Published in final edited form as:

Curr Opin Chem Biol. 2009 October ; 13(4): 485–491. doi:10.1016/j.cbpa.2009.06.019.

Mechanistic Advances in Plant Natural Product Enzymes

Aimee R. Usera and **Sarah E. O'Connor**

Massachusetts Institute of Technology, Department of Chemistry, 18-592, 77, Massachusetts Avenue, Cambridge, MA 02139, USA

Summary of Recent Advances

The biosynthetic pathways of plant natural products offer an abundance of knowledge to scientists in many fields. Synthetic chemists can be inspired by the synthetic strategies that nature uses to construct these compounds. Chemical and biological engineers are working to reprogram these biosynthetic pathways to more efficiently produce valuable products. Finally, biochemists and enzymologists are interested in the detailed mechanisms of the complex transformations involved in construction of these natural products. Study of biosynthetic enzymes and pathways therefore has a wide-ranging impact. In recent years, many plant biosynthetic pathways have been characterized, particularly the pathways that are responsible for alkaloid biosynthesis. Here we highlight recently studied alkaloid biosynthetic enzymes that catalyze production of numerous complex medicinal compounds, as well as the specifier proteins in glucosinolate biosynthesis, whose structure and mechanism of action are just beginning to be unraveled.

Introduction

Many natural products derived from plants are highly successful pharmaceuticals (Figure 1). ^{1–4} *Taxus brevifolia* (Pacific Yew) and *Catharanthus roseus* (Madagascar periwinkle) produce the anticancer compounds taxol⁵ and vincristine,^{6–7} while *Artemisia annua* (sweet wormwood) yields the antimalarial artemisinin.⁸ However, these products are frequently produced in low yield in the plants, and the complex structure of these compounds often makes total synthesis costly or impractical on a large scale. Many plant derived compounds are therefore extremely expensive or inaccessible. Understanding the biosynthetic pathways of plant natural product metabolism opens up new approaches for production. Organic chemists can formulate new synthetic approaches to the molecules based on nature's method of production. Moreover, biochemists can engineer biosynthetic pathways to increase production of the medicinal compounds. An understanding of the biosynthetic pathways is of obvious importance and the basis of understanding these pathways lies with the enzymes catalyzing the reactions.

As the genetic tools to identify new plant enzymes has improved, enzymology of plant biosynthesis has flourished, though there is still much to be learned. This review explores significant and recent (after 2006) mechanistic advances in enzymes involved in plant natural products. Specifically, the biosynthetic enzymes involved in the production of nitrogen containing natural products- alkaloids- have been a particularly fruitful area of study. We

© 2009 Elsevier Ltd. All rights reserved.

Corresponding author: Sarah E. O'Connor (soc@mit.edu).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

examine the alkaloid biosynthetic enzymes strictosidine synthase, norcoclaurine synthase, and berberine bridge enzyme, and specifier proteins utilized in glucosinolate biosynthesis.

Strictosidine Synthase

Strictosidine synthase,⁹⁻¹¹ originally detected in *Catharanthus roseus* and *Rauwolfia serpentina* 30 years ago,^{12,13} is one of the most well-characterized alkaloid biosynthetic enzymes. Strictosidine synthase catalyzes the first committed step of monoterpene indole alkaloid biosynthesis by catalyzing a Pictet-Spengler condensation¹⁴⁻¹⁵ between tryptamine and secologanin to yield strictosidine (Figure 2a). This central biosynthetic intermediate is converted into many alkaloids including vincristine (Figure 1). The crystal structure of strictosidine synthase was recently solved,¹⁶⁻¹⁷ and this structure has paved the way for mechanistic studies, as well as protein design efforts. Structures of strictosidine synthase (*R. serpentina*) complexed with tryptamine, secologanin, and strictosidine have all been reported^{16,18} and the residues of the substrate binding pockets have been clearly identified.^{16,18}

