

# Chemistry and biology of monoterpene indole alkaloid biosynthesis

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Monoterpene indole alkaloids exhibit a diverse array of structures and biological activities. The biosynthetic pathways for several representative terpene indole alkaloids are described in detail.

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## 1 Introduction

The terpene indole alkaloids are a diverse class of natural products, comprising over 2000 members. These complex natural products possess a range of chemical structures and a wealth of biological activities (Fig. 1).<sup>1,2</sup> Terpene indole alkaloids are used as anti-cancer, anti-malarial and anti-arrhythmic agents (Fig. 1).<sup>3</sup> The biosynthetic pathways of some classes of terpene indole alkaloids are well understood. In certain cases, many of the enzymes that are responsible for biosynthesis have been cloned and mechanistically studied *in vitro*. In other cases, the biosynthetic pathway is only proposed based on the results of feeding studies with isotopically labeled substrates and from the structures of isolated biosynthetic intermediates.

Early studies of plant alkaloid biosynthesis relied on administration of isotopically labeled starting materials to differentiated plants or plant cell cultures, followed by isolation and structural characterization of the labeled products. Additionally, chemical reactions with isolated biosynthetic intermediates allowed predictions of chemically reasonable transformations. However, with recent advances in molecular biology, the biosynthetic pathways of plant alkaloid natural products have been subject to study at the enzymatic level.<sup>4,5</sup> A number of enzymes involved

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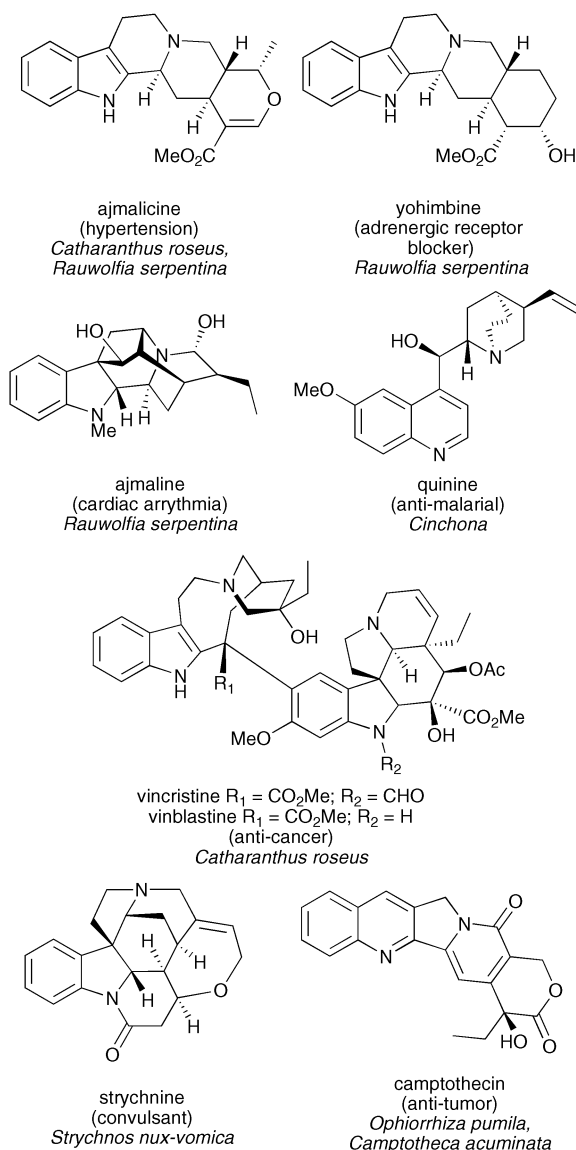


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**Fig. 1** Representative terpene indole alkaloids, with corresponding biological function and species of plants from which they are isolated.

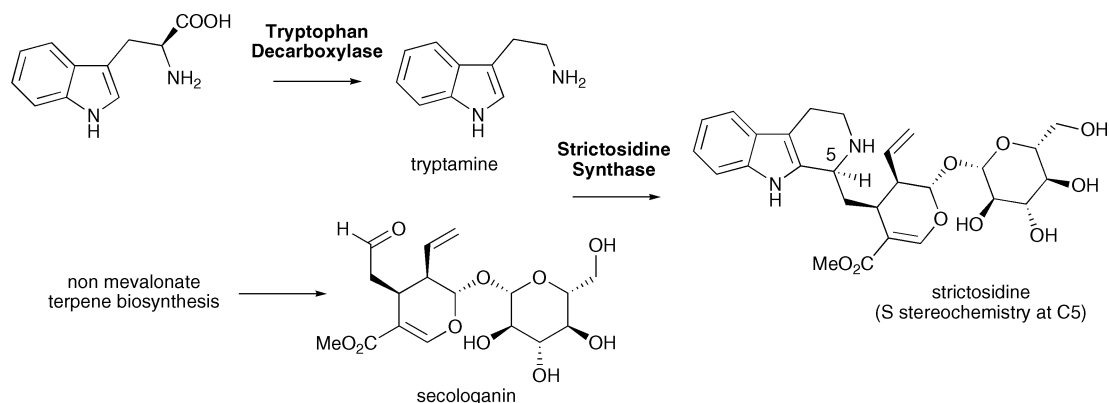
in plant alkaloid biosynthesis have been successfully cloned, and many more enzymes have been purified from alkaloid producing plants or cell lines.<sup>6</sup>

Plant biosynthetic pathways are much less well understood than prokaryotic metabolic pathways since the genes expressing complete plant pathways are usually not clustered. The study of plant-derived secondary metabolites typically requires that each plant enzyme of a pathway be individually isolated and cloned independently of one another. Many plant enzymes have been characterized by “reverse genetics” in which the enzymes are isolated from plants or plant cell culture by traditional biochemical chromatography techniques.<sup>6</sup> After purification, the protein may be partially sequenced, and this sequence information is then used to identify the corresponding gene from a plant cDNA library. More recently, plant cDNA libraries have been successfully screened for well known classes of enzymes such as P450 enzymes or acetyl transferase homologues.<sup>7,8</sup> However, this homology-based cloning method is limited to identification of enzymes with regions of high sequence conservation. Alternatively, plant cell lines can be stimulated with an elicitor to produce alkaloids. Genes that are upregulated in the elicited strain are likely to be involved in alkaloid biosynthesis.<sup>9,10</sup>

In this review we highlight a few well characterized alkaloid biosynthetic pathways for representative members of the terpene indole alkaloid family. Specifically, we discuss the biosynthetic pathways of the corynanthe group (ajmalicine, serpentine, yohimbine), the iboga group (catharanthine), the aspidosperma group (tabersonine, vindoline), and the quinoline group (camptothecin, quinine).

## 2 Biosynthesis of terpene indole alkaloids

All terpene indole alkaloids are derived from tryptophan and the iridoid terpene secologanin (Scheme 1). Tryptophan decarboxylase, a pyridoxal dependent enzyme,<sup>11–13</sup> converts tryptophan to tryptamine.<sup>14</sup> The involvement of an iridoid monoterpene in these indole alkaloid pathways was first proposed after the structures of several iridoid terpenes were elucidated.<sup>15–17</sup> Secologanin was subsequently identified as the specific iridoid precursor.<sup>18–20</sup>



**Scheme 1** First steps of terpene indole alkaloid biosynthesis.

Secologanin is itself a natural product, and the biosynthetic pathway for this molecule has not been fully elucidated. Isopentenyl diphosphate (IPP), the precursor for all terpenoids, is produced by either the mevalonate biosynthetic pathway or the more recently discovered triose phosphate/pyruvate pathway.<sup>21</sup> Feeding studies with *Catharanthus roseus* cell suspension cultures and <sup>13</sup>C-glucose strongly suggest that secologanin is ultimately derived from the triose phosphate/pyruvate or “non-mevalonate” pathway (Scheme 2).<sup>22</sup> Feeding studies with cultures of *Ophiorrhiza pumila* were also consistent with the utilization of the non-mevalonate pathway in secologanin biosynthesis.<sup>23</sup> Several enzymes involved in the biosynthesis of IPP-DXP synthase, DXP reductoisomerase, and MEP synthase have been cloned from *Catharanthus roseus*.<sup>24,25</sup> In the first committed step of iridoid terpene biosynthesis, geraniol, derived from IPP, is hydroxylated by geraniol-10-hydroxylase. Geraniol-10-hydroxylase (G10H) has been heterologously expressed in yeast and shown to hydroxylate geraniol *in vitro*.<sup>26,27</sup> Feeding experiments with <sup>3</sup>H-labeled terpene intermediates suggest that 10-hydroxygeraniol, iridodial and iridotrial are intermediates in the secologanin biosynthetic pathway (Scheme 2).<sup>28,29</sup> Oxidation of the iridotrial aldehyde to the carboxylic acid is followed by esterification and glucosylation to yield deoxyloganin; subsequent hydroxylation of deoxyloganin yields loganin. Secologanin is then generated by oxidative cleavage of loganin by the enzyme secologanin synthase (SLS). This NADPH dependent P450 oxidase was isolated from a cDNA library of an alkaloid producing *C. roseus* cell culture,<sup>30</sup> and was shown to convert loganin to secologanin *in vitro*, presumably through a radical mediated reaction mechanism.<sup>31</sup> Recent data from precursor feeding studies suggest that the biosynthesis of secologanin,<sup>32,33</sup> and perhaps the conversion of loganin to

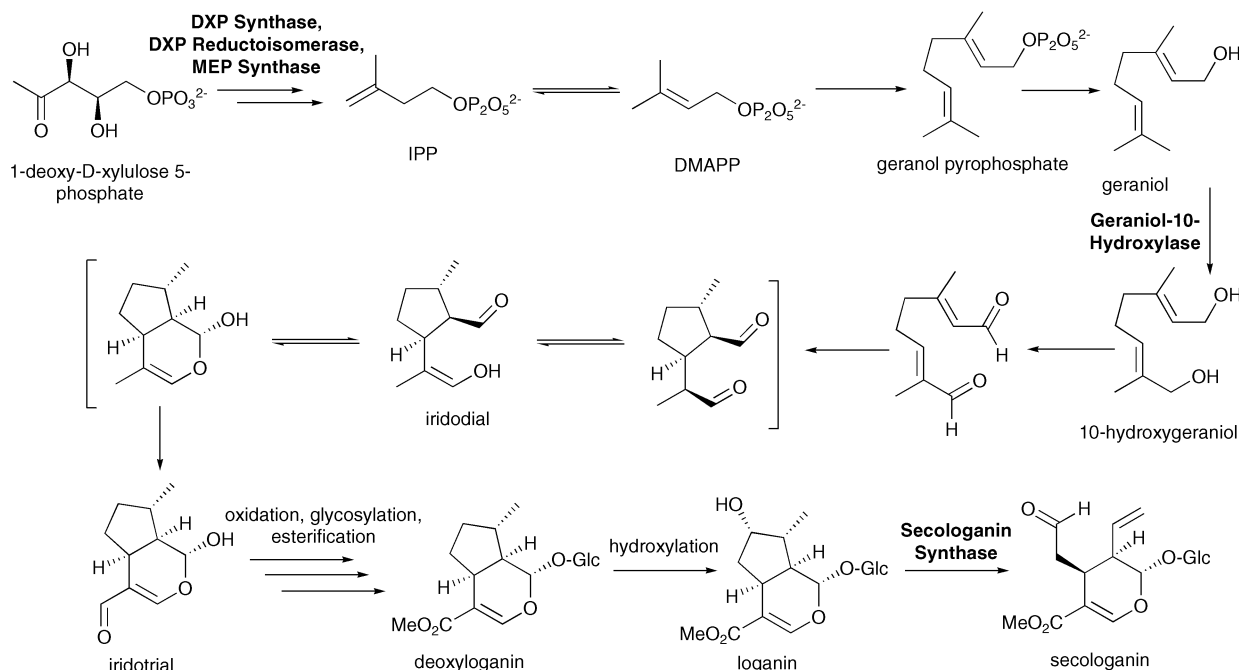
secologanin,<sup>34</sup> may be the rate-limiting step in indole alkaloid biosynthesis. Therefore, overexpression of secologanin synthase (SLS) in alkaloid-producing plants could potentially improve the yield of secologanin-derived alkaloids.

Tryptamine and secologanin are utilized in the first committed step of terpene indole alkaloid biosynthesis. In this step, the enzyme strictosidine synthase catalyzes a stereoselective Pictet–Spengler condensation<sup>35,36</sup> between tryptamine and secologanin to yield strictosidine (iso-vincoside) (*S* stereochemistry at C5, Scheme 1).<sup>37–42</sup>

Strictosidine synthase<sup>43</sup> has been isolated and cloned from the plants *Catharanthus roseus*,<sup>44–49</sup> *Rauwolfia serpentina*,<sup>50–57</sup> and, recently, *O. pumila*.<sup>58</sup> A crystal structure of strictosidine synthase from *R. serpentina* has recently been reported.<sup>59</sup> Notably, a second “Pictet–Spenglerase”, norcoclaurine synthase (involved in tetrahydroisoquinoline biosynthesis in *Thalictrum flavum*), has dramatically different substrate specificity and shows no sequence homology to strictosidine synthase.<sup>60</sup>

Strictosidine synthase tolerates a variety of substitutions on the indole ring of tryptophan, as well as benzofuran and benzothiofene heterocycles.<sup>61</sup> However, tryptophan, phenethylamine, and pyrrole derivatives are not accepted. Although strictosidine synthase does not accept other naturally occurring iridoid aldehydes, the enzyme does accept certain semi-synthetic derivatives of secologanin (Fig. 2).<sup>51,61,62</sup>

The Apocynaceae, Loganiaceae, Rubiaceae and Nyssaceae families of plants each produce terpene indole alkaloids with dramatically diverse structures (Fig. 3). The mechanisms and control of the processes by which strictosidine rearranges into these diverse families of products remain one of the most fascinating problems in secondary metabolism. The following four



**Scheme 2** Biosynthesis of secologanin. The isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) precursors are synthesized by the non-mevalonate pathway. Three of the enzymes involved in IPP and DMAPP synthesis have been cloned from *C. roseus* (DXP synthase, DXP reductoisomerase, MEP synthase). Geraniol is hydroxylated to 10-hydroxygeraniol by geraniol-10-hydroxylase (G10H). Loganin is converted to secologanin by the P450 oxidase, secologanin synthase (SLS).

