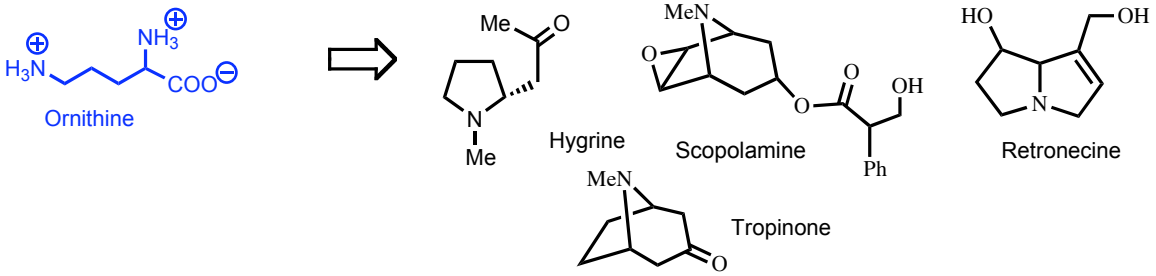
5. Biosynthesis of Alkaloid Natural Products

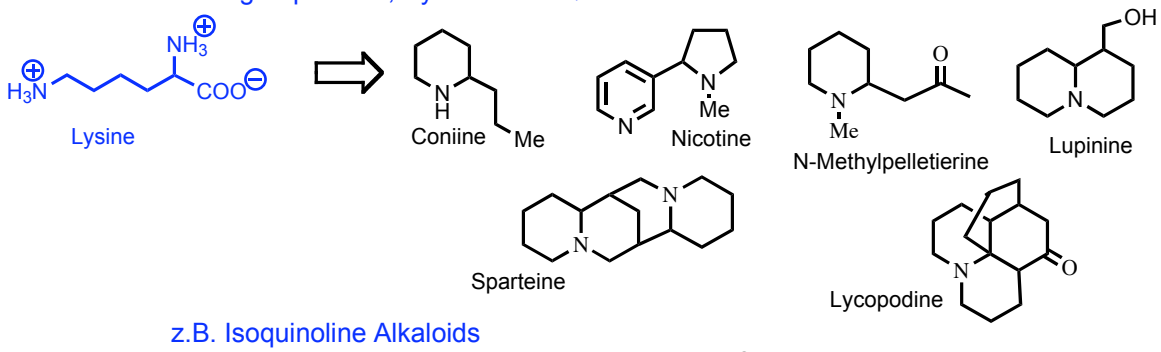
5.1. Alkaloids are derived from amino acids

Nitrogen-containing compounds, with a slightly basic character, have been isolated from many different organisms, mostly plants and microorganisms, and are biosynthesized from amino acids - these are called **alkaloids**. There are probably over 10'000 known alkaloids, having very diverse structures. They can nevertheless be classified into families, on the basis of structural similarities and the amino acids that are used for their biosynthesis Some alkaloids are also produced using building blocks derived from other secondary metabolic pathways, such as terpenoids, polyketides and peptides. Some of the important classes of alkaloid are shown below:

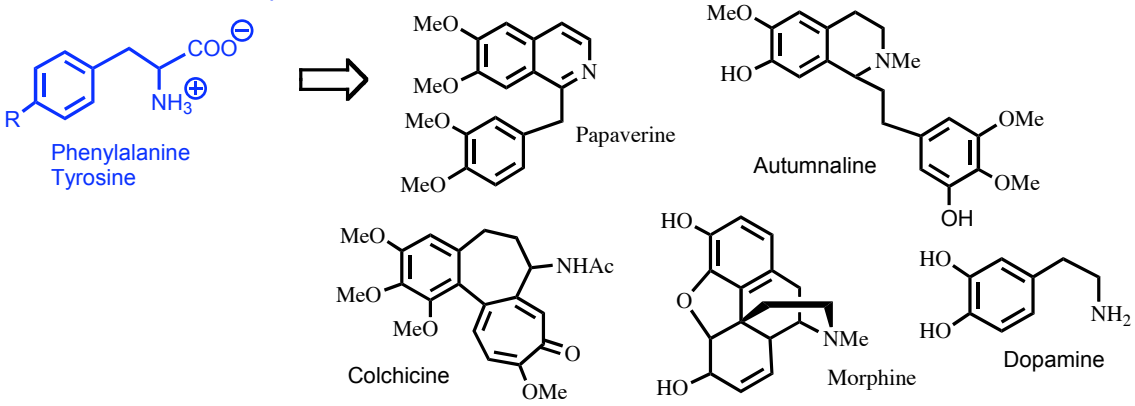
e.g. Pyrrolidine, Pyrrolizidine and Tropane Alkaloids



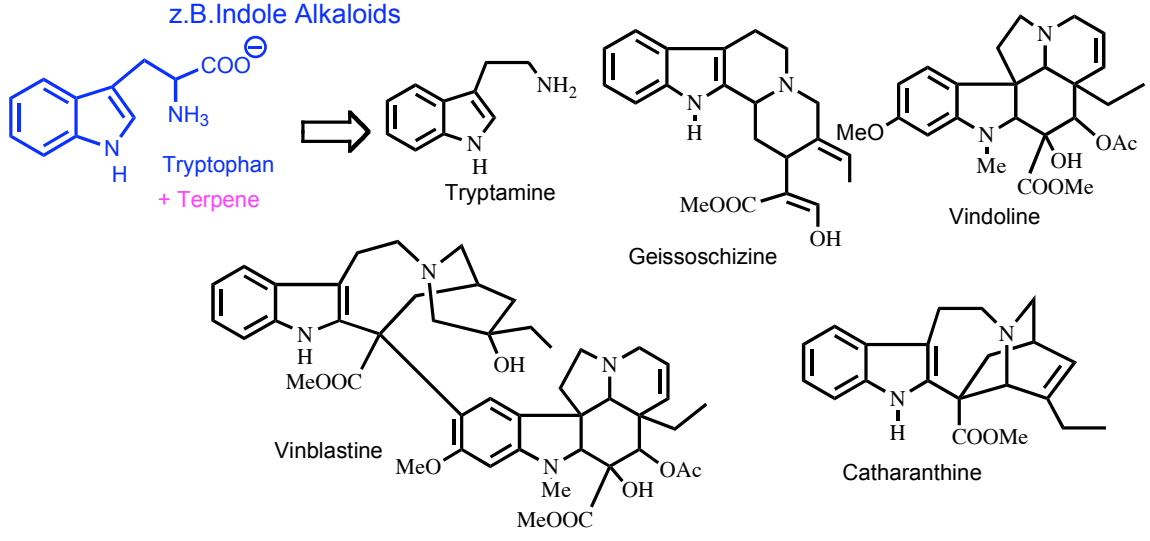
e.g. Piperidine, Pyridine und Quinolizidine Alkaloids