Glu309 (*R. serpentina*) has been shown by both this structural analysis and by site-directed mutagenesis experiments to be the key residue required for acid-base catalysis in the Pictet-Spengler reaction.^{16,19,20} Glu309 likely hydrogen-bonds to the primary amino group of tryptamine, ensuring that it is neutral and therefore nucleophilic. This primary amine attacks the aldehyde of secologanin to form an iminium ion (Figure 2a). To form the strictosidine product, the indole moiety then attacks at the iminium in an electrophilic aromatic substitution reaction creating the (*S*)-stereocenter. In one possible reaction pathway (Pathway A), the C-2 carbon of tryptamine attacks the iminium to form a six-membered ring and a tertiary carbocation. Glu309 then deprotonates, restoring aromaticity, and creating strictosidine. (Figure 2a)

Alternatively (Pathway B), the C-3 carbon attacks the iminium to form a spirocyclic intermediate, which would then rearrange to form a six-membered ring (Figure 2a). *Ab initio* calculations find that the transition state of the spirocyclic intermediate is higher in energy than the transition state for direct C-2 attack.²⁰ Moreover, the rearrangement of the spirocyclic intermediate to the six-membered ring was found to be energetically unfavorable.²⁰ Therefore, if the spirocyclic intermediate does form, it is likely a non-productive intermediate, and will simply revert back to the iminium starting material. A tryptamine substrate with a deuterium label at the C-2 position displayed a primary kinetic isotope effect, indicating that all steps prior to re-aromatization are reversible.²⁰

Several substrate specificity and redesign studies for strictosidine synthase have been carried out.^{3,16-23} Strictosidine synthase will turn over only a few variants of the secologanin substrate, though the enzyme accepts a number of substituted tryptamine analogs with modest steric and electronic perturbations.^{19,22,23} The active site of this enzyme can be mutated to accommodate more sterically demanding substrate analogues.^{19,23} These substrates, which are not turned over by the natural strictosidine synthase enzyme, are nevertheless turned over by downstream biosynthetic enzymes,¹⁹ suggesting that strictosidine synthase is a “gate-keeper” of substrate specificity in this pathway. Engineered variants of strictosidine synthase can therefore be incorporated into the biosynthetic pathway to produce new alkaloid analogs.²⁴

Norcoclaurine Synthase

Norcoclaurine synthase is utilized in the first committed step of the benzyloisoquinoline biosynthetic pathway.²⁵⁻²⁷ This enzyme, like strictosidine synthase, catalyzes a Pictet-Spengler condensation. Norcoclaurine synthase uses dopamine and 4-hydroxyphenylacetaldehyde (4-HPAA) to yield (*S*)-norcoclaurine, a central biosynthetic

intermediate in the benzyloquinoline pathway (Figure 2b).²⁵⁻²⁷ Norcoclaurine synthase has been recently cloned from both *Thalictrum flavum*²⁸⁻²⁹ and *Coptis japonica*.³⁰ Notably, in *C. japonica*, two entirely different enzymes catalyzing the reaction were cloned and isolated.³⁰ One shows strong sequence similarity to the enzyme identified from *T. flavum*, while the other appears to be similar to a 2-oxoglutarate-dependent dioxygenases requiring ferrous ion.³⁰ It remains to be determined what specific *in planta* roles these two norcoclaurine enzymes have. Notably, neither of the norcoclaurine synthase sequences is at all similar to strictosidine synthase,³¹ despite the fact that all of these enzymes catalyze a Pictet-Spengler reaction.

The enzyme identified from *T. flavum*, which shows homology to Bet v 1 allergen and pathogenesis-related (PR)10 proteins,^{29,31-33} is the best-characterized of the norcoclaurine synthase enzymes. Norcoclaurine synthase displays sigmoidal saturation kinetics with the dopamine substrate, indicating that there is positive cooperativity in the binding of dopamine.²⁹ Enzymes exhibiting this type of kinetic property often have a regulatory role in metabolism, suggesting that norcoclaurine synthase may play a role in regulating the metabolic flux of the benzyloquinoline pathway.