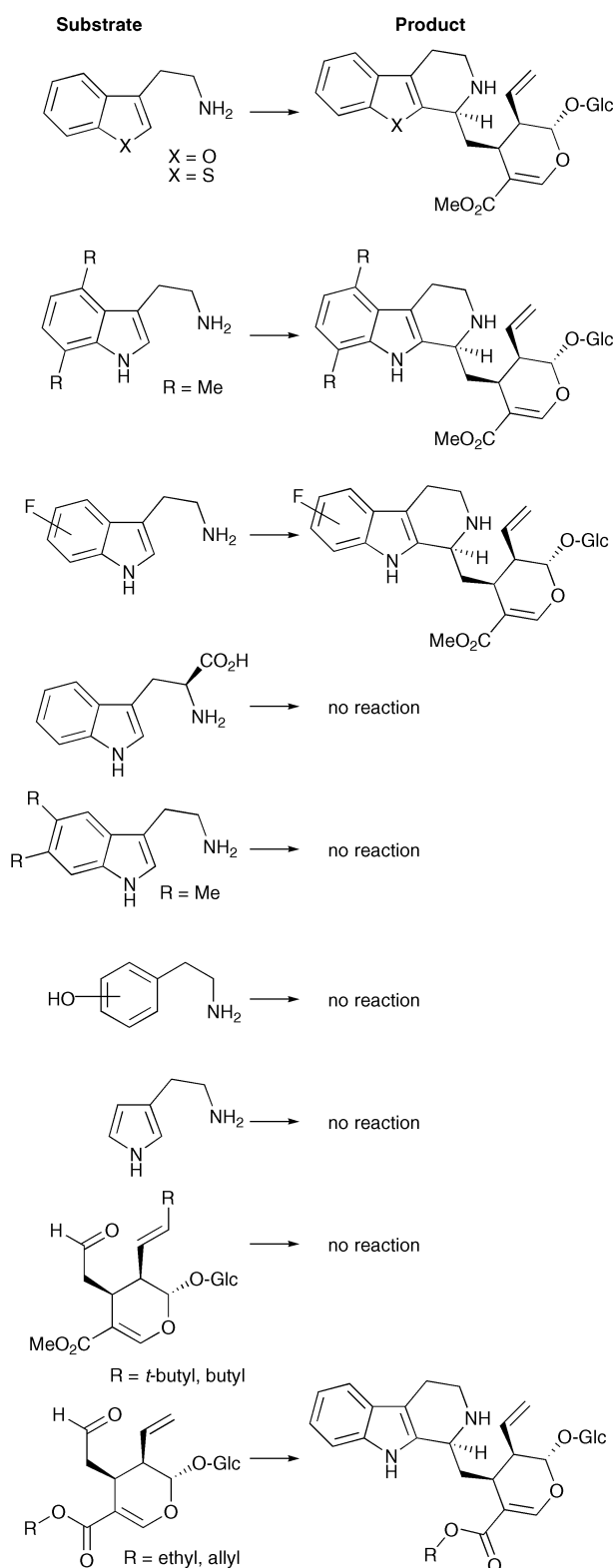


Fig. 2 Substrate specificity of strictosidine synthase.

sections highlight what is known about terpene indole alkaloid biosynthesis downstream of strictosidine formation in *Rauwolfia serpentina*, *Catharanthus roseus*, *Ophiorrhiza pumila*, *Camptotheca acuminata* and *Cinchona*.

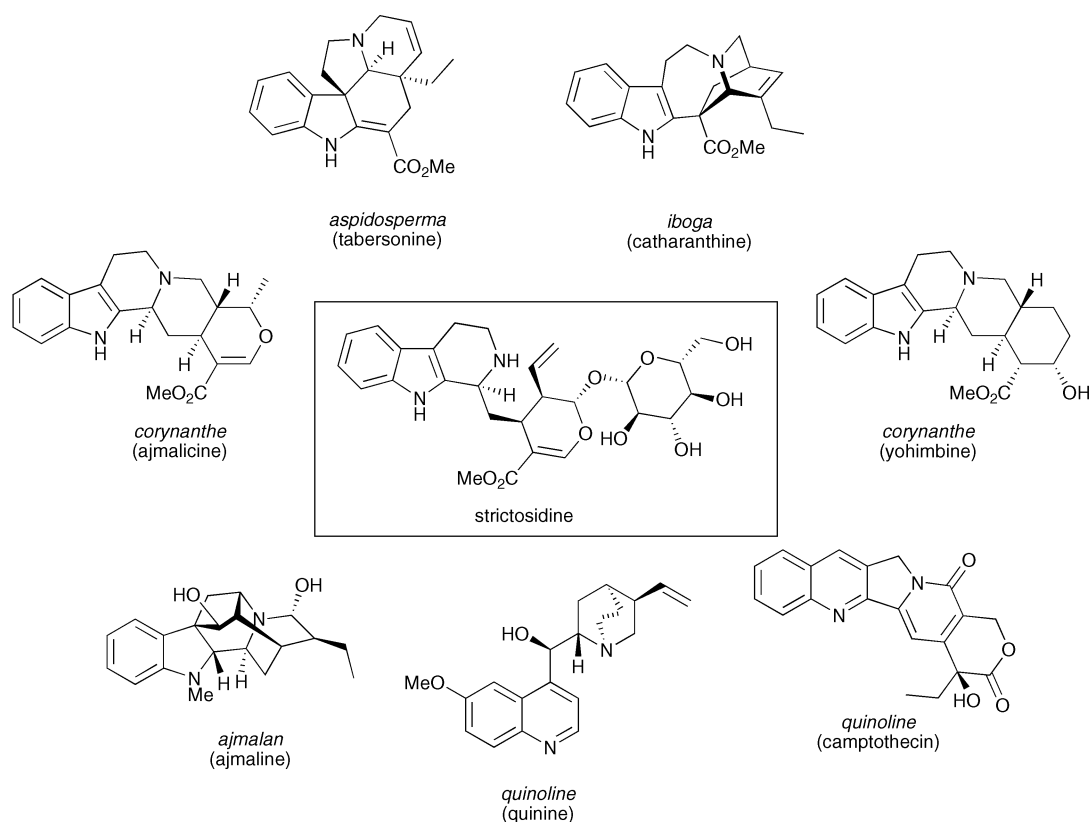
## 2.1 *Rauwolfia serpentina*

*R. serpentina*, along with other *Rauwolfia* species, produces numerous terpene indole alkaloids such as ajmaline, yohimbine and ajmalicine (Fig. 4). Since *Rauwolfia* is slow-growing and difficult to cultivate, experiments in *Rauwolfia* biosynthesis were facilitated by the development of cell suspension and hairy root cultures.<sup>63</sup> The biosynthetic pathway for ajmaline in *Rauwolfia serpentina* is one of the best characterized terpene indole alkaloid pathways. Over the last 10 years, remarkable progress has been made in identifying the enzymes responsible for ajmaline biosynthesis. Much of this progress has been detailed in a recent extensive review.<sup>63</sup>

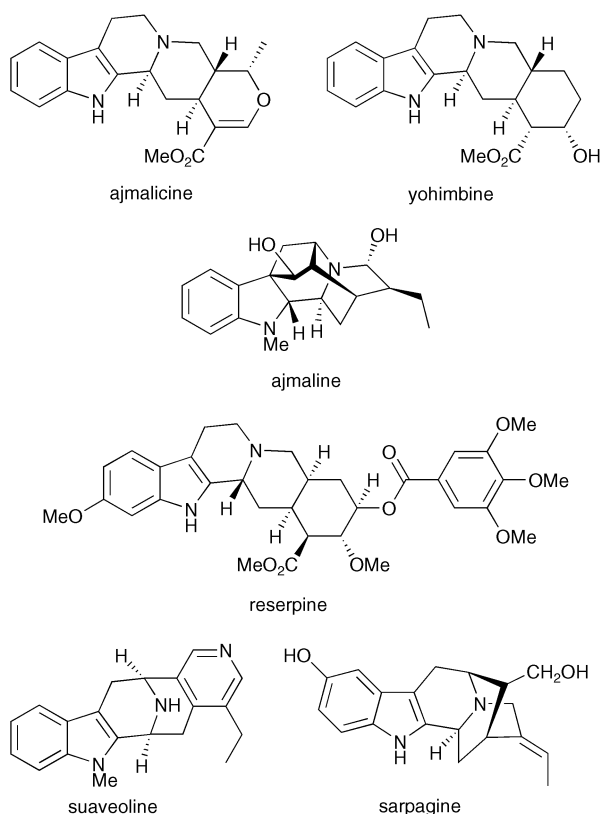
**2.1.1 Sarpagan and ajmalan type: ajmaline.** Like all other terpene indole alkaloids ajmaline, an antiarrhythmic drug with potent sodium channel blocking properties,<sup>64</sup> is derived from strictosidine. Strictosidine is deglycosylated by a dedicated  $\beta$ -glucosidase, converting it to a reactive hemiacetal intermediate.<sup>65</sup> This hemiacetal opens to form a dialdehyde intermediate, which then reacts with the secondary amine of the strictosidine framework to yield 4,21-dehydrocorynantheine aldehyde (Scheme 3). Alternatively, this dialdehyde can form vallesiachotamine,<sup>66</sup> a less common intermediate of terpene indole alkaloid biosynthesis. Dehydrocorynantheine aldehyde then undergoes allylic isomerization and enolization to produce either the enol or keto forms of dehydrogeissoschizine. The enol form of dehydrogeissoschizine undergoes 1,4 conjugate addition to produce the heteroyohimbine cathenamine.<sup>67</sup> Cathenamine and dehydrogeissoschizine have been observed in equilibrium *in vitro*.<sup>68,69</sup>

The dedicated glucosidase, strictosidine- $\beta$ -glucosidase, has been cloned from *Rauwolfia serpentina*.<sup>65</sup> Substrates structurally similar to strictosidine were accepted by this glucosidase, though the diastereomer of strictosidine, vincoside (*R* stereochemistry at C5, see Scheme 1), was not. Trapping experiments with this glucosidase and hydride reducing agents further support that dehydrocorynantheine aldehyde and cathenamine are observed in this reaction pathway (Fig. 5A). Deglycosylation of *N*- $\beta$ -methyl strictosidine (dolichantoside) yielded the product 3-isocorreaentine A, suggesting that reaction of the aldehyde with the amine to form dehydrocorynantheine aldehyde occurs after product release from the enzyme (Fig. 5B). Therefore, the rearrangements that occur after strictosidine deglycosylation are most likely spontaneous, or substrate driven. In essence, the glucose moiety serves as a protecting group to mask a reactive species, a strategy that is utilized in other plant natural products such as the cyanogenic glucosides and the glucosinolates.<sup>70</sup> Strictosidine is also deglycosylated by non-specific bacterial glucosidases.<sup>66</sup> A crystal structure of strictosidine- $\beta$ -glucosidase enzyme from *R. serpentina* is currently in progress.<sup>71</sup>

At least eight enzymes are predicted to catalyze the subsequent steps of ajmaline biosynthesis that occur after strictosidine deglycosylation (Scheme 4A). The sarpagan type alkaloid, polyneuridine aldehyde, is a known early intermediate of the ajmaline pathway. Feeding experiments suggest 4,21-dehydrogeissoschizine may be a precursor for polyneuridine aldehyde.<sup>72</sup> A possible mechanism by which the sarpagan bridge enzyme transforms an isomer of 4,21-dehydrogeissoschizine to polyneuridine aldehyde is shown.<sup>73</sup> However, detailed study of the sarpagan bridge enzyme is necessary before a mechanism of biosynthesis can be proposed.<sup>63</sup>



