SOLUTION OF THE MULTI-STEP ASSEMBLY OF *CATHARANTHUS ROSEUS* ANTICANCER ALKALOIDS.

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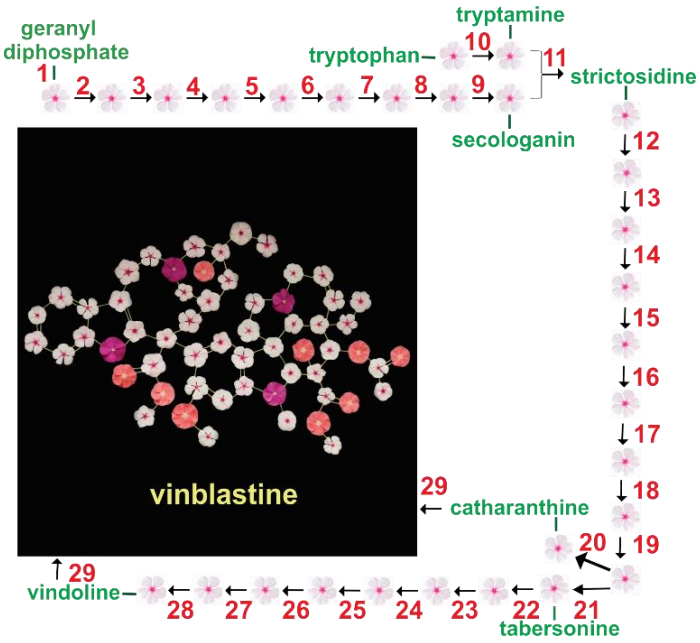
Catharanthus roseus, also known as the Madagascar periwinkle, produces low levels of unique dimeric monoterpenoid indole alkaloids (MIAs) that are harvested and used as anticancer drugs to treat Hodgkin's disease and other cancers. The trace levels of dimers occurring in *C. roseus* have made them very expensive to isolate. It is well known that *C. roseus* leaves are the main sites of biosynthesis of the precursors, catharanthine and vindoline, and that each MIA is localized in different leaf cell types, making it difficult to understand how dimer formation takes place. Research efforts in the past 6 years on the assembly of these monoterpenoid indole alkaloids (MIAs) in *C. roseus* has led to the molecular and biochemical characterization of the remaining genes involved in the 29-step pathway required for their biosynthesis from geraniol diphosphate. The formation of strictosidine from geraniol and tryptophan involves 9-steps, most of which have recently been solved and have led to prototype strictosidine expressing pathways in yeast. Enzyme-mediated hydrolysis of strictosidine leads to formation of precursors used in the biosynthesis of several thousand MIAs, including these well-known anticancer drugs. We recently reported the discovery and functional characterization of 10 remaining genes to complete the description of enzymes for tabersonine and catharanthine assembly [*PNAS* (2018) **115**: 3180-3185; *Planta* (2018) 247:625-634; *Plant J.*, (2108) **97:** 257-266]. These discoveries with our recent successful assembly of vindoline from tabersonine [*PNAS* (2015) **112**: 6224-6229) completed the description of the vindoline and catharanthine pathways from geraniol and tryptophan and has set the stage for developing biological systems for synthesis of many different biologically active MIAs. The basic tools and developments leading to these discoveries will be discussed and analyzed. The impacts of elucidation of the vinblastine/vincristine pathways will also be discussed in relation to future discoveries of MIA pathways of biological and biomedical importance.



The *Catharathus roseus* MIA, vinblastine (depicted as a floral diagram), is a dimer of catharanthine and vindoline. The formation of secologanin involves 1) geraniol synthase, 2) geraniol-8-hydroxylase, 3) 8- hydroxygeraniol oxidase), 4) iridoid synthase, 5) 7- deoxyloganetic acid synthase), 6) 7-deoxyloganetic acid glucosyltransferase, 7) loganic acid synthase, 8) loganic acid O-methyltransferase and 9) secologanin synthase. The 10) tryptophan decarboxylase-mediated formation of tryptamine is condensed with secologanin to generate the central intermediate strictosidine via 11) strictosidine synthase. Hydrolysis of thus MIA by 12) strictosidine-β-glucosidase generates the equilibrium mixture of cathenamine/4,21-dehydrogeissoschizine and 13) geissoschizine synthase generates19*E*-geissoschizine. The formation of stemmadenine involves 14) geissoschizine oxidase, 15) Redox1 and 16) Redox 2. Formation of *O*-acetylstemmadenine by 17) stemmadenine-*O*-acetyl-transferase stabilizes the molecule for subsequent transformations involving 18) geissoschizine synthase, 19) *O*-acetyl-stemmadenine oxidase. A reactive intermediate is acted upon by 20) hydrolase 1 to form catharanthine, and by 21) hydrolase 2 to form tabersonine. Tabersonine if converted to vindoline by the action of 22) tabersonine-16-hydroxylase, 23) 16-O-methytransferase, 24) 3-oxidase, 25) 3-reductase, 26) N-methyltransferase, 27) 4-hydroxylase and 28) O-acetyltransferase. 29) peroxidase may then catalyzed the coupling reaction to form vinblastine