z.B. Isoquinoline Alkaloids

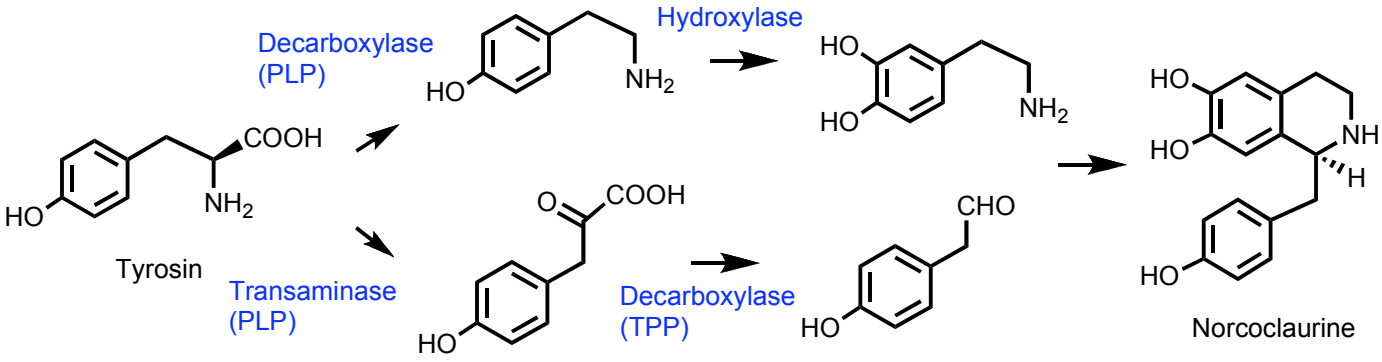


z.B.Indole Alkaloids

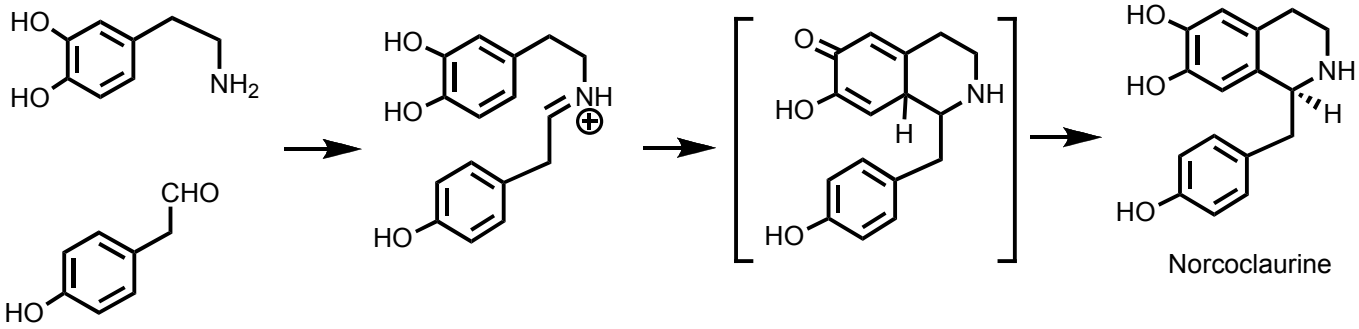


5.2. Benzylisoquinoline Alkaloids

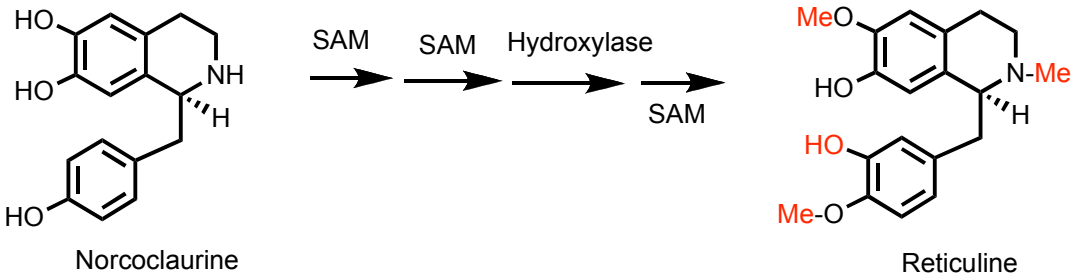
Of special interest within the family of isoquinoline alkaloids are those containing the 1-benzyl(tetrahydro)isoquinoline skeleton, which are found in many different plants. Studies on the biosynthesis of these compounds made progress as soon as radioactively labelled compounds (^{14}C and ^{3}H) became available. Potential precursors could be fed to intact plants, and later the natural prodicts could be isolated from the plants, and then analyzed chemically to detemine whether, and if so, where the radioactive labels had been incorporated. In this way, it was shown that the benzylisoquioline alkaloids are constructed from two molecules of tyrosine:



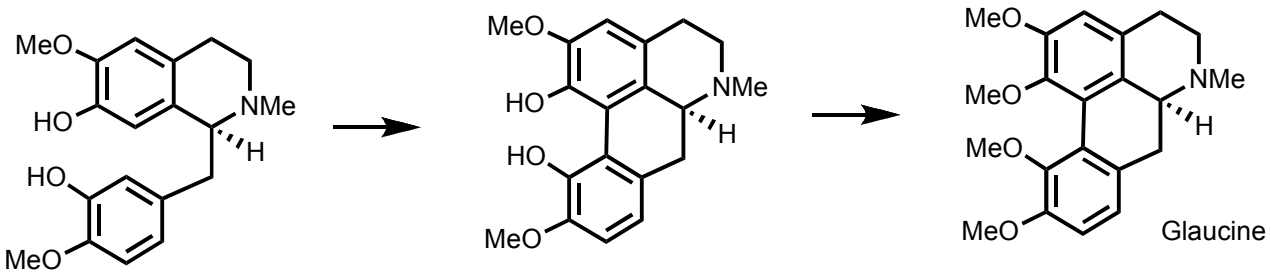
The formation of norcoclaurine is catalyzed by an enzyme, which in effect catalyzes a Pictet-Spengler-Reaction. The reaction shown actually occurs spontaneously in aqueous solution, but then slowly gives racemic product, whereas the enzymic reaction runs much faster and gives optically pure product:



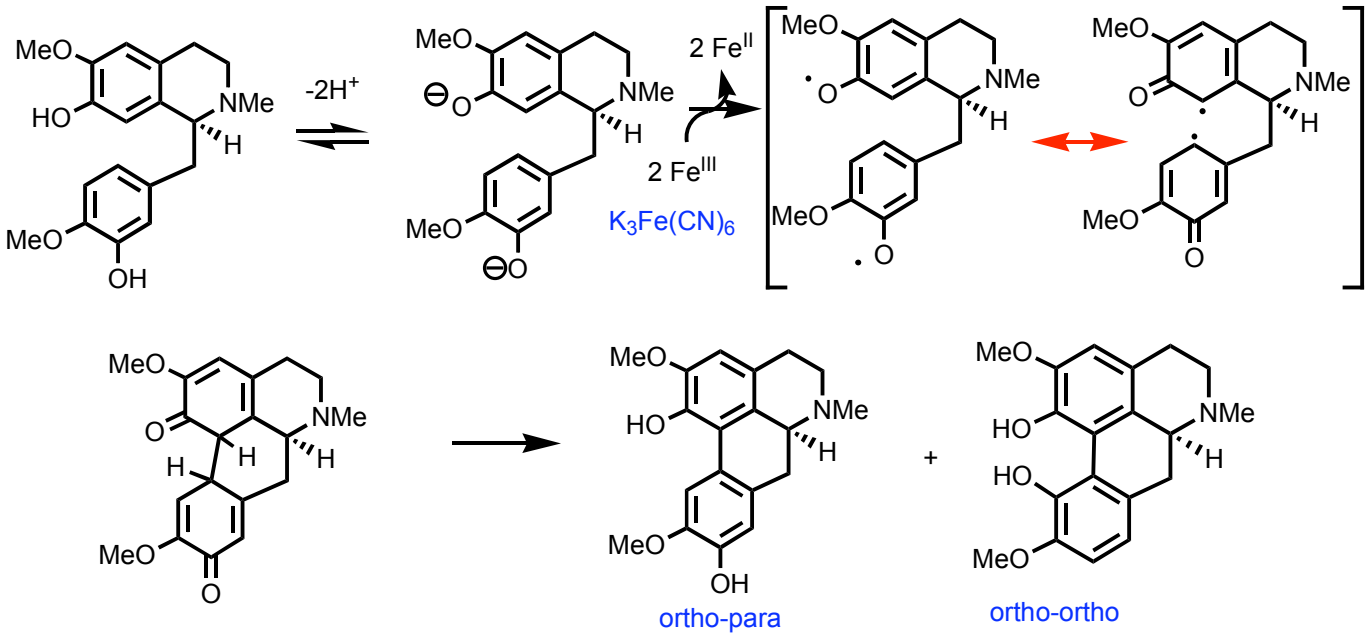
Next, the norcoclaurine is converted into (S)-reticuline :



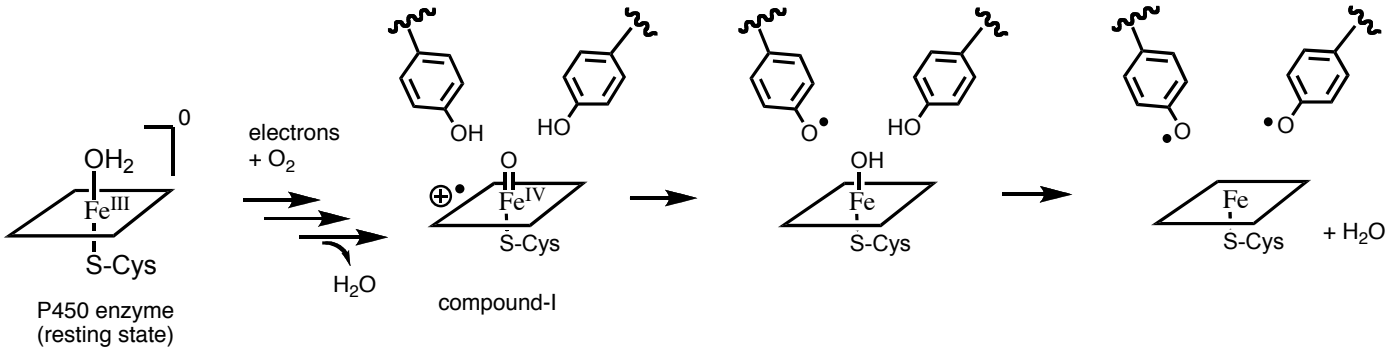
Reticuline is used for the biosynthesis of many other benzylisoquinoline alkaloids, amongst others, the so-called aporphine alkaloids, e.g.:



An important step here is the formation of a direct aryl-aryl bond. This occurs in an oxidative phenol coupling reaction. Nature has evolved a series of hemoproteins of the cytochrome P450 family that catalyze specific oxidative phenol coupling reactions (not hydroxylations, compare earlier). Such coupling reactions are well known in synthetic chemistry, where they can be carried out with phenolic compounds, under basic conditions, using $K_3Fe(CN)_6$ as oxidizing agent, e.g.:



Such reactions tend to produce mixtures of products, because the free radical intermediates can often couple in more than one way. The enzymes, however, catalyze only one pathway specifically. The mechanisms of the enzymic reactions are not well understood, but require molecular oxygen as well as the hemoprotein (P450). The oxidizing power of compound-I is used to drive the coupling reaction, e.g.:



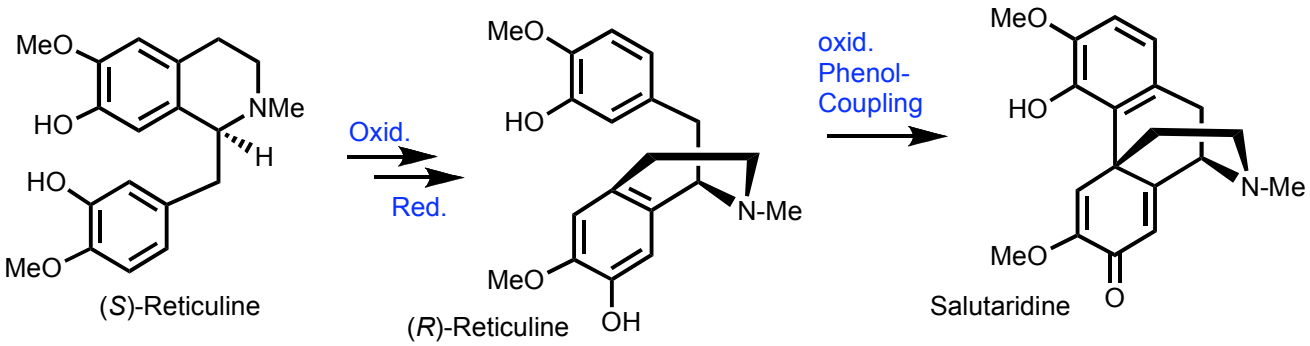
Oxidative phenol coupling reactions are often found in alkaloid biosynthesis. Perhaps the best-known example occurs during the biosynthesis of morphine.