**Fig. 3** Major classes of terpene indole alkaloids derived from strictosidine.

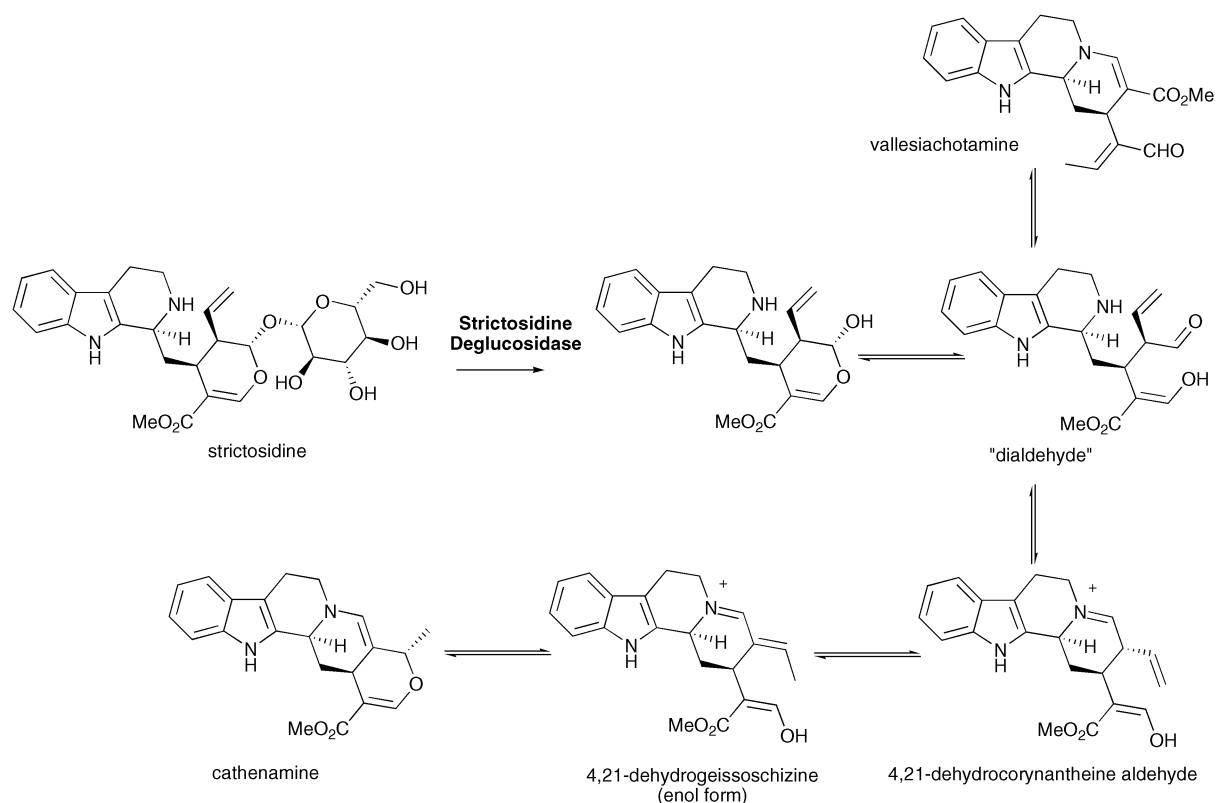


**Fig. 4** Representative alkaloids of *Rauwolfia serpentina*.

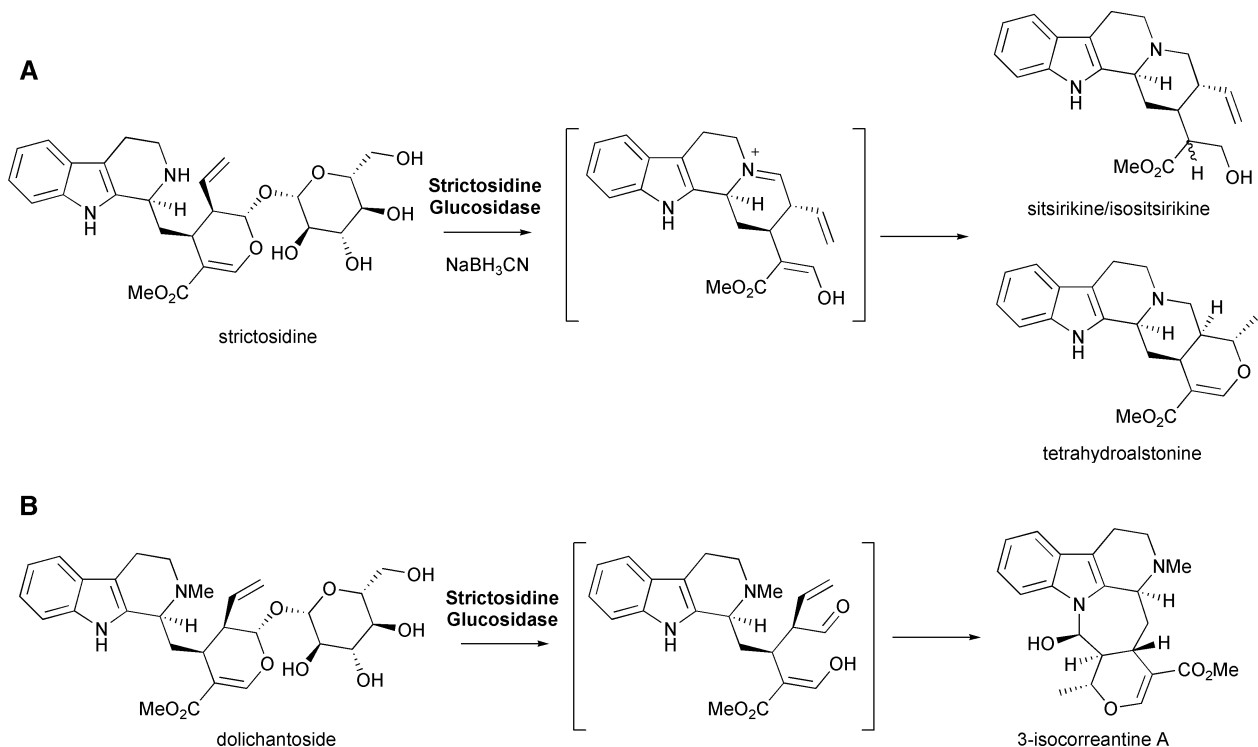
A membrane-protein fraction of an *R. serpentina* extract transformed labeled strictosidine into sarpgan type alkaloids. The enzyme activity was shown to be dependent on NADPH and molecular oxygen, suggesting that the sarpgan bridge enzyme may be a cytochrome P450 enzyme.<sup>63,74</sup> Isolation of this enzyme will yield further insight into this key step that commits the deglycosylated strictosidine intermediate to the sarpgan and ajmalan type alkaloid pathways.

The next steps of ajmaline biosynthesis are well characterized at the enzymatic level (Scheme 4A). Polyneuridine aldehyde esterase hydrolyzes the polyneuridine aldehyde methyl ester, generating an acid which decarboxylates, perhaps spontaneously, to yield epi-vellosamine. Purification of this esterase from *Rauwolfia* cell cultures and sequencing of protein fragments enabled a clone of polyneuridine aldehyde esterase to be isolated from a *Rauwolfia* cDNA library. This enzyme has been overexpressed in *E. coli* and subjected to detailed mechanistic studies. Polyneuridine aldehyde esterase appears to be a member of the  $\alpha/\beta$  hydrolase super family and contains a Ser, His, Asp catalytic triad.<sup>75–78</sup> Site directed mutagenesis indicates that each residue of the catalytic triad is required for activity.

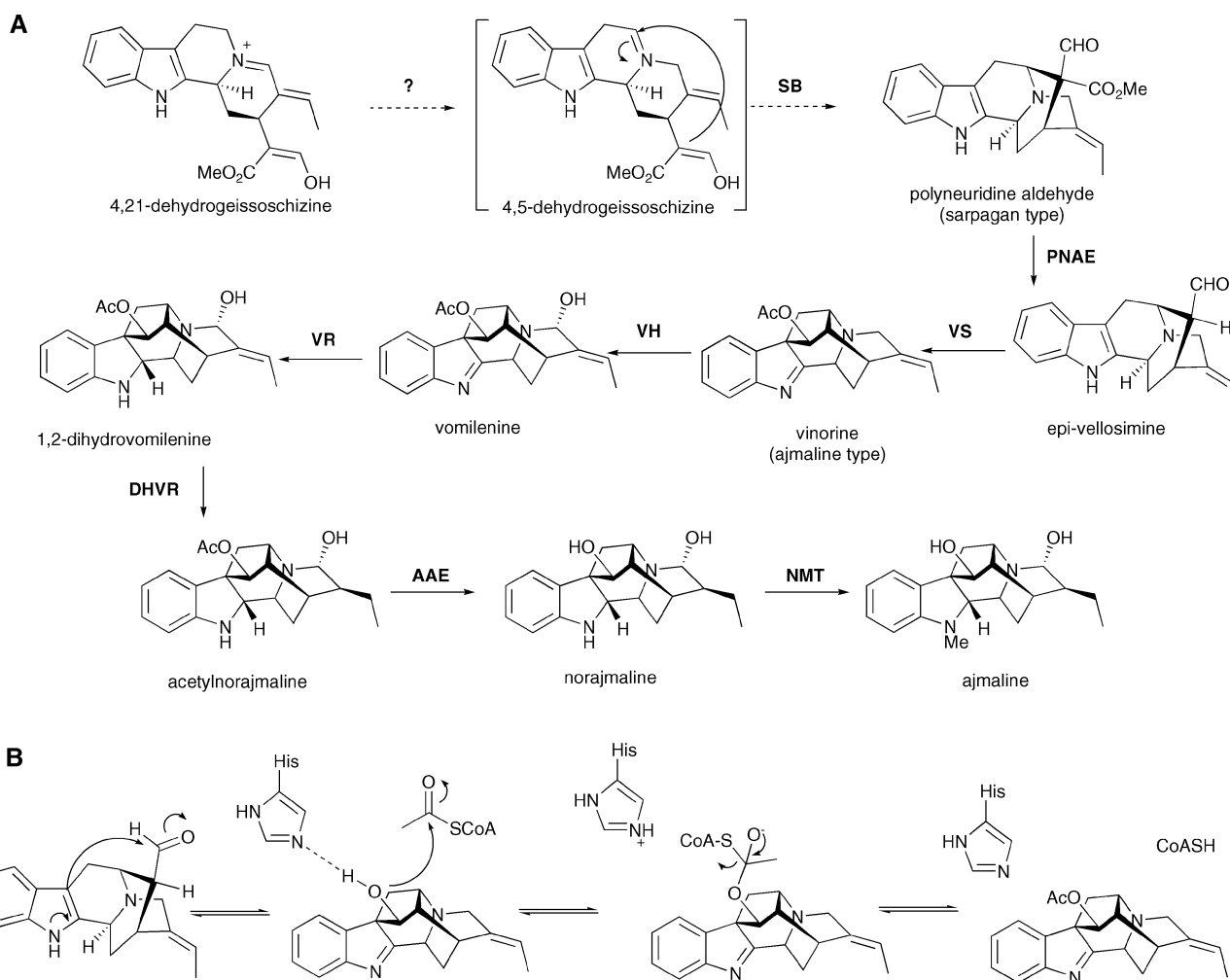
In the next step of the ajmaline pathway, vinorine synthase transforms the sarpgan alkaloid epi-vellosamine to the ajmalan alkaloid vinorine.<sup>79</sup> Vinorine synthase is therefore responsible for constructing the ajmalan backbone from a sarpgan type intermediate. Vinorine synthase has also been purified from *Rauwolfia* cell culture, subjected to protein sequencing and cloned from a cDNA library.<sup>80,81</sup> The enzyme, which appears to be an acetyl transferase homologue, has been heterologously expressed



**Scheme 3** Deglycosylation of strictosidine reveals a reactive intermediate.



**Fig. 5** (A) Products isolated after incubation of strictosidine glucosidase and  $\text{NaBH}_3\text{CN}$ . (B) The product resulting from deglycosylation of dolichantaside.



**Scheme 4** (A) Ajmaline biosynthesis from deglycosylated strictosidine. The mechanism of polyneuridine formation remains unclear; one possible mechanism is shown.<sup>73</sup> SB, sarpagan bridge enzyme; PNAE, polyneuridine aldehyde reductase; VS, vinorine synthase; VH, vinorine hydroxylase; VR, vomilenine reductase; DHVR, dihydrovomilenine reductase; AAE, 17-*O*-acetyl-ajmalanesterase; NMT, norajmaline-*N*-methyltransferase. (B) Proposed mechanism for the formation of the ajmalan backbone from the sarpagan alkaloid polyneuridine aldehyde.

in *E. coli*. Crystallization and site directed mutagenesis studies of this protein have led to the proposed mechanism of catalysis shown in Scheme 4B.<sup>82</sup>

Vinorine hydroxylase then hydroxylates vinorine to vomilenine.<sup>83</sup> Vinorine hydroxylase is a P450 enzyme that requires an NADPH dependent reductase. Since this P450 enzyme could not be purified directly from plant material in active form, seven full length cytochrome P450 clones were isolated from a *Rauwolfia* cDNA library by homology cloning and then heterologously expressed in combination with a *Rauwolfia* reductase. None of these clones exhibited vinorine hydroxylation activity, though expression of these clones in different cell systems may prove more successful.<sup>63</sup>

Two reduction steps follow the formation of vomilenine. First, the indolenine bond is reduced by an NADPH dependent reductase to yield 1,2-dihydrovomilenine. A second enzyme, 1,2-dihydrovomilenine reductase, then reduces this product to acetylnorajmaline. Partial protein sequences have been obtained for both of the purified reductases. Although several putative clones encoding these proteins have been isolated, the activity of these clones has not been verified.<sup>63,84,85</sup>

An acetyltransferase then hydrolyzes the acetyl linkage of acetylnorajmaline to yield norajmaline. This esterase was purified from *R. serpentina* cell suspension cultures, partial amino acid sequences were obtained and a full length clone was isolated from a cDNA library. Expression of the gene in tobacco leaves successfully yielded protein with the expected enzymatic activity.<sup>86</sup>

In the final step of ajmaline biosynthesis, an *N*-methyl transferase introduces a methyl group at the indole nitrogen of norajmaline. Although this enzymatic activity has been detected in crude cell extracts, the enzyme has not been further characterized.<sup>87</sup>

In summary, the enzymatic activities for all steps of ajmaline biosynthesis have been detected. Five of the enzymes, strictosidine synthase, strictosidine glucosidase, polyneuridine aldehyde esterase, vinorine synthase and 17-*O*-acetyl-ajmalanesterase have been cloned. Putative clones for vinorine hydroxylase, vomilenine reductase, and 1,2-dihydrovomilenine reductase have been isolated. *N*-Methyl-transferase activity and sarpagan bridge enzyme activities have only been detected in crude cell extracts.

**2.1.2 Yohimbine.** Yohimbine, also isolated from *Rauwolfia*, is an  $\alpha 2$ -adrenoceptor antagonist with potential clinical applications in erectile dysfunction.<sup>88</sup> The enzymes that convert deglycosylated strictosidine to yohimbine have not been identified. However, a direct biosynthetic route may involve homoallylic isomerization of the keto dehydrogeissoschizine followed by 1,4 conjugate addition (Scheme 5).<sup>89</sup>

## 2.2 *Catharanthus roseus*

*C. roseus* is a rich source of terpene indole alkaloids, and a list of alkaloids produced by *C. roseus* has been compiled.<sup>90</sup> A diversity of alkaloids including aspidosperma, corynanthe, iboga, and bisindole types have each been isolated from this single plant (Fig. 6). Therefore, *C. roseus* is a system well suited to mechanistic study of the divergence of multiple alkaloid families from the common intermediate strictosidine. Since the 1960's, the biosynthetic pathways responsible for alkaloid production in *C. roseus* have been studied in mature plants, seedlings, cell cultures, and hairy roots (see references below).