The crystal structure of *T. flavum* norcoclaurine synthase has recently been solved in complex with dopamine and a nonreactive aldehyde, 4-hydroxybenzaldehyde.³¹ The enzyme appears to be a homodimer, where each monomer contains an active site in which the dopamine and aldehyde substrates form a stacked configuration. Interactions of the phenolic hydroxyl groups of the substrates with tyrosine and aspartate residues appear to keep the substrates oriented.

The placement of Lys122, Tyr108, and Glu110 relative to the orientation of the substrates in the active site³¹ suggests that these residues may be involved in the catalytic mechanism. Although mutations of Tyr108 and Glu110 result in some loss of enzyme catalysis, mutation of Lys122 to Ala results in a complete loss of activity,³⁴ suggesting that Lys122 is the key catalytic residue of the enzyme. Positively charged Lys122 is proposed to polarize the carbonyl of the aldehyde substrate, making it more susceptible to nucleophilic attack by the primary amine. Lys122 may also protonate the resulting carbinolamine, driving the loss of water to form the iminium species (Figure 2b). Following iminium ion formation, the dopamine moiety is deprotonated at the C-2 hydroxyl group, which helps drive the attack on the iminium ion to form the norcoclaurine skeleton. Formation of this phenolate ion has been proposed to be mechanistically essential since analogues lacking a C-2 hydroxyl group are not substrates.³⁴ However, the residues involved in C-2 hydroxyl group deprotonation, or stabilization of the resulting phenolate ion, have not been definitively identified, though Tyr108 may be involved. Glu110 or water could then deprotonate at C-5 to rearomatize the intermediate to form (S)-norcoclaurine. As with strictosidine synthase, a primary kinetic isotope effect with deuterated dopamine was also noted.³⁴

Berberine Bridge Enzyme

The berberine bridge enzyme also acts in the benzyloquinoline alkaloid pathway, and serves to channel (S)-reticuline into the berberine type alkaloids,³⁵ rather than the morphine type alkaloids (Figure 1). This enzyme catalyzes the regioselective formation of a carbon-carbon bond between the N-methyl group and the C2' carbon of the phenolic ring of (S)-reticuline to create (S)-scoulerine (Figure 3).^{35,36} Notably, this reaction is not readily accomplished by synthetic means. Berberine bridge enzyme (*Eschscholzia californica*) has been mechanistically studied in detail, and has been recently shown to contain covalent linkages to the FAD co-factor at residues His104 and Cys166 (Figure 3).^{37,38} Although mutation of His104 to Ala or Thr failed to yield soluble enzyme for mechanistic analysis, the Cys166Ala mutant was readily isolated.³⁸ This Cys166A mutant has a significantly decreased oxidation potential compared

to the wild type enzyme, indicating that the bicovalent attachment tunes the redox properties of the enzyme.³⁸

The X-ray structure of the berberine bridge enzyme in complex with (S)-reticuline was recently elucidated.³⁶ A variety of protein-substrate interactions were noted, but most importantly Glu417 appears to hydrogen bond to the C3' OH group of (S)-reticuline.³⁶ Since substrates lacking the C3'-OH group are not turned over by the enzyme,³⁹ the C3' hydroxyl group likely plays a critical role in the mechanism. It is proposed that Glu417 deprotonates the C3'-OH group, thereby enhancing the nucleophilicity of the C2' carbon so that it can attack the N-methyl group and form the "berberine bridge".³⁶ Consistent with this hypothesis, the Glu417Gln mutant displays a 1500 fold decrease in the rate of (S)-scoulerine formation.³⁶ In addition to the attack of the C2' carbon on the N-methyl group, the enzyme also catalyzes transfer of a hydride from the N-methyl to FAD, thereby reducing the flavin cofactor. The Glu417Gln mutant does not appear to catalyze flavin reduction,³⁶ suggesting that the attack of C2' onto the N-methyl and the transfer of hydride to the flavin are coupled processes. Following berberine bridge formation, an unidentified base deprotonates at the C-2 position to restore aromaticity and form (S)-scoulerine (Figure 3).