Morphine is a highly-potent opiate analgesic drug and is the principal active agent in opium and the prototypical opioid. It is also a natural endocrine product in humans and other animals. Like other opiates, e.g., diacetylmorphine (heroin), morphine acts directly on the central nervous system (CNS) to relieve pain, and at synapses of the *nucleus accumbens* in particular. Studies done on the efficacy of various opioids have indicated that, in the management of severe pain, no other narcotic analgesic is more effective or superior to morphine. Morphine is highly addictive when compared to other substances; tolerance, physical and psychological dependences develop very rapidly. The word "morphine" is derived from Morpheus, one of the Greek gods of dreams.

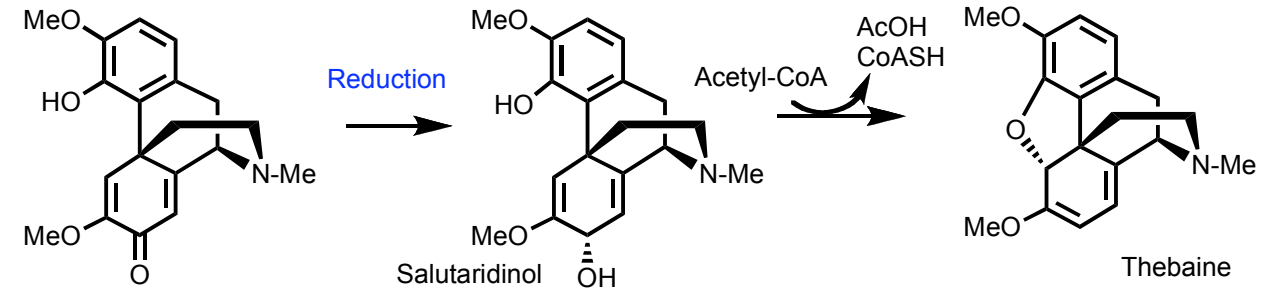
The opium poppy is *Papaver somniferum*.

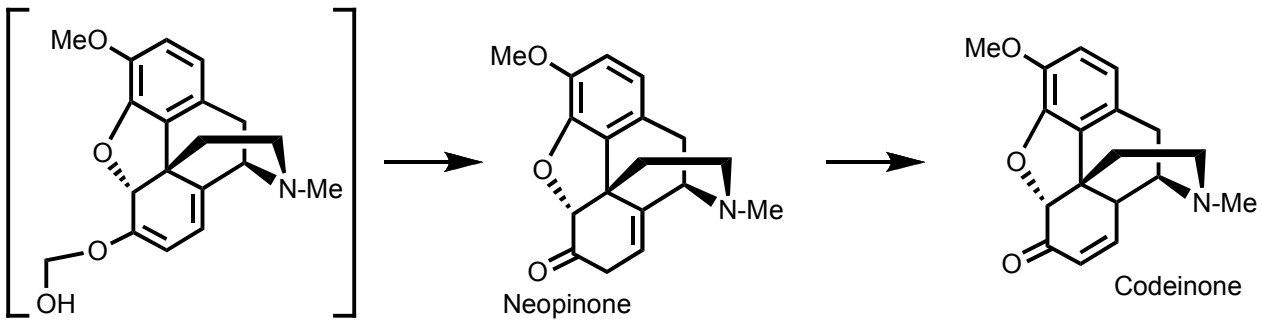


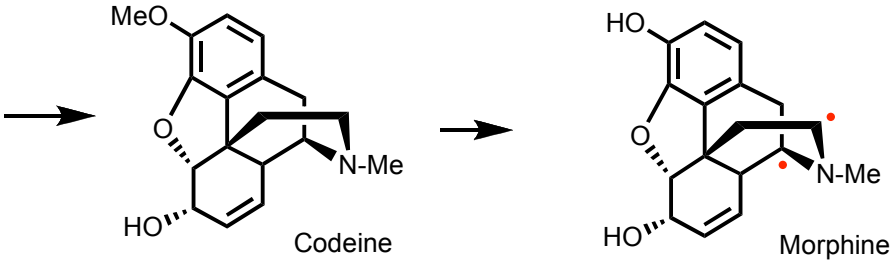
(*R*)-Reticuline is an important intermediate in the biosynthesis of morphine, and is produced by racemization of (*S*)-reticuline in a redox process, as shown below:



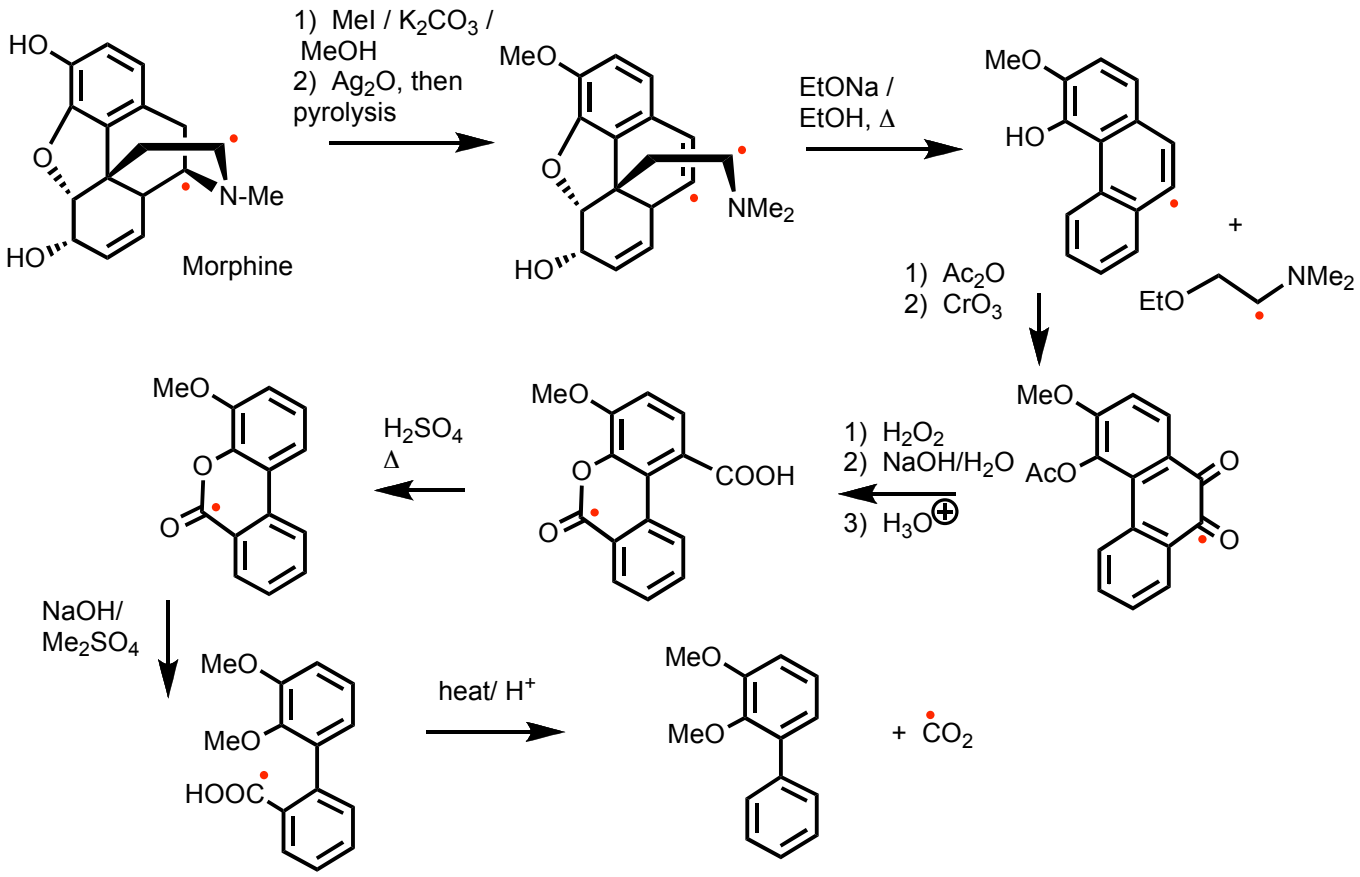
Salutaridine is found as a minor alkaloid constituent in the opium poppy:







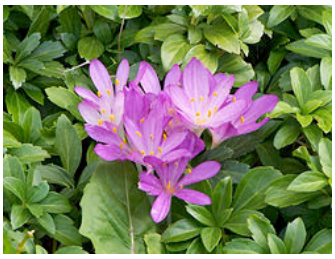
The biosynthesis of morphine in the opium poppy was one of the first alkaloid pathways to be elucidated with the aid of ^{14}C-labelled precursors. It was shown that [2-^{14}C]-tyrosine is incorporated into morphine, with the ^{14}C label appearing at the positions indicated above. This was proven, by degrading the ^{14}C-labelled morphine in the following way:



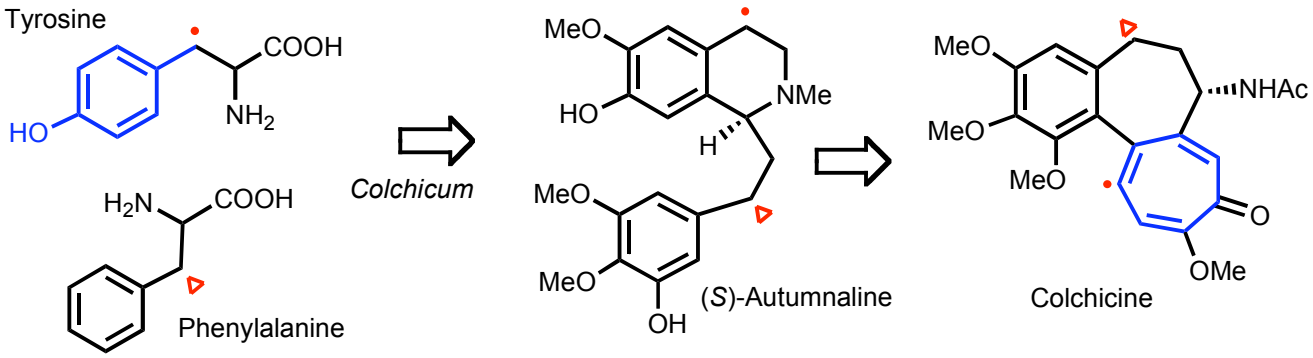
Another interesting benzylisoquinoline alkaloid is colchicine. Colchicine was originally extracted from plants of the genus *Colchicum* (Autumn crocus, *Colchicum autumnale*, also known as the "Meadow saffron"). Originally used to treat rheumatic complaints and especially gout, it was also prescribed for its cathartic and emetic effects. Its present medicinal use is mainly in the treatment of gout; it is also being investigated for its potential use as an anti-cancer drug.