As in *Rauwolfia*, strictosidine is deglycosylated by a dedicated glucosidase. This glucosidase from *C. roseus* has been isolated and cloned,<sup>91–94</sup> and it shares high sequence homology with the *Rauwolfia* enzyme described in Section 2.1. Both glucosidase enzymes yield cathenamine from strictosidine *in vitro*, suggesting that the downstream divergence into different alkaloid classes is not glucosidase dependent in *C. roseus* and *R. serpentina* plants.<sup>65,91</sup> Strictosidine glucosidase enzyme from *C. roseus* has broad enough substrate specificity to deglycosylate the enzymatically generated strictosidine analogues shown in Fig. 2.<sup>61,62</sup>

**2.2.1 Corynanthe type: ajmalicine, tetrahydroalstonine and serpentine.** The heteroyohimbine cathenamine is the major product isolated after reaction of strictosidine with strictosidine- $\beta$ -glucosidase *in vitro*.<sup>65,91</sup> A pathway to the corynanthe skeleton from deglycosylated strictosidine might simply entail reduction of the cathenamine intermediate.<sup>67,95–100</sup> A partially purified NADPH-dependent reductase isolated from a tetrahydroalstonine producing *C. roseus* cell line, catalyzed the conversion of cathenamine to tetrahydroalstonine *in vitro* (Scheme 6).<sup>101</sup> A second *C. roseus* cell line yielded an additional reductase that produces ajmalicine and

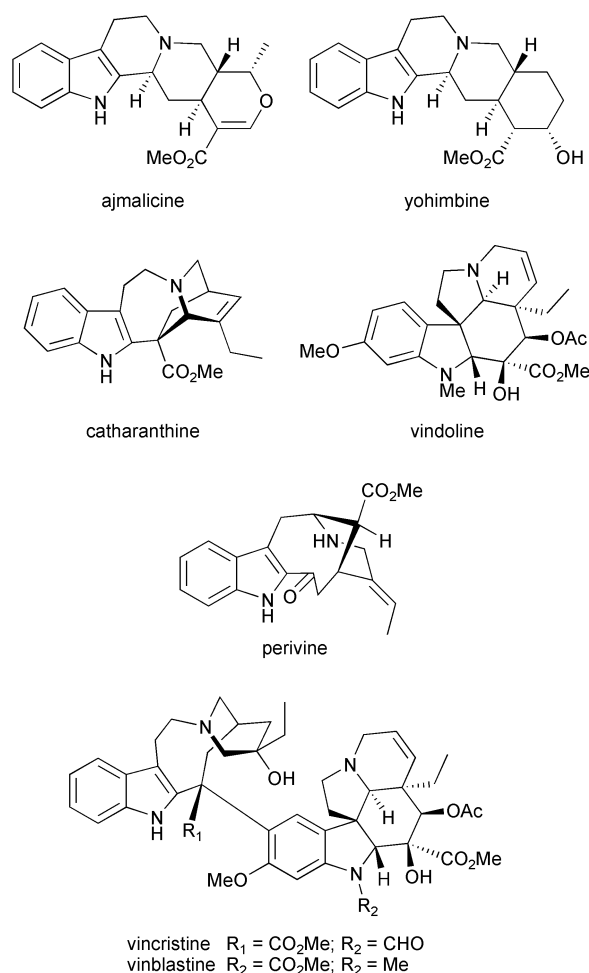
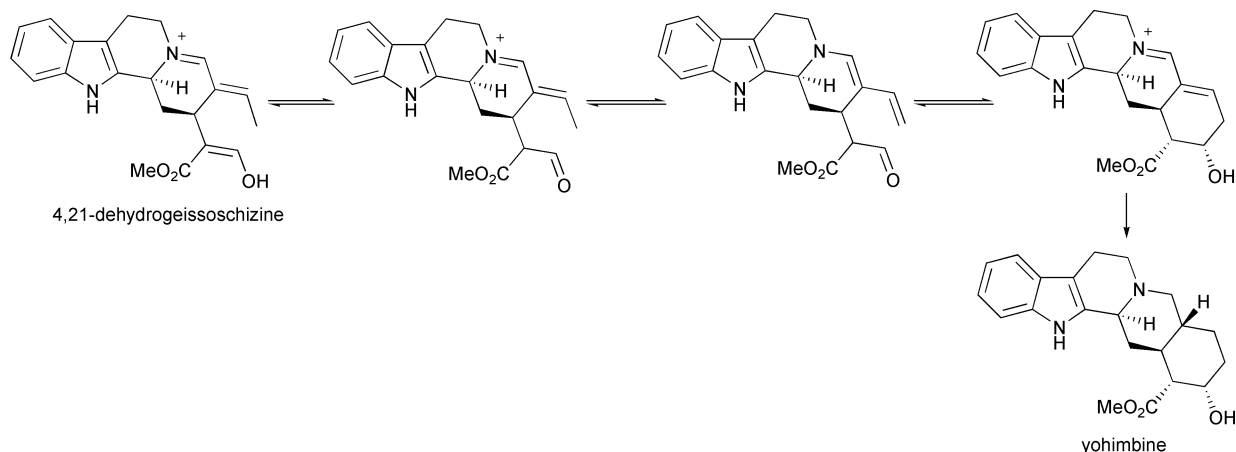


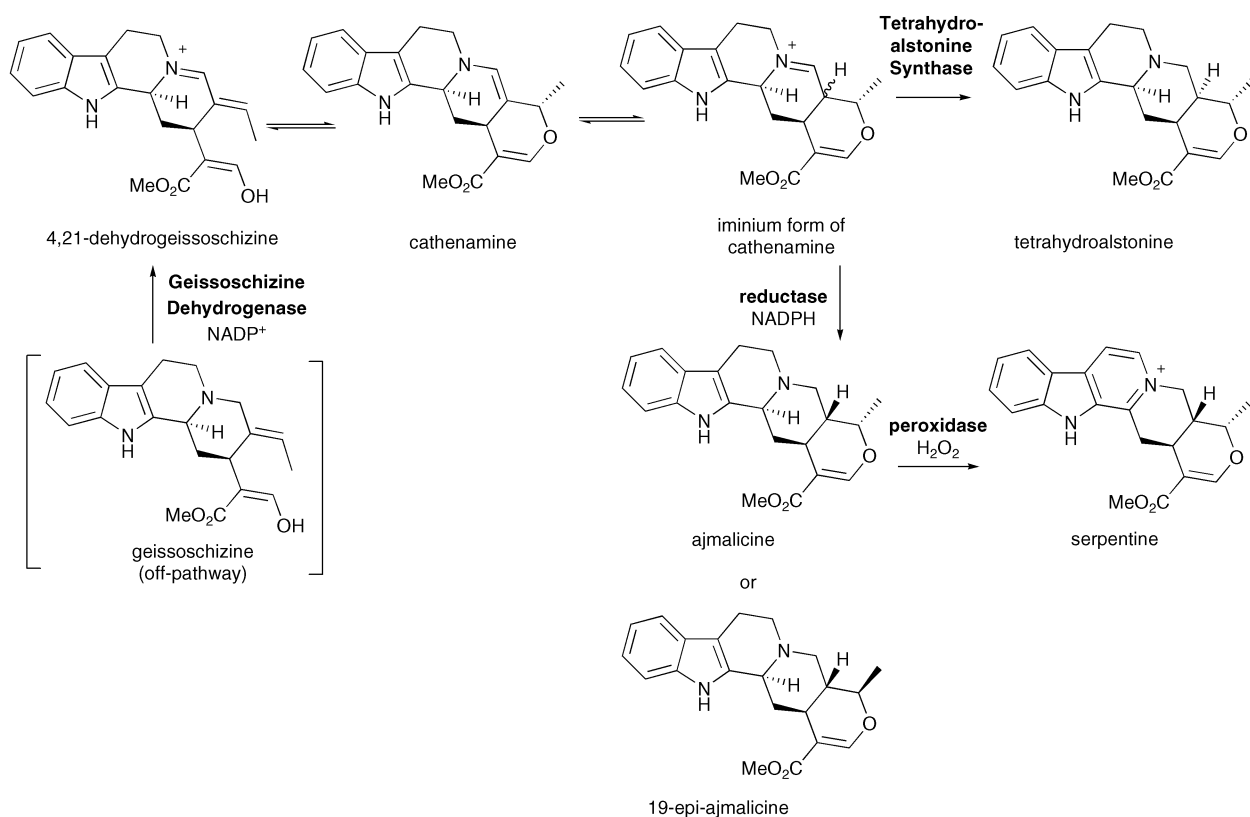
Fig. 6 Representative alkaloids of *Catharanthus roseus*.

19-epi-ajmalicine from cathenamine (Scheme 6). Labeling studies performed with crude *C. roseus* cell extracts in the presence of D<sub>2</sub>O or NADPD support a mechanism in which the reductase acts on the iminium form of cathenamine.<sup>102</sup> Although some early feeding studies suggested that geissoschizine, the reduced form of 4,21-dehydrogeissoschizine, is the precursor for the



Scheme 5 Proposed yohimbine biosynthetic pathway.





**Scheme 6** Corynanthe biosynthesis from dehydrogeissoschizine.

corynanthe alkaloids, subsequent data indicate that dehydrogeissoschizine is in fact the central intermediate for corynanthe alkaloid biosynthesis (Scheme 6).<sup>103,104</sup> A dehydrogenase enzyme isolated from *C. roseus* catalyzes the oxidation of geissoschizine to dehydrogeissoschizine, indicating that conversion of geissoschizine to dehydrogeissoschizine occurs *in vivo*.<sup>105</sup> Ajmalicine has been further oxidized to yield serpentine *in vitro*. Although a dedicated enzyme responsible for this oxidation has not been cloned, conversion of ajmalicine to serpentine from peroxidases present in the plant vacuoles has been observed.<sup>106,107</sup> Even though these pathways were elucidated in *C. roseus*, ajmalicine and serpentine have been isolated from *Rauwolfia* species as well, and are presumably produced by similar mechanisms.

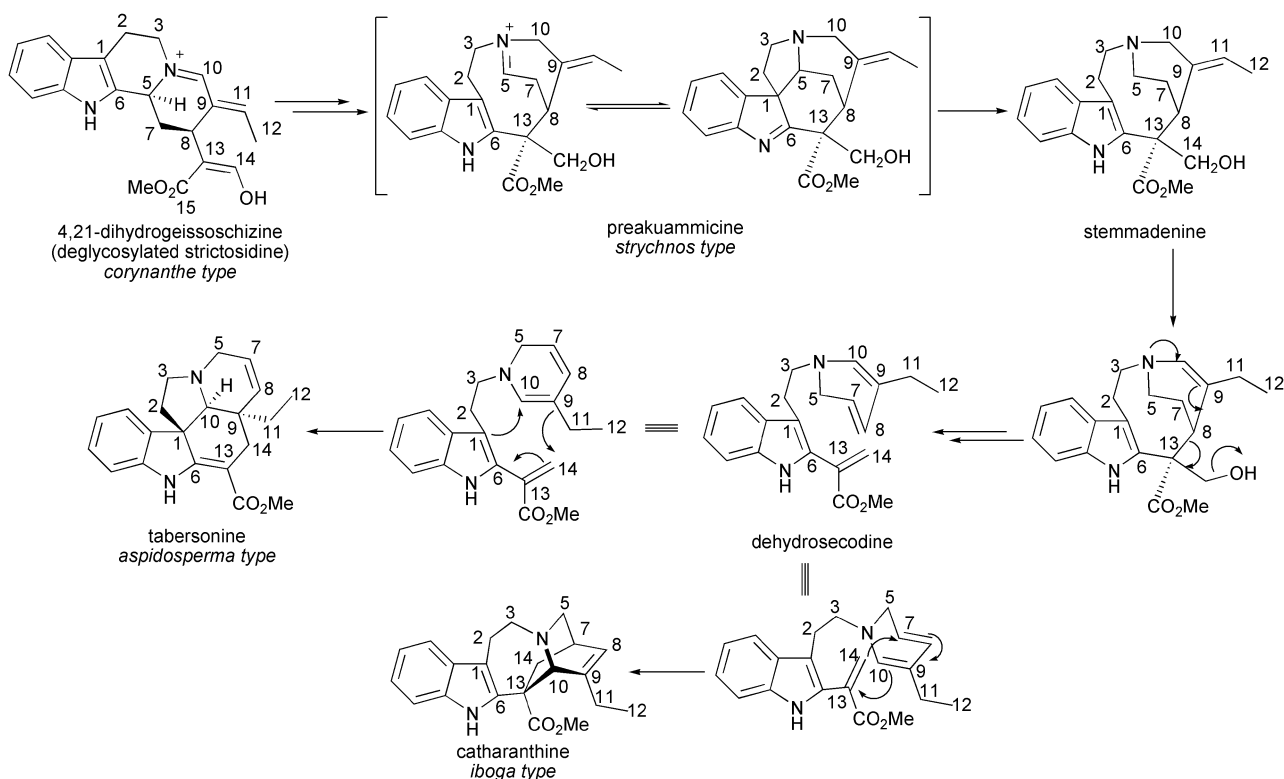
The corynanthe alkaloids display numerous biological activities. Ajmalicine (raubasine) affects smooth muscle function and is used to help prevent strokes,<sup>108</sup> serpentine is a type II topoisomerase inhibitor,<sup>109</sup> and tetrahydroalstonine exhibits antipsychotic properties.<sup>110</sup>

**2.2.2 Strychnos, aspidosperma, and iboga type: preakuammicine, vindoline, catharanthine.** It is believed that the structurally more complex aspidosperma, iboga, and strychnos alkaloids are derivatives of the corynanthe alkaloids.<sup>111</sup> This hypothesis is indirectly supported by observation that the corynanthe alkaloids are produced early in the lifetime of the *Catharanthus roseus* plant, while the aspidosperma and iboga alkaloids appear mainly in older plants.<sup>111</sup> Studies by numerous groups in the 1960's and 1970's enabled detailed proposals of the interrelationships and biosynthetic pathways for the strychnos, iboga, and aspidosperma

type alkaloids in *C. roseus* (Scheme 7). (Some key references are listed.<sup>112–122</sup>) These proposed pathways are based on feeding studies of isotopically labeled substrates to seedlings or shoots, isolation of discrete intermediates from plant materials, and from biomimetic model reactions. However, no enzymes responsible for the construction of the strychnos, aspidosperma, or iboga backbones are known.