Specifier Proteins

Specifier proteins are enzymes that work in concert with myrosinases to produce products derived from glucosinolate natural products.⁴⁰ Glucosinolates are deglucosylated by myrosinases to yield a reactive aglycone intermediate (Figure 4).⁴¹ In the absence of specifier proteins, the deglucosylated glucosinolates undergo a spontaneous Lossen-type rearrangement⁴² to yield isothiocyanates,^{43,44} which are believed to protect plants from herbivore and pathogen attack.^{43,45-48} In the presence of specifier proteins however, the glucosinolate hydrolysis intermediate is directed away from isothiocyanate formation towards the production of epithionitriles, nitriles, and thiocyanates, depending on the structure of the specific glucosinolate (Figure 4).^{40,43} The biological functions of the epithionitriles, nitriles, and thiocyanates are not currently known, but are assumed to have defensive functions.^{43,45,46,48} Importantly, the specifier proteins influence the enzymatic reaction by producing different products, but these specifier proteins do not display hydrolytic activity, and can only function in the presence of myrosinase.^{40,43}

Rigorous identification and biochemical characterization of the specifier proteins has only recently begun. Two iron-dependent specifier proteins, epithiospecifier protein⁴⁹⁻⁵² and thiocyanate-forming protein,^{53,54} which share high levels of sequence identity, have so far been identified from *Arabidopsis thaliana*,⁵¹ *Brassica napus*,⁵⁰ *Brassica oleracea*,⁵² and *Lepidium sativum*.^{43,53} Each specifier protein contain Kelch motifs^{52,55} that are known to play a role in protein-protein interactions.⁵⁶ It is therefore logical to speculate that the specifier proteins physically interact with the myrosinases to direct formation of alternative products from the glucosinolate aglycone. However, stable interactions among myrosinases and specifier proteins have not been detected.⁴⁸ Evaluation of how the specifier protein:myrosinase ratio affects the ratio and rate of product formation suggests that the specifier proteins may play a catalytic role, and do not simply act as cofactors for myrosinase.⁵⁵ The pronounced substrate specificities of the different specifier proteins⁴⁰ also suggest that these proteins may be catalytic enzymes. However, much work is needed to understand, on the molecular level, exactly how the specifier proteins control product formation. These specifier proteins, which both control and expand the diversity of products that result from glucosinolate hydrolysis, represent an exciting area in plant-derived natural product biosynthesis.

Conclusions

The alkaloid biosynthetic enzymes and the specifier proteins of the glucosinolates represent two distinct classes of plant proteins involved in nitrogen containing natural product biosynthesis. Strictosidine synthase, norcoclaurine synthase, and berberine bridge enzyme are each well-studied proteins with explicitly defined catalytic functions. Recent structural analyses and mechanistic studies of these three enzymes have spectacularly demonstrated how nature catalyzes complex biochemical transformations in alkaloid natural product biosynthesis. In contrast, the catalytic function of the specifier proteins remains cryptic, though these proteins are clearly required for correct product formation. Additional mechanistic studies using site-directed mutagenesis, structural analysis and isotope labeling will undoubtedly elucidate the chemical mechanisms of these intriguing enzymes. The selected examples described in this review demonstrates the impressive progress that has been made in plant biosynthetic studies in recent years, but also highlights the complexities in plant natural product biosynthesis that remain to be elucidated.