Colchicine inhibits microtubule polymerization by binding to tubulin, one of the main constituents of microtubules. Tubulin is essential for mitosis, and therefore colchicine effectively functions as a "mitotic poison" or spindle poison. Since one of the defining characteristics of cancer cells is a significantly increased rate of mitosis, this means that cancer cells are significantly more vulnerable to colchicine poisoning than are normal cells. However, the therapeutic value of colchicine against cancer is (as is typical with chemotherapy agents) limited by its toxicity against normal cells.

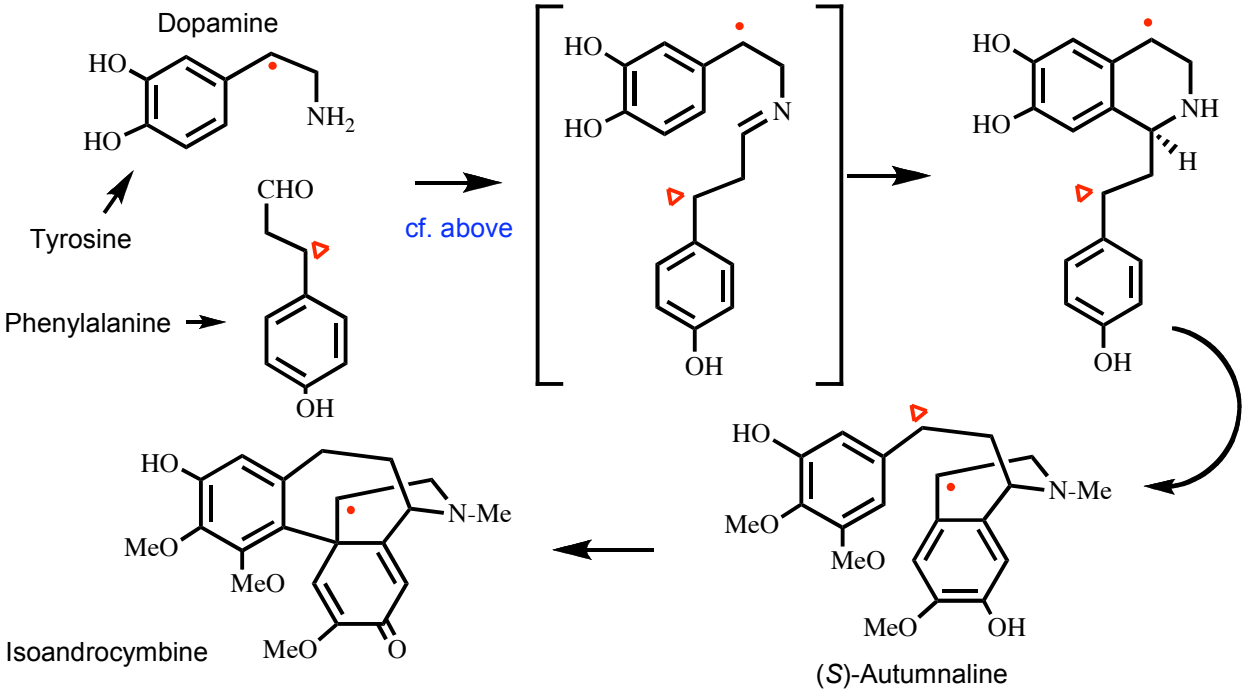
In 2008, the Botanic Gardens Conservation International (representing botanic gardens in 120 countries) stated that "400 medicinal plants are at risk of extinction, from over-collection and deforestation, threatening the discovery of future cures for disease." These included Yew trees (the bark is used for the cancer drug taxol (paclitaxel)); Hoodia (from Namibia, source of weight loss drugs); half of Magnolias (used as Chinese medicine for 5,000 years to fight cancer, dementia and heart disease); and Autumn crocus (for gout). The group also found that 5 billion people benefit from traditional plant-based medicine for health care.



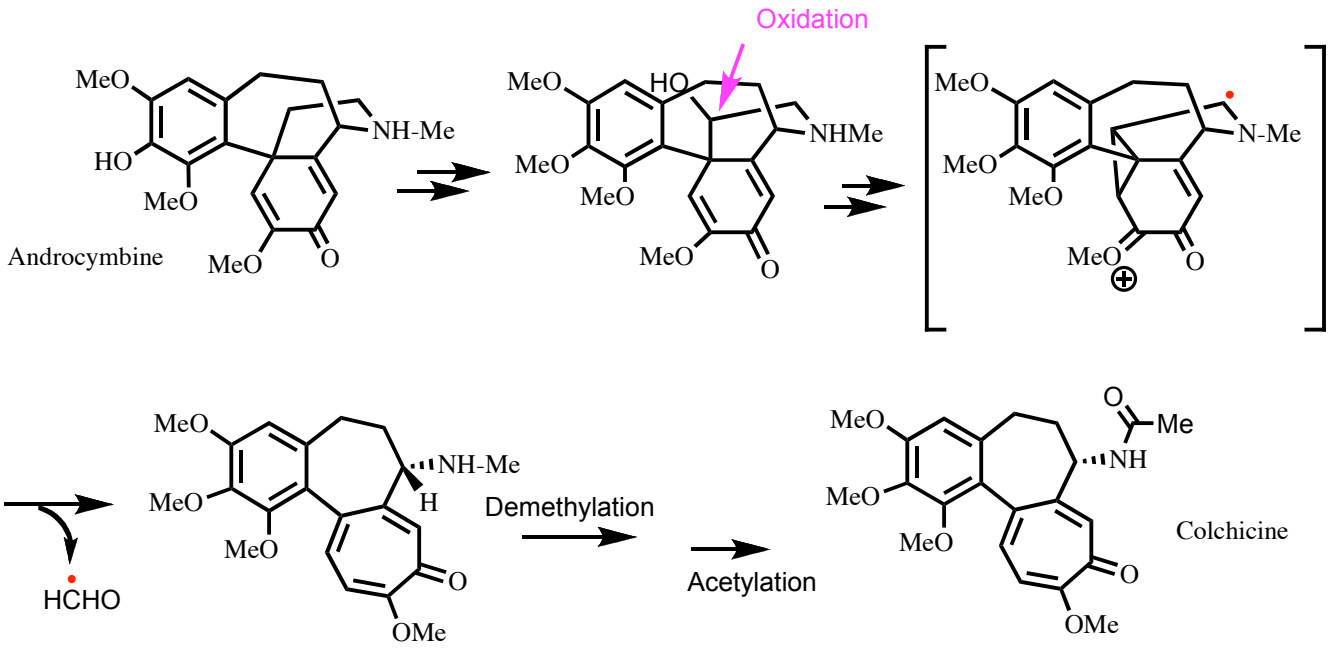
Early labelling experiments showed that tyrosine and phenylalanine are required for colchicine biosynthesis, and that autumnaline is a key intermediate. However, the Phe provides a C_6C_3 unit rather than a C_6C_2 fragment:



The seven membered tropolone ring was shown by labelling experiments to originate by ring expansion of the tyrosine-derived aromatic ring, including the adjacent benzylic carbon atom.

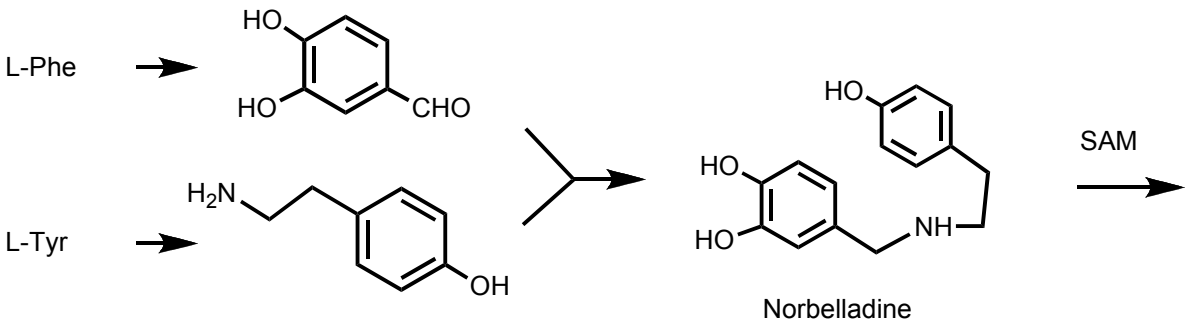


Androcymbine has been isolated from *Androcymbium melanthioides*. The later steps have not been proven, but may involve the following reactions:

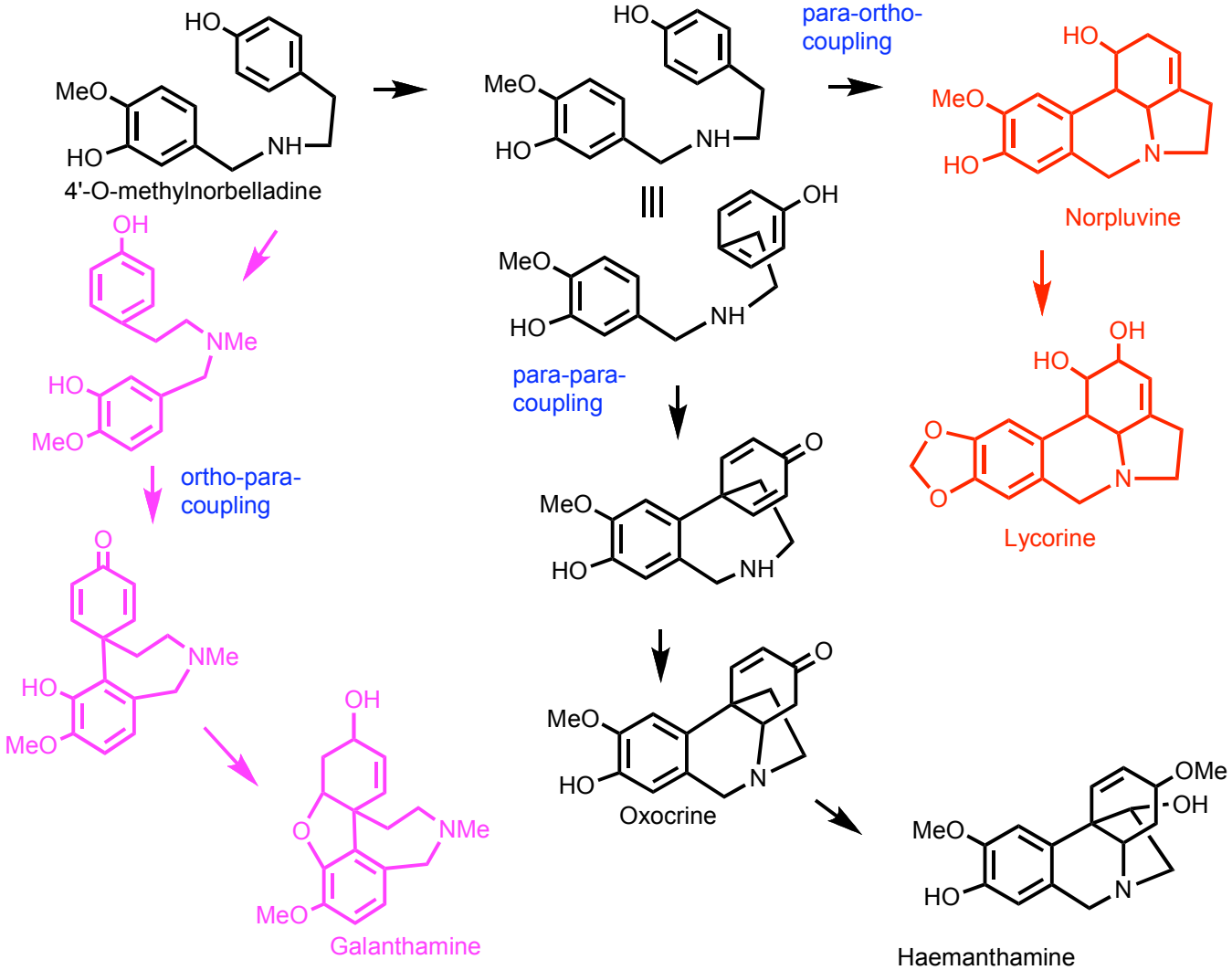


Various types of alkaloids are encountered in the daffodil family, called the Amaryllidaceae alkaloids (*Amaryllidaceae* is the botanical name of a family of flowering plants. The plants are herbaceous perennials that grow from bulbs, often with showy flowers). The *Amaryllidaceae* family includes *Amarylis*, *Narcissus* and *Galanthus*, and the alkaloid content of bulbs from most members makes them toxic. However, galanthamine from daffoldils and snowdrops is currently an important drug for the treatment of the symptoms of Alzheimer's disease. The natural sources of galanthamine are certain species of daffodil and because these species are scarce and because the isolation of galanthamine from daffodil is expensive (a 1996 figure specifies 50,000 US $ per kilogram; the yield from daffodil is 0.1-0.2% dry weight) alternative synthetic sources have been developed. Galanthamine acts as a competitive inhibitor of acetylcholinesterase, and enhances cognitive functions by raising acetylcholine levels in brain areas lacking cholinergic neurons. It does not cure the condition, but merely slows the rate of cognitive decline.

Phe and Tyr are again the starting materials used for the biosynthesis of the Amaryllidaceae alkaloids:

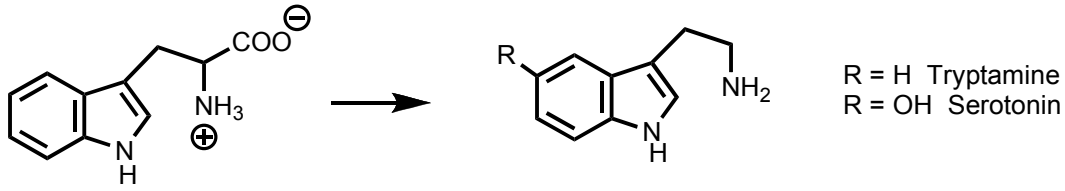


Thereafter, three different modes of phenol coupling are seen:



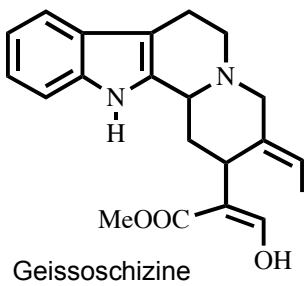
5.3. Indole Alkaloids (see *Nat. Prod. Rep.* 2006, *23*, 532)

The simplest representative of the indole alkaloids are the natural amines tryptamine und serotonin, which are biosynthesized from the amino acid tryptophan (Trp):

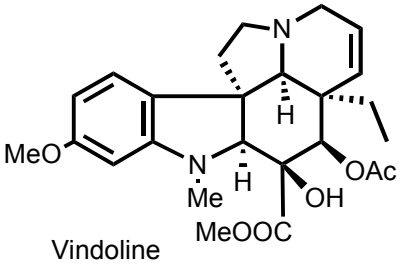


Serotonin is a monoamine neurotransmitter synthesized in serotonergic neurons in the central nervous system (CNS), and enterochromaffin cells in the gastrointestinal tract of animals including humans. Serotonin is also found in many mushrooms and plants, including fruits and vegetables. Serotonin is believed to play an important role as a neurotransmitter, in the modulation of anger, aggression, body temperature, mood, sleep, sexuality, and appetite as well as stimulating vomiting.

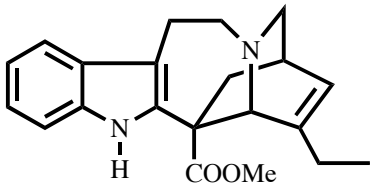
The **vinca alkaloids** are a very interesting class of indole alkaloids, and include vinblastine, vincristine, vindesine and vinorelbine. These alkaloids are produced by plants of the genus *Catharanthus*. *Catharanthus* (Madagascar Periwinkle) is a genus of eight species of herbaceous perennial plants, seven endemic to the island of Madagascar, the eighth native to the Indian subcontinent in southern Asia. One species, *C. roseus*, has been widely cultivated, and after introduction has become an invasive species in some areas. *C. roseus* has also gained interest from the pharmaceutical industry; the alkaloids vincristine and vinblastine from its sap have been shown to be an effective treatment for leukaemia. Although the sap is poisonous if ingested, some 70 useful alkaloids have been identified from it. In Madagascar, extracts have been used for hundreds of years in herbal medicine for the treatment of diabetes, as hemostatics and tranquilizers, to lower blood pressure, and as disinfectants. The extracts are not without their side effects, however, which include hair loss.



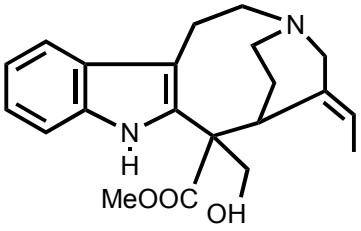
Geissoschizine



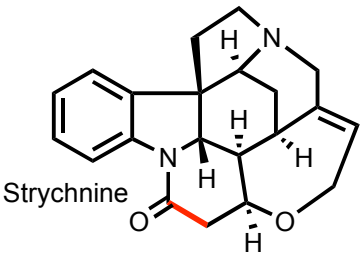
Vindoline



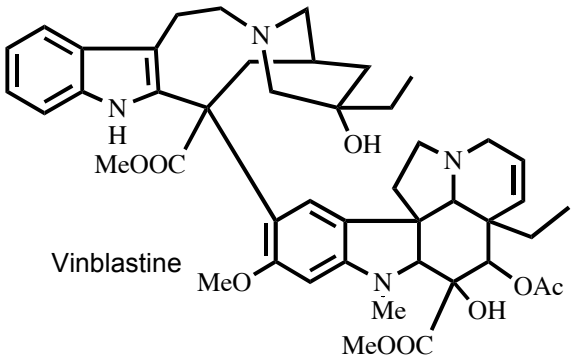
Catharanthine



Stemmadenine

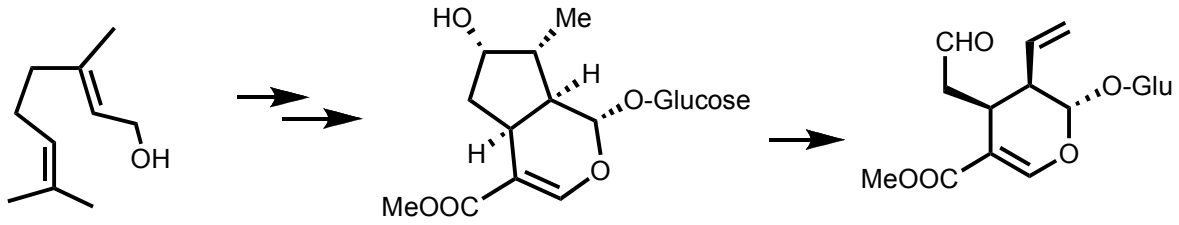


Strychnine



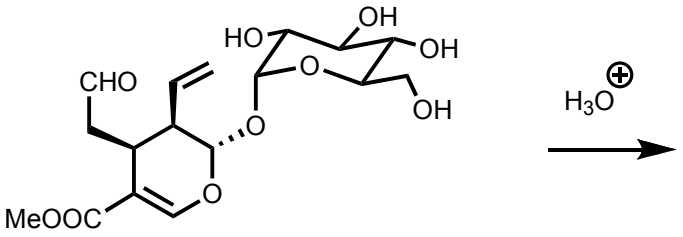
Vinblastine

The structures of these alkaloids reveal that not only Trp is required for the biosynthesis. A C10 fragment is also needed, and is provided from **terpene metabolism**. Strychnine biosynthesis also incorporates one acetate unit (in red above). The important C10 fragment is produced from geraniol, and is called secologanin:

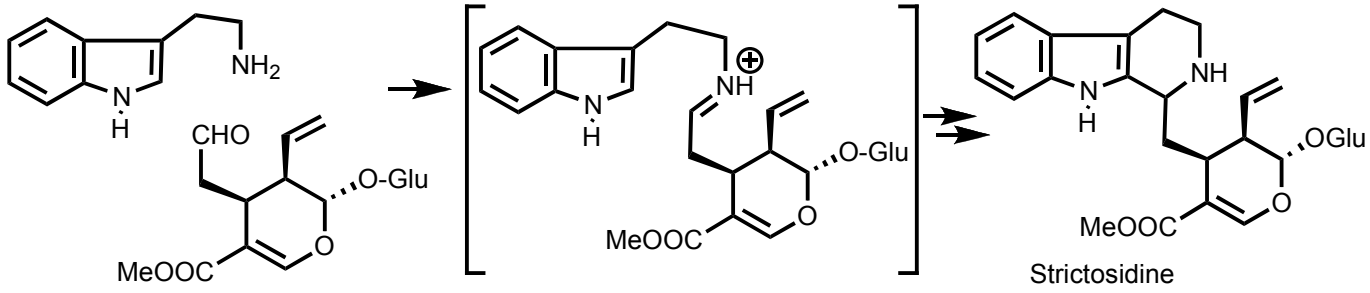


Geraniol Loganin Secologanin

Secologanin is a glucoside, which can be cleaved by hydrolysis under acidic conditions:

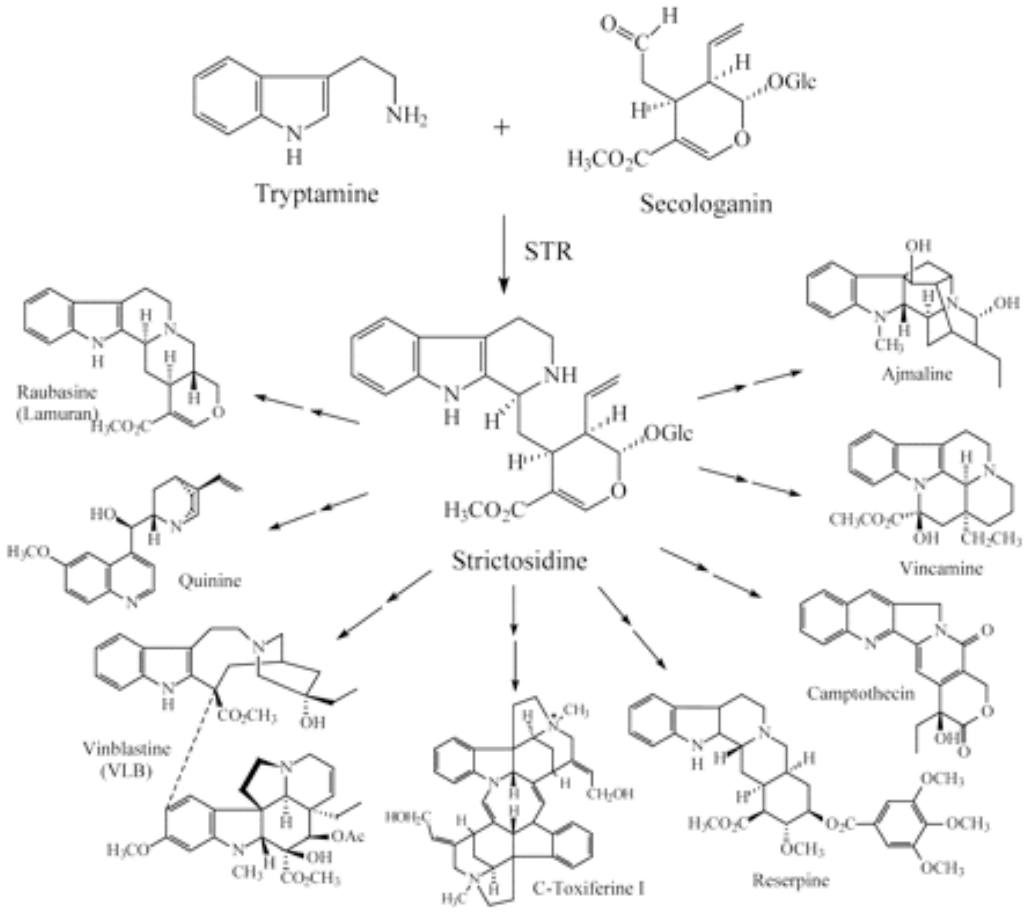


The formation of the indole alkaloids begins with the condensation of tryptamine and secologanin, catalyzed by strictosidine synthase (STR, see below):

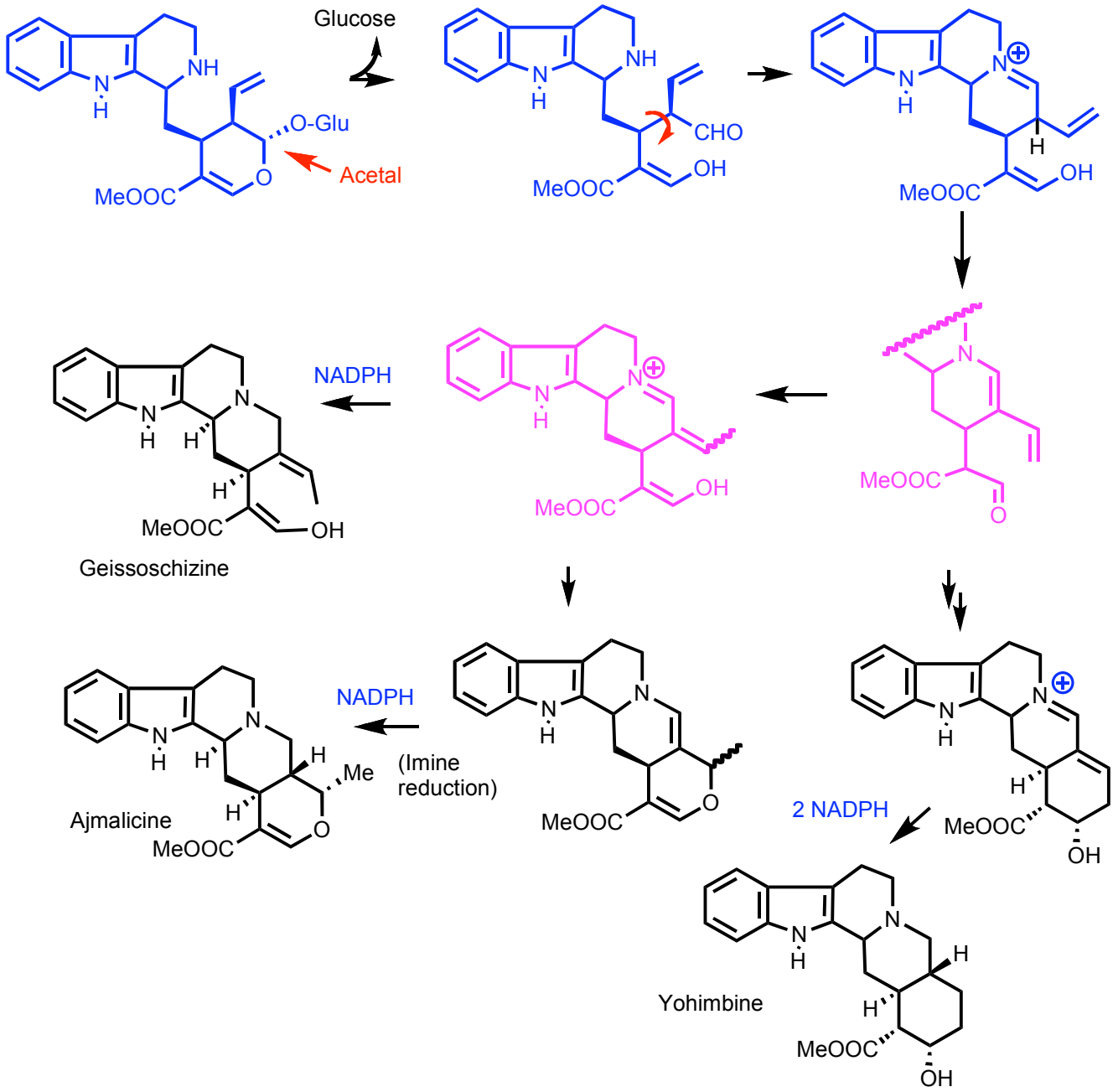


Strictosidine

Strictosidine is then a key intermediate in the formation of over 1000 different indole-terpene alkaloids.