The strictosidine derivative preakuammicine (strychnos-type intermediate) is the common precursor for the aspidosperma, strychnos and iboga alkaloids. Although several mechanisms to explain the formation of preakuammicine from geissoschizine have been proposed,<sup>123,124</sup> the actual mechanism and physiological precursor for preakuammicine remain unknown (summarized in ref. 123). Due to its lability, preakuammicine has not been isolated from plant material. Reduction of preakuammicine yields stemmadenine, a productive intermediate in the pathway. *C. roseus* cell cultures rapidly consume stemmadenine present in cell culture media.<sup>68</sup> Stemmadenine rearranges to form the acrylic ester dehydrosecodine<sup>125–127</sup> which serves as a common intermediate for the aspidosperma and the iboga skeletons. Although it is possible that the iboga type alkaloid catharanthine and the aspidosperma type alkaloid tabersonine are formed from a Diels–Alder reaction of dehydrosecodine, there is no evidence for this reaction in the plant.<sup>128</sup> Some of these findings are reviewed in references.<sup>129–135</sup>

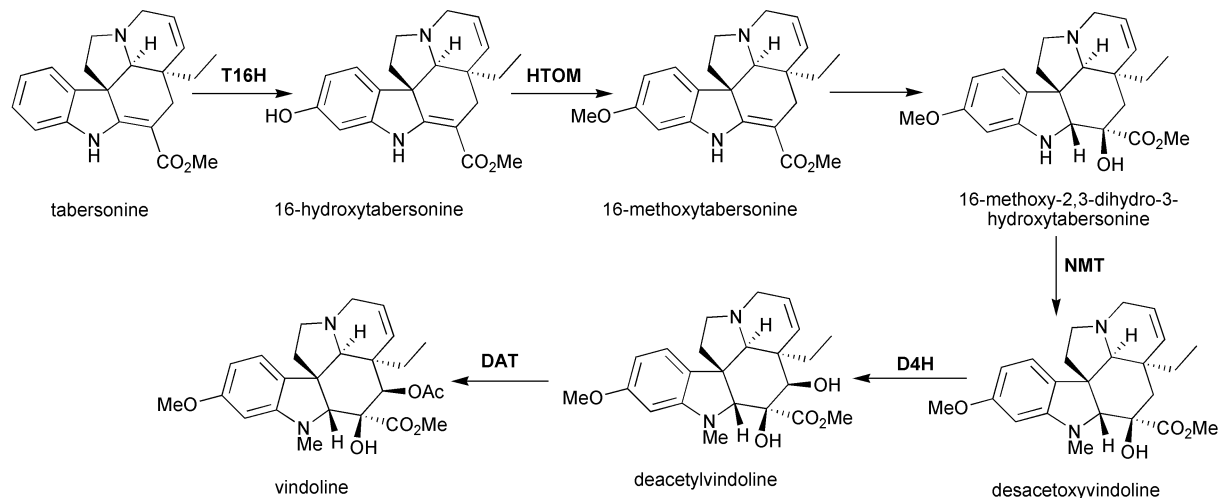
More details are known about the six steps that catalyze the elaboration of tabersonine to vindoline (Scheme 8).<sup>6,136</sup> The cytochrome P450 monooxygenase (tabersonine-16-hydroxylase, T16H) responsible for hydroxylating tabersonine to 16-hydroxy-tabersonine in the first step of this sequence has been cloned.<sup>137,138</sup>



**Scheme 7** Proposed biosynthetic pathway of aspidosperma and iboga alkaloids. No enzymatic information is available for the steps proposed in this scheme.

This hydroxyl group is then methylated by a SAM dependent *O*-methyltransferase to yield 16-methoxy-tabersonine; this enzyme (16-hydroxytabersonine-16-*O*-methyltransferase, HTOM) has been purified, but not cloned.<sup>139</sup> In the next step, hydration of a double bond by an uncharacterized enzyme produces 16-methoxy-2,3-dihydro-3-hydroxytabersonine. Transfer of a methyl group to the indole nitrogen by an *N*-methyl transferase (NMT) yields desacetoxyvindoline. This methyl transferase activity has

been detected only in differentiated plants, not in plant cell cultures.<sup>140</sup> The penultimate intermediate, deacetylvindoline, is produced by the action of the 2-oxoglutarate-dependent dioxygenase desacetoxyvindoline 4-hydroxylase (D4H). This enzyme has been cloned and is also absent from plant cell cultures.<sup>141</sup> In the last step, deacetylvindoline is acetylated by deacetylvindoline *O*-acetyltransferase (DAT). This enzyme, also absent from non-differentiated plant material, has been successfully cloned.<sup>142</sup>



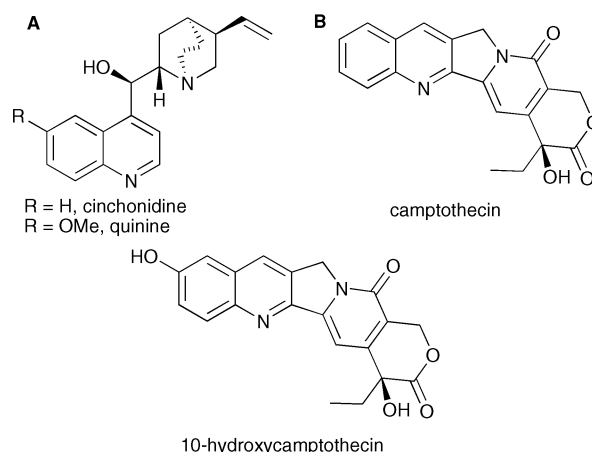
**Scheme 8** Vindoline biosynthesis from tabersonine. T16H, tabersonine-16-hydroxylase; HTOM, 16-hydroxytabersonine 16-*O*-methyltransferase; NMT, *N*-methyltransferase; D4H, desacetoxyvindoline 4-hydroxylase; DAT, deacetylvindoline *O*-acetyltransferase. Tabersonine 16-hydroxylase, desacetoxyvindoline 4-hydroxylase and deacetylvindoline *O*-acetyltransferase have been cloned.

**2.2.3 Bisindole type: vinblastine.** Vinblastine and the structurally related vincristine are highly effective anti-cancer agents currently used clinically against leukemia, Hodgkin's lymphoma and other cancers.<sup>143,144</sup> Early feeding experiments with isotopically labeled loganin also indicated that the bisindole alkaloids belong to the terpene indole alkaloid family.<sup>145</sup> Inspection of these bisindole alkaloids suggests that they are derived from dimerization of vindoline and catharanthine. The dimerization of catharanthine and vindoline is believed to proceed *via* the formation of an iminium intermediate with catharanthine (Scheme 9). This iminium intermediate is reduced to form anhydrovinblastine, a naturally occurring compound in *C. roseus* plants.<sup>146</sup> In support of this mechanism, anhydrovinblastine is incorporated into vinblastine and vincristine in feeding studies with cell free extracts.<sup>106,147–149</sup>

Peroxidase-containing fractions of plant extracts were found to catalyze the formation of the bisindole dehydrovinblastine from catharanthine and vindoline.<sup>150,151</sup> The peroxidase CRPRX1 ( $\alpha$ -3',4'-anhydrovinblastine synthase), purified and cloned from *C. roseus* leaves, has been demonstrated to convert vindoline and catharanthine to anhydrovinblastine *in vitro*.<sup>152,153</sup> It is proposed that catharanthine is oxidized to an iminium ion, which then reacts with the relatively nucleophilic vindoline.<sup>154</sup> Although this peroxidase is not highly substrate specific for catharanthine and vindoline, localization studies suggest that CRPRX1 is the dedicated peroxidase required for bisindole formation.<sup>106,153,154</sup> Finally, after formation of dehydrovinblastine, hydroxylation of the double bond yields vinblastine, and oxidation of the *N*-methyl group yields vincristine.

## 2.3 *Ophiorrhiza pumila*, *Camptotheca acuminata*

**2.3.1 Quinoline type: camptothecin.** *Ophiorrhiza pumila* and *Camptotheca acuminata* both produce the quinoline alkaloid camptothecin (Fig. 7). Camptothecin is a topoisomerase inhibitor and analogues of this compound are used as anti-cancer agents. The biological activity and biosynthesis of camptothecin has been recently reviewed.<sup>155</sup>

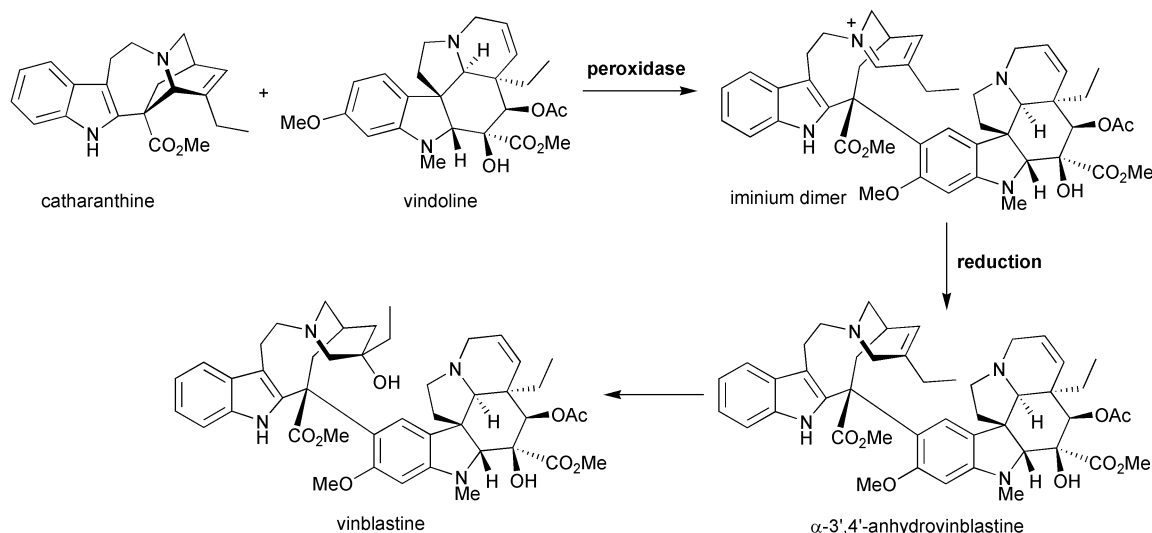


**Fig. 7** Representative quinoline alkaloids from (A) *Ophiorrhiza pumila* and *Camptotheca acuminata* and (B) *Cinchona*.

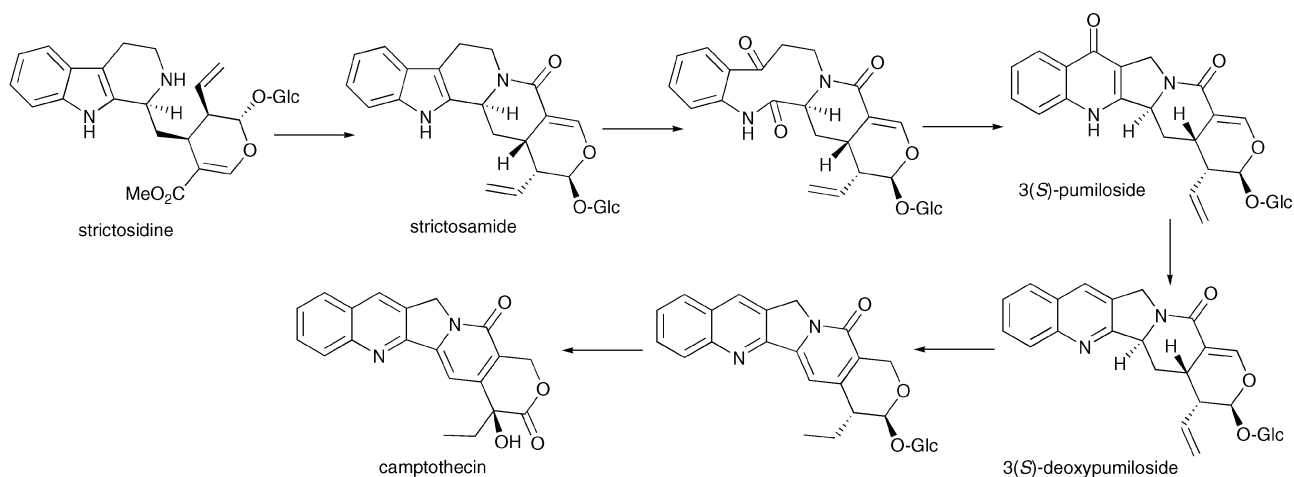
Although camptothecin is a quinoline alkaloid lacking the basic indole structure, early proposals suggested that camptothecin might be part of the terpene indole alkaloid family.<sup>156</sup> Feeding of labeled tryptamine<sup>157</sup> and strictosidine to *C. acuminata* plants verified this hypothesis, and established these compounds as intermediates in the camptothecin pathway.<sup>158</sup> As noted in Section 2, *O. pumila* strictosidine synthase has recently been cloned from hairy root cultures.<sup>58,159</sup>

The biosynthesis of camptothecin is unique among terpene indole alkaloids because strictosidine is not immediately deglycosylated. Instead, a lactam is formed between the amine of strictosidine and the methyl ester derived from the secologanin moiety to yield the intermediate strictosamide (Scheme 10). Incorporation of labeled strictosamide into camptothecin validates that strictosamide is a productive intermediate.<sup>160,161</sup>

The steps following strictosamide formation remain somewhat speculative. A series of chemically reasonable transformations have been proposed (Scheme 10), though there is little experimental evidence for these steps.<sup>160,162</sup> However, two of these



**Scheme 9** Dimerization of vindoline and catharanthine by the peroxidase  $\alpha$ -3',4'-anhydrovinblastine synthase to produce vinblastine.



