of thebaine and oxycodone from yeast starting with glucose.[19] They succeeded! ■

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