For example, the *Corynanthe* alkaloids:



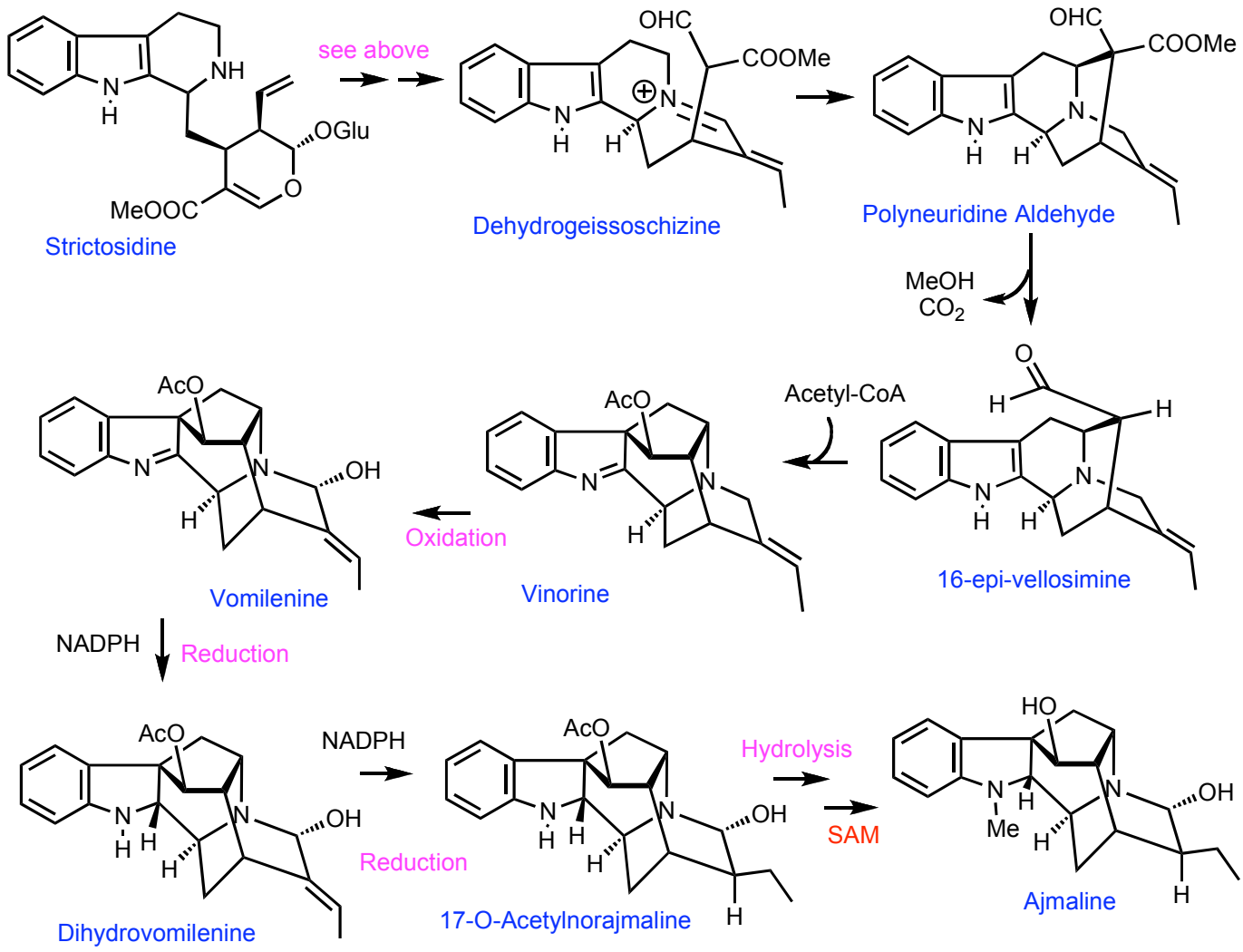
Yohimbine is the principal alkaloid of the bark of the West-African evergreen *Pausinystalia yohimbe* Pierre (formerly *Corynanthe yohimbe*), family *Rubiaceae* (Madder family). There are 31 other yohimbane alkaloids found in Yohimbe. In Africa, yohimbine has traditionally been used as an aphrodisiac. Yohimbine hydrochloride is a standardized form of yohimbine that is available as a prescription drug in the United States, and has been shown to be effective in the treatment of male impotence. Yohimbine hydrochloride has also been used for the treatment of sexual side effects caused by some antidepressants, female hyposexual disorder, as a blood pressure boosting agent in autonomic failure, xerostomia, and as a probe for noradrenergic activity.

Ajmaline was first isolated from the roots of *Rauwolfia serpentina*, a species of flowering plant in the family *Apocynaceae*. It is one of the 50 fundamental herbs used in traditional Chinese medicine, where it has the name shé ē n mù (蛇根木) or yìndù shémù (印度蛇木). The extract of the plant has also been used for millenia in India — it was reported that Mahatma Gandhi took it as a tranquilizer during his lifetime. Ajmaline is a class Ia antiarrhythmic agent, a group of pharmaceuticals that are used to suppress fast rhythms of the heart (cardiac arrhythmias), such as atrial fibrillation, atrial flutter, ventricular tachycardia,

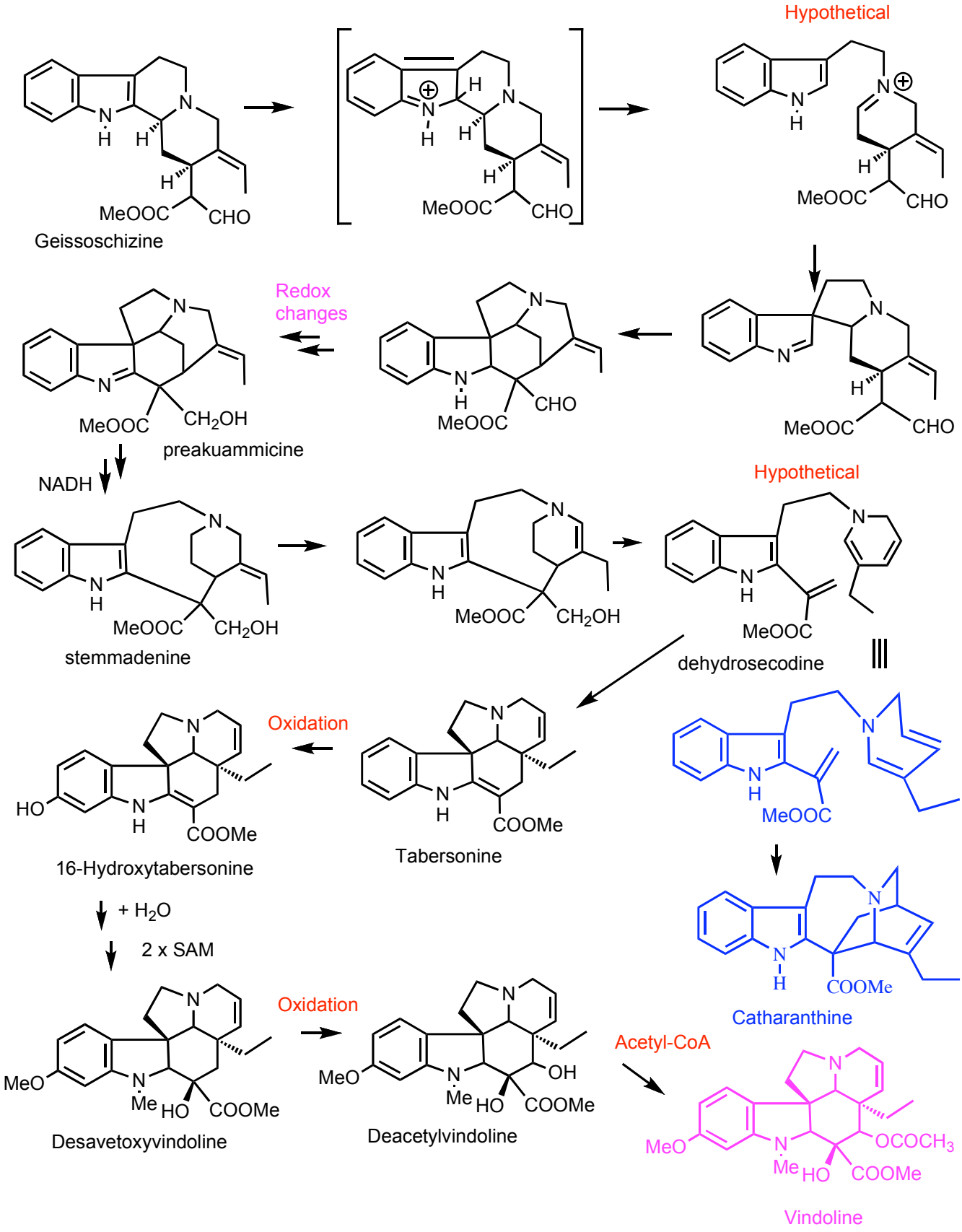
and ventricular fibrillation. Ajmaline functions by blocking Na-channels in cell membranes.

Rauwolfia caffra is the South African quinine tree. *Rauwolfia serpentina*, or Indian Snakeroot or Sarpagandha, contains a number of bioactive chemicals, including ajmaline, deserpidine, rescinnamine, serpentinine, and yohimbine. Reserpine is an alkaloid first isolated from *R. serpentina*, and was widely used as an antihypertensive drug. It had drastic psychological side effects and has been now replaced by blood-pressure-lowering drugs that lack such adverse effects. But in herbal use it is a safe and effective resource for hypertensive patients. The pharmaceutical companies have stopped producing this drug as reserpine or deserpedine. It is only available currently in the U.S. as a herbal medicine over the Internet.