Scheme 10 Camptothecin biosynthesis.

potential biosynthetic intermediates, 3(*S*)-pumiloside and 3(*S*)-deoxypulminoside, have been isolated from *O. pumila*.<sup>163–165</sup>

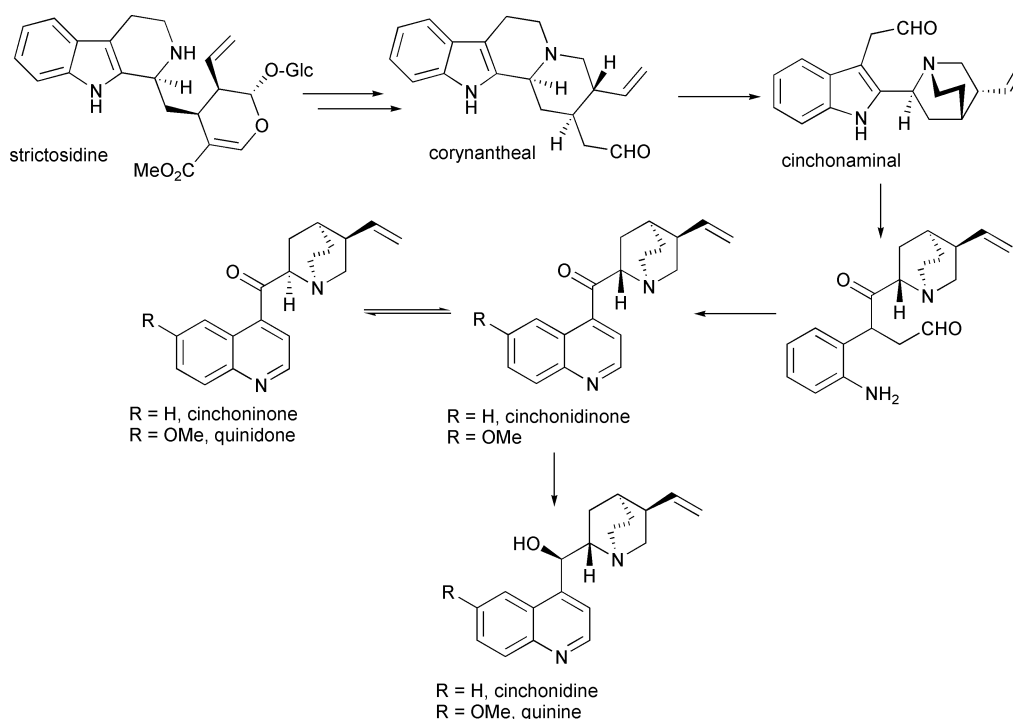
## 2.4 Cinchona

**2.4.1 Quinoline type: quinine.** A variety of quinoline alkaloids are produced by the *Cinchona* species (*C. robusta*, *C. officinalis*, *C. ledgeriana*) (Fig. 7).<sup>166</sup> Quinine, the best known of the Cinchona alkaloids, is a highly effective anti-malarial agent.<sup>167</sup> Feeding studies with radiolabeled tryptophan,<sup>168–170</sup> monoterpenes,<sup>168,171–174</sup> and strictosidine<sup>170</sup> indicate that the *Cinchona* quinoline alkaloids are derived from strictosidine. Strictosidine synthase has been purified from cell cultures of *C. robusta*.<sup>175</sup>

and strictosidine glucosidase activity in *C. robusta* has been detected.<sup>176</sup>

Feeding experiments in *C. ledgeriana* with labeled precursors have led to a proposed biosynthetic pathway (Scheme 11).<sup>177,178</sup> Incorporation of the corynantheal intermediate shown in Scheme 11 suggests that the methoxycarbonyl group is lost at an early stage.

Two isoforms of one enzyme involved in the later stages of the quinine biosynthetic pathway have been purified from cell suspension cultures of *C. ledgeriana*.<sup>179</sup> One isoform of this NADPH-dependent oxidoreductase catalyzes the reduction of cinchoninone (which equilibrates with its epimer cinchonidinone) to give a mixture of cinchonine and cinchonidine, while a



Scheme 11 Proposed quinine biosynthetic pathway.

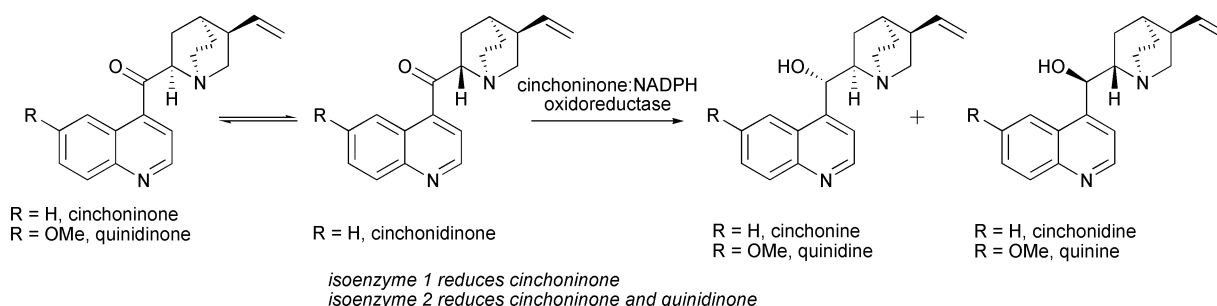


Fig. 8 Oxidoreductase involved in quinoline alkaloid biosynthesis.

second isoform catalyzes the reduction of both cinchoninone and quinidinone (Fig. 8).

### 3 Localization of terpene indole alkaloid biosynthetic enzymes

Localization of biosynthetic enzymes is an important mechanism of control in plant pathways.<sup>180</sup> Enzyme localization in terpene indole alkaloid biosynthesis has been extensively studied in *C. roseus* (reviewed in ref. 5,180). The coexistence of multiple pathways—corynanthe, aspidosperma and iboga—makes *Catharanthus* an intriguing system to monitor the localization of biosynthetic enzymes. For enzymes where the gene sequence is known, the localization is typically deduced by immunolocalization or *in situ* RNA hybridization. Alternatively, enzyme activity can be localized to a particular cellular compartment based on isolation of a given cellular organelle.

Enzymes of plant secondary metabolic pathways are localized to separate subcellular compartments and expression levels vary by tissue and cell type. At the cellular level, enzymes may be found in the cytosol, endoplasmic reticulum, vacuole or chloroplast (or plastid). The compartmentalization of terpene indole alkaloid biosynthesis is no exception (Fig. 9).<sup>181</sup>

As with all terpenoids derived from non-mevalonate pathways, secologanin biosynthesis (Scheme 2) begins in the plastid where the geranyl intermediate is generated.<sup>21</sup> Geraniol is then exported to the cytosol where it is hydroxylated by geraniol-10-hydroxylase (G10H) which is believed to be associated with the vacuolar membrane (Scheme 2).<sup>26,182</sup> Secologanin synthase (SLS) (Scheme 2) is also an endomembrane associated protein.<sup>183</sup> Tryptamine is

generated by the action of tryptophan decarboxylase (TDC) in the cytosol (Scheme 1).<sup>184,185</sup>

In the first committed step of terpene indole alkaloid synthesis, strictosidine synthase (STR) acts on secologanin and tryptamine in the vacuole (Scheme 1).<sup>185,186</sup> Both secologanin and tryptamine substrates traverse the vacuole membrane (the tonoplast) from the cytosol. The product, strictosidine, is then exported out of the vacuole into the cytosol for reaction with strictosidine glucosidase (SGD), which is associated with the membrane of the endoplasmic reticulum.<sup>91,185</sup>

The steps immediately following strictosidine glucosidase define the branch point for the diversification of the strictosidine aglycone. Localization of the steps immediately following strictosidine deglycosylation has not been studied since no enzymes for these steps have been isolated (see Section 2.2). However, conversion of tabersonine to vindoline is well understood (Scheme 8). T16H, which acts on tabersonine in the first step of vindoline biosynthesis is associated with the endoplasmic reticulum membrane.<sup>137</sup> *N*-Methyl transferase activity (16-methoxy-2,3-dihydro-3-hydroxy tabersonine *N*-methyl transferase, NMT) is believed to be associated with the thylakoid, a structure located within the chloroplast.<sup>140,184</sup> The methylated intermediate, desacetoxyvindoline is transported to the cytosol, where it is hydroxylated and acetylated by the two cytosolic enzymes vindoline-4-hydroxylase (D4H) and deacetylvindoline *O*-acetyltransferase (DAT).<sup>184,187</sup> The peroxidase (PER) which catalyzes bisindole alkaloid formation is localized in the vacuole.<sup>153</sup> Additionally, the peroxidase (PER) responsible for oxidation of ajmalicine to serpentine (Scheme 6) is also believed to be in the vacuole.<sup>106</sup> Overall, extensive subcellular trafficking of biosynthetic intermediates is

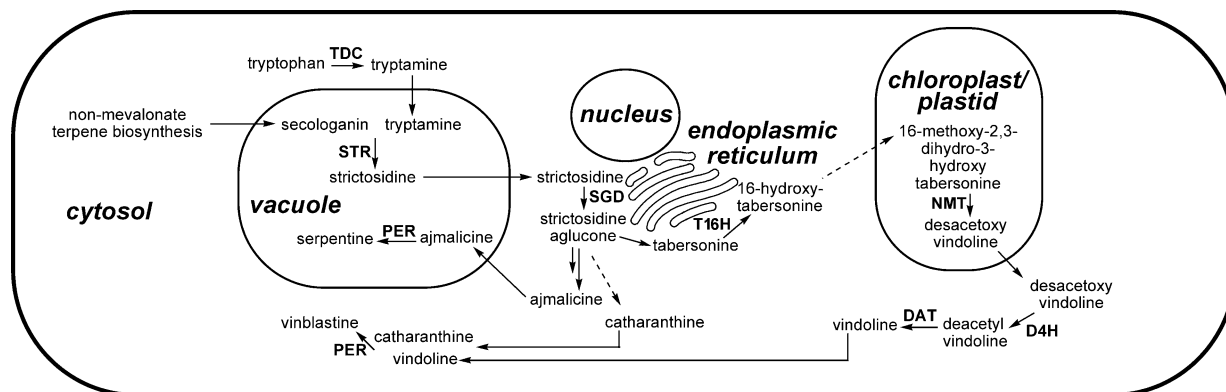


Fig. 9 Sub-cellular localization of known *C. roseus* enzymes in terpene indole alkaloid biosynthesis (adapted from ref. 90).

**Table 1** Cell type and associated alkaloid biosynthetic enzyme. Geraniol-10-hydroxylase (G10H); secologanin synthase (SLS); tryptophan decarboxylase (TDC); strictosidine synthase (STR); strictosidine glucosidase (SGD); *N*-methyltransferase (16-methoxy-2,3-dihydro-3-hydroxytabersonine *N*-methyl transferase (NMT)); vindoline 4-hydroxylase (D4H); desacetylvindoline *O*-acetyltransferase (DAT)

Enzyme	Transformation	Cell type
DXP synthase	Scheme 2	Vascular, epidermal
DXP reductoisomerase	Scheme 2	Vascular, epidermal
MEP synthase	Scheme 2	Vascular, epidermal
G10H	Scheme 2	Vascular, epidermal
SLS	Scheme 2	Vascular, epidermal
TDC	Scheme 1	Epidermal
STR	Scheme 1	Epidermal
T16H	Scheme 8	Epidermal
D4H	Scheme 8	Laticifers and idioblasts
DAT	Scheme 8	Laticifers and idioblasts

required for terpene indole alkaloid biosynthesis. Although uptake of ajmalicine and serpentine into the vacuole has been studied, in general the mechanisms of transport remain unclear.<sup>107,188</sup>

Aside from sub-cellular compartmentalization, specific cell types are apparently required for the biosynthesis of some terpene alkaloids; notably, cell suspension cultures of *C. roseus* fail to produce vindoline.<sup>188</sup> Studies of the localization of vindoline biosynthetic enzymes strongly suggest that the early part of the vindoline pathway (TDC and STR) takes place in epidermal cells (cells on the surface) of leaves and stems (Table 1). However, the later steps catalyzed by D4H and DAT take place in specialized cells, the laticifers and idioblasts.<sup>189</sup> Moreover, several enzymes involved in secologanin terpene biosynthesis, DXP synthase, DXP reductoisomerase, MEP synthase, geraniol-10-hydroxylase (G10H) and secologanin synthase (SLS), are observed in both vascular cells (cells involved in transport) and epidermal cells.<sup>190,191</sup> Vascular cells are believed to be specialized “terpene factories”.<sup>190</sup> In short, vindoline biosynthesis requires at least two distinct cell types and a mechanism of intercellular transport of intermediates.

Finally, enzyme activity is often restricted to a certain region of the plant or seedling. For example, TDC and STR are most abundant in roots but are also found in photosynthetic organs.<sup>192</sup> T16H, D4H and DAT are found in young leaves and shoots.<sup>137,141,142</sup> Vindoline and the bisindole alkaloids vinblastine and vincristine are found in the leaves and the stems, while the iboga alkaloid catharanthine is distributed throughout most tissues.<sup>193,194</sup> The corynanthe alkaloids appear primarily in the roots.<sup>195</sup>

## 4 Conclusion

After half a century of study, the chemistry of terpene indole alkaloid biosynthesis is still not completely understood. Elucidation of enzymatic pathways that construct ajmaline (*R. serpentina*) and vindoline (*C. roseus*) alkaloids has been particularly successful. Localization of alkaloid enzymes suggests that a complex network of intra- and inter-cellular trafficking of biosynthetic intermediates occurs throughout the course of alkaloid biosynthesis. Advances in molecular biology will undoubtedly lead to further insights into the chemistry, biochemistry, and biology of this complex set of biosynthetic pathways.

## 5 References

- 1 J. Leonard, *Nat. Prod. Rep.*, 1999, **16**, 319–338.
- 2 E. J. Saxton, *Nat. Prod. Rep.*, 1997, **14**, 559–590.
- 3 G. A. Cordell, *The Alkaloids: Chemistry and Biology*, Academic Press, San Diego, 1998, vol. 50, p. 260.
- 4 V. De Luca and P. T. Laflamme, *Curr. Opin. Plant Biol.*, 2001, **4**, 225–233.
- 5 P. J. Facchini, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 2001, **52**, 29–66.
- 6 T. Hashimoto and Y. Yamada, *Curr. Opin. Biotechnol.*, 2003, **14**, 163–168.
- 7 T. M. Kuchan, *Recent Adv. Phytochem.*, 2002, **36**, 163–178.
- 8 D. J. Hoelscher, D. C. Williams, M. R. Wildung and R. Croteau, *Phytochemistry*, 2003, **62**, 1081–1086.
- 9 T. M. Kuchan and J. Schroder, *Methods Enzymol.*, 2002, **36**, 370–381.
- 10 A. Goossens, S. T. Haekkinen, I. Laakso, T. Seppanen-Laakso, S. Biondi, V. De Sutter, F. Lammertyn, A. M. Nuutila, H. Soederlund, M. Zabeau, D. Inze and K.-M. Oksman-Caldentey, *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 8595–8600.
- 11 V. De Luca, C. Marneau and N. Brisson, *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 2582–2586.
- 12 P. J. Facchini, K. L. Huber-Allanach and L. W. Tari, *Phytochemistry*, 2000, **54**, 121–138.
- 13 S.-B. Hong, C. A. M. Peebles, J. V. Shanks, K.-Y. San and S. I. Gibson, *J. Biotechnol.*, 2006, **122**, 28–38.
- 14 E. Leete, *Tetrahedron*, 1961, **14**, 35–41.
- 15 E. Wenkert, *J. Am. Chem. Soc.*, 1962, **84**, 98–102.
- 16 R. Thomas, *Tetrahedron Lett.*, 1961, **16**, 544–553.
- 17 T. Money, I. G. Wright, F. McCapra and A. I. Scott, *Proc. Natl. Acad. Sci. USA*, 1965, **53**, 901–903.
- 18 A. R. Battersby, A. R. Burnett and P. G. Parsons, *Chem. Commun.*, 1968, 1280–1281.
- 19 A. R. Battersby, A. R. Burnett and P. G. Parsons, *J. Chem. Soc. C*, 1969, 1187–1192.
- 20 A. R. Battersby, J. C. Byrne, R. S. Kapil, J. A. Martin, T. G. Payne, D. Arigoni and P. Loew, *Chem. Commun.*, 1968, 951–953.
- 21 W. Eisenreich, A. Bacher, D. Arigoni and F. Rohdich, *Cell. Mol. Life Sci.*, 2004, **61**, 1401–1426.
- 22 A. Contin, R. van der Heijden, A. W. M. Lefebvre and R. Verpoorte, *FEBS Lett.*, 1998, **434**, 413–416.
- 23 Y. Yamazaki, M. Kitajima, M. Arita, H. Takayama, H. Sudo, M. Yamazaki, N. Aimi and K. Saito, *Plant Physiol.*, 2004, **134**, 161–170.
- 24 K. Chahed, A. Oudin, N. Guivarc’h, S. Hamdi, J.-C. Chenieux, M. Rideau and M. Clastre, *Plant Physiol. Biochem.*, 2000, **38**, 559–566.
- 25 B. Veau, M. Courtois, A. Oudin, J.-C. Chenieux, M. Rideau and M. Clastre, *Biochim. Biophys. Acta*, 2000, **1517**, 159–163.
- 26 G. Collu, N. Unver, A. M. G. Peltenburg-Looman, R. van der Heijden, R. Verpoorte and J. Memelink, *FEBS Lett.*, 2001, **508**, 215–220.
- 27 G. Collu, A. Garcia, R. van der Heijden and R. Verpoorte, *Plant Sci.*, 2002, **162**, 165–172.
- 28 S. K. Uesato, A. Iida, H. Inouye and M. H. Zenk, *Phytochemistry*, 1986, **25**, 839–842.
- 29 S. Uesato, S. Matsuda and H. Inouye, *Chem. Pharm. Bull.*, 1984, **32**, 1671–1674.
- 30 S. Irmeler, G. Schroder, B. St. Pierre, N. P. Crouch, M. Hotze, J. Schmidt, D. Strack, U. Matern and J. Schroder, *Plant J.*, 2000, **24**, 797–804.
- 31 H. Yamamoto, N. Katano, A. Ooi and K. Inoue, *Phytochemistry*, 2000, **53**, 7–12.
- 32 J. A. Morgan and J. V. Shanks, *J. Biotechnol.*, 2000, **79**, 137–145.
- 33 S. Whitmer, C. Canel, D. Hallard, C. Goncalves and R. Verpoorte, *Plant Physiol.*, 1998, **116**, 853–857.
- 34 A. Contin, R. van der Heijden and R. Verpoorte, *Plant Cell, Tissue Organ Cult.*, 1999, **56**, 111–119.
- 35 A. Pictet and T. Spengler, *Ber. Dtsch. Chem. Ges.*, 1911, **44**, 2030–2036.
- 36 E. D. Cox and J. M. Cook, *Chem. Rev.*, 1995, **95**, 1797–1842.
- 37 A. I. Scott, S. L. Lee, P. de Capite, M. G. Culver and C. R. Hutchinson, *Heterocycles*, 1977, **7**, 979–984.
- 38 A. R. Battersby, N. G. Lewis and J. M. Tippet, *Tetrahedron Lett.*, 1978, **48**, 4849–4852.
- 39 A. H. Heckendorf and C. R. Hutchinson, *Tetrahedron Lett.*, 1977, **48**, 4153–4154.
- 40 N. Nagakura, M. Ruffer and M. H. Zenk, *J. Chem. Soc., Perkin Trans. I*, 1979, 2308–2312.

- 41 J. Stockigt and M. H. Zenk, *J. Chem. Soc., Chem. Commun.*, 1977, 646–648.
- 42 R. T. Brown, J. Leonard and S. K. Sleight, *Phytochemistry*, 1978, **78**, 899–900.
- 43 T. M. Kutchan, *Phytochemistry*, 1993, **32**, 493–506.
- 44 H. Mizukami, H. Nordlov, S.-L. Lee and A. I. Scott, *Biochemistry*, 1979, **18**, 3760–3763.
- 45 U. Pfizner and M. H. Zenk, *Planta Med.*, 1989, **55**, 525–530.
- 46 T. D. McKnight, C. A. Roessner, R. Devagupta, A. I. Scott and C. Nessler, *Nucleic Acids Res.*, 1990, **18**, 4939.
- 47 A. de Waal, A. H. Meijer and R. Verpoorte, *Biochem. J.*, 1995, **306**, 571–580.
- 48 C. A. Roessner, R. Devagupta, M. Hasan, H. J. Williams and I. A. Scott, *Protein Expression Purif.*, 1992, **3**, 295–300.
- 49 A. Geerlings, F. J. Redondo, A. Contin, J. Memelink, R. van der Heijden and R. Verpoorte, *Appl. Microbiol. Biotechnol.*, 2001, **56**, 420–424.
- 50 J. F. Treimer and M. H. Zenk, *FEBS Lett.*, 1979, **97**, 159–162.
- 51 J. F. Treimer and M. H. Zenk, *Eur. J. Biochem.*, 1979, **101**, 225–233.
- 52 U. Pfizner and M. H. Zenk, *Methods Enzymol.*, 1987, **136**, 342–350.
- 53 T. M. Kutchan, N. Hampp, F. Lottspeich, K. Beyreuther and M. H. Zenk, *FEBS Lett.*, 1988, **237**, 40–44.
- 54 T. M. Kutchan, *FEBS Lett.*, 1989, **257**, 127–130.
- 55 E. J. Pennings, R. A. van den Bosch, R. van der Heijden, L. H. Stevens, J. A. Duine and R. Verpoorte, *Anal. Biochem.*, 1989, **176**, 412–415.
- 56 D. Bracher and T. M. Kutchan, *Arch. Biochem. Biophys.*, 1992, **294**, 717–723.
- 57 T. M. Kutchan, A. Bock and H. Dittich, *Phytochemistry*, 1994, **35**, 353–360.
- 58 Y. Yamazaki, H. Sudo, M. Yamazaki, N. Aimi and K. Saito, *Plant Cell Physiol.*, 2003, **44**, 395–403.
- 59 X. Ma, S. Panjikar, J. Koepke, E. Lovis and J. Stöckigt, *Plant Cell*, 2006, **18**, 907–920.
- 60 N. Samanani, D. K. Liscombe and P. J. Facchini, *Plant J.*, 2004, **40**, 302–313.
- 61 E. McCoy, M. C. Galan and S. E. O'Connor, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 2475–2478.
- 62 M. C. Galan and S. E. O'Connor, *Tetrahedron Lett.*, 2006, **47**, 1563–1565.
- 63 M. Ruppert, X. Ma and J. Stoeckigt, *Curr. Org. Chem.*, 2005, **9**, 1431–1444.
- 64 S. Rolf, H.-J. Bruns, T. Wichter, P. Kirchhof, M. Ribbing, K. Wasmer, M. Paul, G. Breithardt, W. Haverkamp and L. Eckardt, *Eur. Heart J.*, 2003, **24**, 1104–1112.
- 65 I. Gerasimenko, Y. Sheludko, X. Ma and J. Stockigt, *Eur. J. Biochem.*, 2002, **269**, 2204–2213.
- 66 Z. Shen, W. Eisenreich and T. M. Kutchan, *Phytochemistry*, 1998, **48**, 293–296.
- 67 C. Kan-Fan and H. P. Husson, *J. Chem. Soc., Chem. Commun.*, 1979, 1015–1018.
- 68 M. El-Sayed, Y. H. Choi, M. Frederich, S. Roytrakul and R. Verpoorte, *Biotechnol. Lett.*, 2004, **26**, 793–798.
- 69 P. Heinsteins, G. Hofle and J. Stockigt, *Planta Med.*, 1979, **37**, 349–357.
- 70 P. M. Dewick, *Medicinal Natural Products: A Biosynthetic Approach*, John Wiley and Sons, Ltd., New York, 2nd edn, 2002.
- 71 L. Barleben, X. Ma, J. Koepke, G. Peng, H. Michel and J. Stoeckigt, *Biochim. Biophys. Acta*, 2005, **1747**, 89–92.
- 72 J. Stockigt, *Stud. Org. Chem.*, 1986, **26**, 497–511.
- 73 D. Schmidt and J. Stockigt, *Planta Med.*, 1995, **61**, 254–258.
- 74 J. Stockigt, *The Alkaloids in Biosynthesis in Rauwolfia serpentina*, ed. G. A. Cordell, Academic Press, San Diego, 1995, vol. 47, p. 115.
- 75 A. Pfizner and J. Stockigt, *J. Chem. Soc., Chem. Commun.*, 1983, 459–460.
- 76 E. Mattern-Dogru, X. Ma, J. Hartmann, H. Decker and J. Stockigt, *Eur. J. Biochem.*, 2002, **269**, 2889–2896.
- 77 A. Pfizner and J. Stockigt, *Planta Med.*, 1983, **48**, 221–227.
- 78 E. Dogru, H. Warzecha, F. Seibel, S. Haebel, F. Lottspeich and J. Stockigt, *Eur. J. Biochem.*, 2000, **267**, 1397–1406.
- 79 A. Pfizner and J. Stoeckigt, *Tetrahedron Lett.*, 1983, **24**, 5197–5200.
- 80 I. Gerasimenko, X. Ma, Y. Sheludko, R. Mentle, F. Lottspeich and J. Stockigt, *Bioorg. Med. Chem.*, 2004, **12**, 2781–2786.
- 81 A. Bayer, X. Ma and J. Stockigt, *Bioorg. Med. Chem.*, 2004, **12**, 2787–2795.
- 82 X. Ma, J. Koepke, S. Panjikar, G. Fritzsche and J. Stoeckigt, *J. Biol. Chem.*, 2005, **280**, 13576–13583.
- 83 H. Falkenhagen, L. Polz, H. Takayama, M. Kitajima, S. Sakai, N. Aimi and J. Stockigt, *Heterocycles*, 1995, **41**, 2683–2690.
- 84 S. Gao, G. von Schumann and J. Stockigt, *Planta Med.*, 2002, **68**, 906–911.
- 85 G. von Schumann, S. Gao and J. Stockigt, *Bioorg. Med. Chem.*, 2002, **10**, 1913–1918.
- 86 M. Ruppert, J. Woll, A. Giritch, E. Genady, X. Ma and J. Stoeckigt, *Planta*, 2005, **222**, 888–898.
- 87 J. Stockigt, A. Pfizner and P. I. Keller, *Tetrahedron Lett.*, 1983, **244**, 2485–2486.
- 88 S. E. Drewes, J. George and F. Khan, *Phytochemistry*, 2003, **62**, 1019–1025.
- 89 C. Kan-Fan and H. P. Husson, *Tetrahedron Lett.*, 1980, **21**, 1463–1466.
- 90 R. van der Heijden, D. I. Jacobs, W. Snoeijer, D. Hallared and R. Verpoorte, *Curr. Med. Chem.*, 2004, **11**, 607–628.
- 91 A. Geerlings, M. M.-L. Ibanez, J. Memelink, R. van der Heijden and R. Verpoorte, *J. Biol. Chem.*, 2000, **275**, 3051–3056.
- 92 T. Hemscheidt and M. H. Zenk, *FEBS Lett.*, 1980, **110**, 187–191.
- 93 A. I. Scott, S. L. Lee and W. Wan, *Biochem. Biophys. Res. Commun.*, 1977, **75**, 1004–1009.
- 94 T. J. C. Luijendijk, L. H. Stevens and R. Verpoorte, *Plant Physiol. Biochem.*, 1998, **36**, 419–425.
- 95 C. Kan-Fan and H. P. Husson, *J. Chem. Soc., Chem. Commun.*, 1978, 618–619.
- 96 H. P. Husson, C. Kan-Fan, T. Sevenet and J.-P. Vidal, *Tetrahedron Lett.*, 1977, **22**, 1889–1892.
- 97 R. T. Brown, J. Leonard and S. K. Sleight, *J. Chem. Soc., Chem. Commun.*, 1977, 636–638.
- 98 R. T. Brown and J. Leonard, *J. Chem. Soc., Chem. Commun.*, 1979, 877–879.
- 99 R. T. Brown and J. Leonard, *Tetrahedron Lett.*, 1977, **48**, 4251–4254.
- 100 J. Stockigt, H. P. Husson, C. Kan-Fan and M. H. Zenk, *J. Chem. Soc., Chem. Commun.*, 1977, 164–166.
- 101 T. Hemscheidt and M. H. Zenk, *Plant Cell Rep.*, 1985, **4**, 216–219.
- 102 J. Stockigt, T. Hemscheidt, G. Hofle, P. Heinsteins and V. Formacek, *Biochemistry*, 1983, **22**, 3448–3452.
- 103 J. Stockigt, G. Hofle and A. Pfizner, *Tetrahedron Lett.*, 1980, **21**, 1925–1926.
- 104 J. Stockigt, *J. Chem. Soc., Chem. Commun.*, 1978, 1097–1099.
- 105 A. Pfizner and J. Stoeckigt, *Phytochemistry*, 1982, **21**, 1585–1588.
- 106 M. Sottomayor, I. L. Cardoso, L. G. Pereira and A. R. Barcel, *Phytochem. Rev.*, 2004, **3**, 159–171.
- 107 T. J. M. Blom, M. Sierra, T. B. Van Vliet, M. E. I. Franke-van Dijk, P. De Koning, F. Van Iren, R. Verpoorte and K. R. Libbenga, *Planta*, 1991, **183**, 170–177.
- 108 S. Li, J. Long, Z. Ma, Z. Xu, J. Li and Z. Zhang, *Curr. Med. Res. Opin.*, 2004, **20**, 409–415.
- 109 L. Dassonneville, K. Bonjean, M.-C. De Pauw-Gillet, P. Colson, C. Houssier, J. Quetin-Leclercq, L. Angenot and C. Bailly, *Biochemistry*, 1999, **38**, 7719–7726.
- 110 L. Costa-Campos, S. C. Dassoler, A. P. Rigo, M. Iwu and E. Elisabetsky, *Pharmacol. Biochem. Behav.*, 2004, **77**, 481–489.
- 111 A. A. Qureshi and A. I. Scott, *Chem. Commun.*, 1968, 948–950.
- 112 A. R. Battersby, A. R. Burnett, E. S. Hall and P. G. Parsons, *Chem. Commun.*, 1968, 1582–1583.
- 113 A. I. Scott, P. C. Cherry and A. A. Qureshi, *J. Am. Chem. Soc.*, 1969, **91**, 4932–4933.
- 114 A. R. Battersby and E. S. Hall, *J. Chem. Soc. D*, 1969, 793–794.
- 115 A. R. Battersby, A. R. Burnett and P. G. Parsons, *J. Chem. Soc. C*, 1969, 1193–1200.
- 116 A. R. Battersby and A. K. Bhatnagar, *Chem. Commun.*, 1970, 193–195.
- 117 A. R. Battersby and K. H. Gibson, *J. Chem. Soc. D*, 1971, 902–903.
- 118 A. I. Scott, P. B. Reichardt, M. B. Slaytor and J. G. Sweeny, *Bioorg. Chem.*, 1971, **1**, 157–173.
- 119 A. I. Scott, *J. Am. Chem. Soc.*, 1972, **94**, 8262.
- 120 A. I. Scott and C. C. Wei, *J. Am. Chem. Soc.*, 1972, **94**, 8263–8264.
- 121 A. I. Scott and C. C. Wei, *J. Am. Chem. Soc.*, 1972, **94**, 8264–8265.
- 122 A. I. Scott and C. C. Wei, *J. Am. Chem. Soc.*, 1972, **94**, 8266–8267.
- 123 A. I. Scott and A. A. Qureshi, *J. Am. Chem. Soc.*, 1969, **91**, 5874–5876.
- 124 E. Wenkert and B. Wickberg, *J. Am. Chem. Soc.*, 1965, **87**, 1580–1589.
- 125 A. I. Scott and C. C. Wei, *Tetrahedron*, 1974, **30**, 3003–3011.
- 126 A. A. Qureshi and A. I. Scott, *Chem. Commun.*, 1968, 945–946.