The pathway to ajmaline has been well documented, although few mechanistic studies have been reported so far on the biosynthetic enzymes:

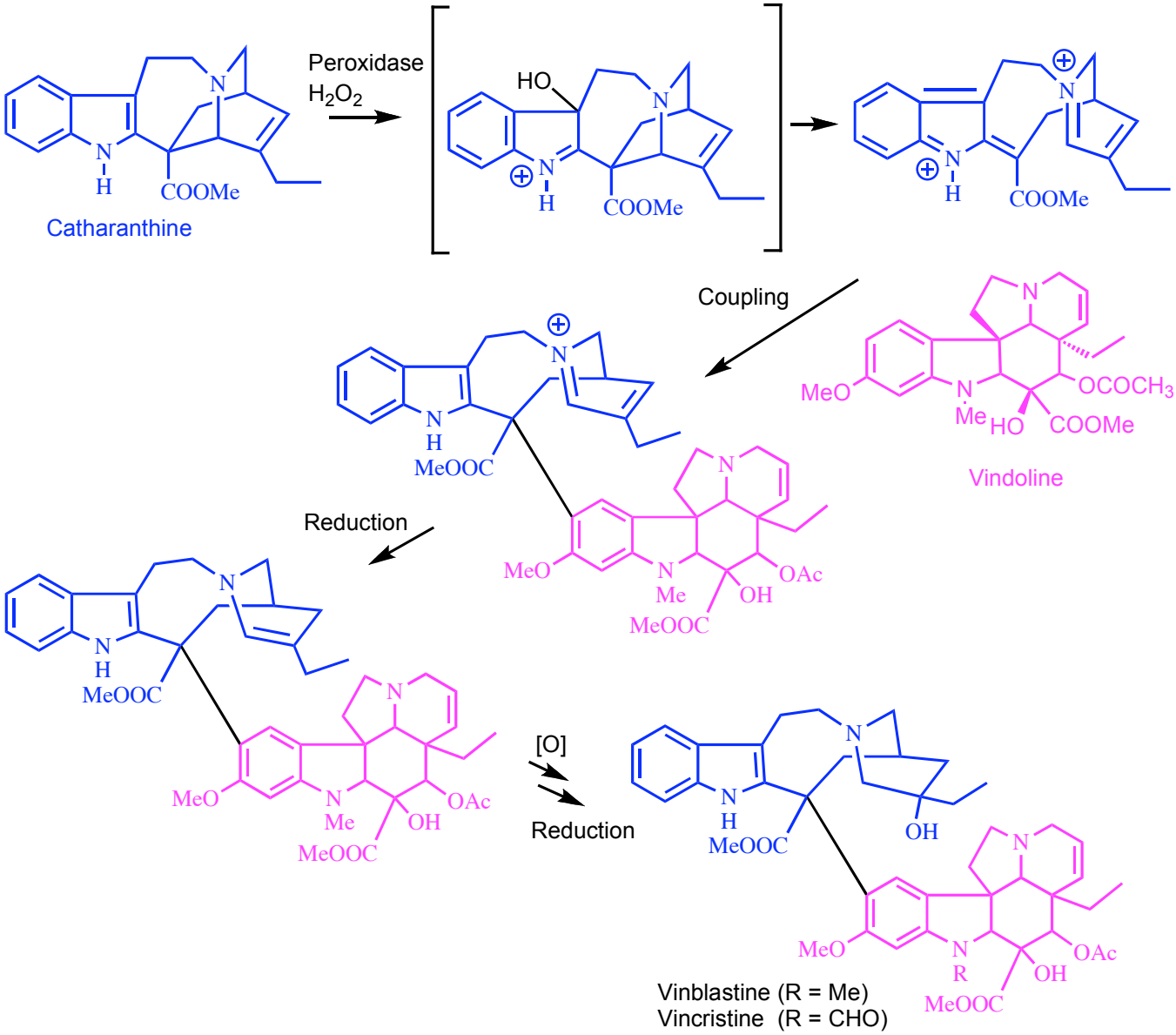


Catharanthine is a member of the so-called *iboga* family of indole alkaloids. It is one of the many alkaloids present in *Catharanthus roseus*. It is produced along with many other Catharanthus alkaloids by factory farming in China. It can be used as a starting material for the synthesis of the anti-tumor drugs, vinblastine and vincristine. Vindoline (an *Aspidosperma* alkaloid) is another important component of the bis-indole alkaloids, typified by vinblastine and vincristine, also produced by *C. roseus*. Some of the biosynthetic steps have been documented, but the enzymes have not yet been studied in detail. A fascinating proposal was made to explain how catharanthine and vindoline might be produced from geissoschizine. Tabersonine is a known intermediate, and the steps from tabersonine have been established; the rest is hypothetical -

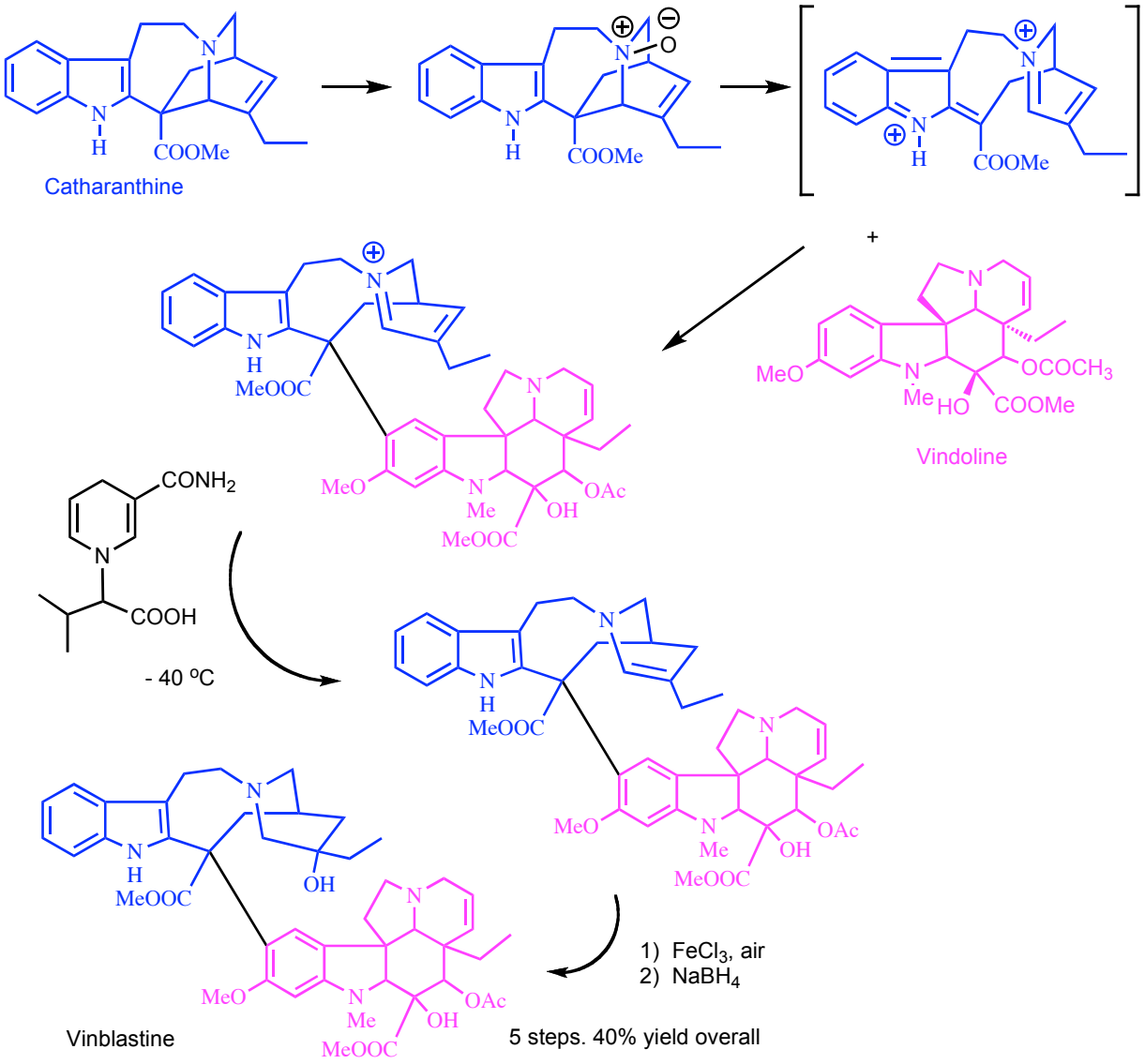


Vinblastine and vincristine are anti-mitotic drugs used to treat certain kinds of cancer, including Hodgkin's lymphoma, non-small cell lung cancer, breast cancer and testicular cancer. They bind to tubulin, thereby inhibiting the assembly of microtubules. They are M phase cell cycle specific, since microtubules are a component of the mitotic spindle and the kinetochore, which are necessary for the separation of chromosomes during anaphase of mitosis. Toxicities include bone marrow suppression (which is dose-limiting), gastrointestinal toxicity, potent vesicant (blister-forming) activity, and extravasation injury (forms deep ulcers).

The coupling of catharanthine and vindoline can be catalyzed by a relatively non-specific peroxidase (a hemoprotein). It is possible that a similar enzyme specifically catalyzes this coupling in *C. roseus*.



Vinblastine is only present at low levels in *C. roseus* (0.0002% of dry leaf wt). Over 500 kg of catharanthus is needed to produce 1g of pure vincristine. Much effort has been put into the synthesis of the dimeric alkaloids, starting from the monomers, which can be isolated from the plant in much higher yields. One example is shown below:



(see also: *J.Am.Chem.Soc.*, **2008**, *130*, 420).

Finally, note that strictosidine is also the precursor to the quinoline alkaloids, including the important anti-malarial drug quinine.