- 127 J. P. Kutney, C. Ehret, V. R. Nelson and D. C. Wigfield, *J. Am. Chem. Soc.*, 1968, **90**, 5929–5930.
- 128 S. Laschat, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 289–291.
- 129 J. P. Kutney, W. J. Cretney, J. R. Hadfield, E. S. Hall, V. R. Nelson and D. C. Wigfield, *J. Am. Chem. Soc.*, 1968, **90**, 3566–3567.
- 130 D. Arigoni, *Biochem. Physiol. Alkaloide, Int. Symp.*, 4th., ed. K. Mothes, Akademie-Verlag GmbH, Berlin, 1972, pp. 317–335.
- 131 A. I. Scott, *Acc. Chem. Res.*, 1970, **3**, 151–157.
- 132 J. P. Kutney, J. F. Beck, C. Ehret, G. Poulton, R. S. Sood and N. D. Westcott, *Bioorg. Chem.*, 1971, **1**, 194–206.
- 133 A. I. Scott and A. A. Qureshi, *Tetrahedron*, 1974, **30**, 2993–3002.
- 134 A. R. Battersby, *Alkaloids*, 1971, **1**, 31–47.
- 135 M. H. Zenk, *J. Nat. Prod.*, 1980, **43**, 438–451.
- 136 V. De Luca, *Recent Adv. Phytochem.*, 2003, **37**, 181–202.
- 137 B. St. Pierre and V. De Luca, *Plant Physiol.*, 1995, **109**, 131–139.
- 138 G. Schroder, E. Unterbusch, M. Kaltenbach, J. Schmidt, D. Strack, V. De Luca and J. Schroder, *FEBS Lett.*, 1999, **458**, 97–102.
- 139 S. Cacace, G. Schroder, E. Wehinger, D. Strack, J. Schmidt and J. Schroder, *Phytochemistry*, 2003, **62**, 127–137.
- 140 M. Dethier and V. De Luca, *Phytochemistry*, 1993, **32**, 673–678.
- 141 F. Vazquez-Flota, E. De Carolis, A. Alarco and V. De Luca, *Plant Mol. Biol.*, 1997, **34**, 935–948.
- 142 B. St. Pierre, P. Laflamme, A. Alarco and V. De Luca, *Plant J.*, 1998, **14**, 703–713.
- 143 M. N. Islam and M. N. Iskander, *Mini-Rev. Med. Chem.*, 2004, **4**, 1077–1104.
- 144 T. Beckers and S. Mahboobi, *Drugs Future*, 2003, **28**, 767–785.
- 145 P. E. Daddona and C. R. Hutchinson, *J. Am. Chem. Soc.*, 1974, **96**, 6806–6807.
- 146 A. I. Scott, F. Gueritte and S. L. Lee, *J. Am. Chem. Soc.*, 1978, **100**, 6253–6255.
- 147 K. L. Stuart, J. P. Kutney, T. Honda and B. R. Worth, *Heterocycles*, 1978, **9**, 1419–1427.
- 148 R. I. Baxter, C. A. Dorschel, S. L. Lee and A. I. Scott, *J. Chem. Soc., Chem. Commun.*, 1979, 257–259.
- 149 F. Gueritte, N. V. Bac, Y. Langlois and P. Potier, *J. Chem. Soc., Chem. Commun.*, 1980, 452–453.
- 150 T. Endo, A. Goodbody, J. Vukovic and M. Misawa, *Phytochemistry*, 1988, **27**, 2147–2149.
- 151 J. L. Smith, E. Amouzou, A. Yamagushi, S. McLean and F. DiCosmo, *Biotechnol. Appl. Bioeng.*, 1988, **10**, 568–575.
- 152 F. Hillou, M. Costa, I. Almeida, I. Lopes Cardoso, M. Leech, A. Ros Barcelo and M. Sottomayor, Cloning of a peroxidase enzyme involved in the biosynthesis of pharmaceutically active terpenoid indole alkaloids in *Catharanthus roseus*, in: *Proc. VI Int. Plant Peroxidase Symp.*, ed. M. Acosta, J. N. Rodriguez-Lopez and M. A. Pedreno, Servicio de Publicaciones, Universidad de Murcia, Spain, 2002, pp. 152–158.
- 153 M. Sottomayor, M. Lopez-Serrano, F. DiCosmo and A. Ros Barcelo, *FEBS Lett.*, 1998, **428**, 299–303.
- 154 M. Sottomayor, F. DiCosmo and A. Ros Barcelo, *Enzyme Microb. Technol.*, 1997, **21**, 543–549.
- 155 A. Lorence and C. L. Nessler, *Phytochemistry*, 2004, **65**, 2735–2749.
- 156 E. Wenkert, I. G. Dave, R. G. Lewis and P. W. Sprague, *J. Am. Chem. Soc.*, 1967, **89**, 6741–6745.
- 157 G. M. Sheriha and H. Rapoport, *Phytochemistry*, 1976, **15**, 505–508.
- 158 A. H. Heckendorf and C. R. Hutchinson, *Tetrahedron Lett.*, 1977, **48**, 4153–4154.
- 159 Y. Yamazaki, A. Urano, H. Sudo, M. Kitajima, H. Takayama, M. Yamazaki, N. Aimi and K. Saito, *Phytochemistry*, 2003, **62**, 461–470.
- 160 C. R. Hutchinson, A. H. Heckendorf, J. L. Straughn, P. E. Daddona and D. E. Cane, *J. Am. Chem. Soc.*, 1979, **101**, 3358–3369.
- 161 C. R. Hutchinson, A. H. Heckendorf, P. E. Daddona, E. Hagaman and E. Wenkert, *J. Am. Chem. Soc.*, 1974, **96**, 5609–5611.
- 162 G. A. Cordell, *Lloydia*, 1974, **37**, 219.
- 163 B. K. Carte, C. DeBrosse, D. Eggleston, M. Hemling, M. Mentzer, B. Poehland, N. Troupe, J. W. Westley and S. M. Hecht, *Tetrahedron*, 1990, **46**, 2747–2760.
- 164 Y. Yamazaki, A. Urano, H. Sudo, M. Kitajima, H. Takayama, M. Yamazaki, N. Aimi and K. Saito, *Phytochemistry*, 2003, **62**, 461–470.
- 165 M. Kitajima, S. Masumoto, H. Takayama and N. Aimi, *Tetrahedron Lett.*, 1997, **38**, 4255–4258.
- 166 E. Leete, *Acc. Chem. Res.*, 1969, **2**, 59–64.
- 167 J. K. Baird, *N. Engl. J. Med.*, 2005, **352**, 1565–1577.
- 168 E. Leete and J. N. Wemple, *J. Am. Chem. Soc.*, 1969, **91**, 2698–2702.
- 169 N. Kowanko and E. Leete, *J. Am. Chem. Soc.*, 1962, **84**, 4919–4921.
- 170 C. A. Hay, L. A. Anderson, J. D. Phillipson, D. Curless and R. T. Brown, *Plant Cell, Tissue Organ Cult.*, 1987, **9**, 197–206.
- 171 H. Inouye, S. Ueda and Y. Takeda, *Tetrahedron Lett.*, 1969, 407–408.
- 172 A. R. Battersby, R. T. Brown, R. S. Kapil, J. A. Knight, J. A. Martin and A. O. Plunkett, *Chem. Commun.*, 1966, 810–812.
- 173 A. R. Battersby, R. T. Brown, R. S. Kapil, J. A. Knight, J. A. Martin and A. O. Plunkett, *Chem. Commun.*, 1966, 888–890.
- 174 E. Leete and J. N. Wemple, *J. Am. Chem. Soc.*, 1966, **88**, 4743–4744.
- 175 L. H. Stevens, C. Giroud, E. J. M. Pennings and R. Verpoorte, *Phytochemistry*, 1993, **33**, 99–106.
- 176 L. H. Stevens, J. Schripsema, E. J. M. Pennings and R. Verpoorte, *Plant Physiol. Biochem.*, 1992, **30**, 675–681.
- 177 A. R. Battersby and R. J. Parry, *J. Chem. Soc., Chem. Commun.*, 1971, 30–31.
- 178 A. R. Battersby and R. J. Parry, *J. Chem. Soc., Chem. Commun.*, 1971, 31–32.
- 179 J. E. Isaac, R. J. Robins and M. J. C. Rhodes, *Phytochemistry*, 1987, **26**, 393–399.
- 180 P. J. Facchini and B. St. Pierre, *Curr. Opin. Plant Biol.*, 2005, **8**, 657–666; T. M. Kuchan, *Curr. Opin. Plant Biol.*, 2005, **8**, 292–300.
- 181 V. De Luca and B. St. Pierre, *Trends Plant Sci.*, 2000, **5**, 168–173.
- 182 K. M. Madyastha, J. E. Ridgway, J. G. Dwyer and C. J. Coscia, *J. Cell Biol.*, 1977, **72**, 303–313.
- 183 H. Yamamoto, N. Katano, A. Ooi and K. Inoue, *Phytochemistry*, 2000, **53**, 7–12.
- 184 V. De Luca and A. J. Cutler, *Plant Physiol.*, 1987, **85**, 1099–1102.
- 185 L. H. Stevens, T. J. M. Blom and R. Verpoorte, *Plant Cell Rep.*, 1993, **12**, 573–576.
- 186 T. D. McKnight, D. R. Bergey, R. J. Burnett and C. Nessler, *Planta*, 1991, **185**, 148–152.
- 187 E. De Carolis, F. Chan, J. Balsevich and V. De Luca, *Plant Physiol.*, 1990, **94**, 1323–1329.
- 188 A. H. Meijer, R. Verpoorte and J. H. C. Hoge, *J. Plant Res.*, 1993, **3**, 145–164.
- 189 B. St. Pierre, F. Vazquez-Flota and V. De Luca, *Plant Cell*, 1999, **11**, 887–900.
- 190 J. Murata and V. De Luca, *Plant J.*, 2005, **44**, 581–594.
- 191 V. Burlat, A. Oudin, M. Courtois, M. Rideau and B. St. Pierre, *Plant J.*, 2004, **38**, 131–141.
- 192 G. Pasquali, O. J. M. Goddijn, A. De Waal, R. Verpoortet, R. A. Schilperoort, J. Hoge, C. Harry and J. Memelink, *Plant Mol. Biol.*, 1992, **18**, 1121–1131.
- 193 P. Westekemper, U. Wiczorek, F. Gueritte, N. Langlois, Y. Langlois, P. Potier and M. H. Zenk, *Planta Med.*, 1980, **39**, 24–37.
- 194 B. Deus-Neumann, J. Stöckigt and M. H. Zenk, *Planta Med.*, 1987, **53**, 184–188.
- 195 M.-P. Bonzom, C. Andary, A. Gueiffier, J.-L. Roussel and A. Gargadennec, *Phytochemistry*, 1997, **46**, 235–239.