Acknowledgements

A. R. U. is supported by an NIH post-doctoral fellowship (GM085930). S.E.O. acknowledges support from NIH (GM074820).

References

1. Dewick, PM. *Medicinal Natural Products: A Biosynthetic Approach*. Vol. 2nd Ed.. John Wiley & Sons, Inc.; 2004.
2. Van der Heijden R, Jacobs DI, Snoeijer W, Didier H, Verpoorte R. The *Catharanthus* alkaloids: pharmacogenosy and biotechnology. *Curr Med Chem* 2004;11:607–628. [PubMed: 15032608]
3. O'Connor SE, Maresh J. Chemistry and biology of monoterpene indole alkaloid biosynthesis. *Nat Prod Rep* 2006;23:532–547. [PubMed: 16874388]
4. DeLuca, V.; Laflamme, PT. *The Alkaloids: Chemistry and Biology*. Academic Press; 1998.
5. Wani M, Taylor H, Wall M, Coggon P, McPhail A. Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. *J Am Chem Soc* 1971;93:2325–2327. [PubMed: 5553076]
6. Johnson IS, Wright HF, Svoboda GH. Experimental basis for clinical evaluation of antitumor principles from *Vinca rosea*. *J Lab Clin Med* 1959;54:830–838.
7. Johnson IS, Armstrong JG, Gorman M, Burnett JP. The vinca alkaloids: a new class of oncolytic agents. *Cancer Res* 1963;23:1390–427. [PubMed: 14070392]
8. Klatman DL. Qinghaosu (artemisinin): an antimalarial drug from China. *Science* 1985;228:1049–1055. [PubMed: 3887571]
9. Stoeckigt J, Zenk MH. Isovincoside (strictosidine), the key intermediate in the enzymatic formation of indole alkaloids. *FEBS Lett* 1977;79:233–237.
10. de Waal A, Meijer AH, Verpoorte R. Strictosidine synthase from *Catharanthus roseus*: purification and characterization of multiple forms. *Biochem J* 1995;306:571–580. [PubMed: 7887913]
11. Kutchan TM, Hampp N, Lottspeich F, Beyreuther K, Zenk MH. The cDNA clone for strictosidine synthase from *Rauvolfia serpentina*. DNA sequence determination and expression in *Escherichia coli*. *FEBS Lett* 1988;237:40–44. [PubMed: 3049153]
12. Treimer JF, Zenk MH. Purification and properties of strictosidine synthase, the key enzyme in indole alkaloid formation. *Eur J Biochem* 1979;101:225–233. [PubMed: 510306]
13. Mizukami, Hajime; Nordlov, Hans; Lee, Siu-Leung; Ian Scott, A. Purification and properties of strictosidine synthetase (an enzyme condensing tryptamine and secologanin) from *Catharanthus roseus* cultured cells. *Biochemistry* 1979;18:3760–3763. [PubMed: 476085]
14. Pictet A, Spengler T. Über die Bildung von Isochinolin-derivaten durch Einwirkung von Methylal auf Phenyl-äthylamin, Phenyl-alanin und Tyrosin. *Chemische Berichte* 1911;44:2030–2036.

15. Cox ED, Cook JM. The Pictet-Spengler condensation: a new direction for an old reaction. *Chem Rev* 1995;95:1800–1802.
16. Ma X, Panjikar S, Koepke J, Loris E, Stöckigt J. The structure of rauwolfia serpentina strictosidine synthase is a novel six-bladed β -propeller fold in plant proteins. *Plant Cell* 2006;18:907–920.920 [PubMed: 16531499]*The crystal structures of strictosidine synthase in complex with natural substrates secologanin and tryptamine are achieved. This accomplishment identifies the residues lining the active site and reveals the structure of a six-bladed beta-propeller fold not previously observed in plant proteins. This structure allows the mechanistic elucidation of a Pictet-Spengler catalyzing enzyme for the first time.
17. Stockigt J, Panjikar S. Structural biology in plant natural product biosynthesis-architecture of enzymes from monoterpene indole and tropane alkaloid biosynthesis. *Nat Prod Rep* 2007;24:1382–1400. [PubMed: 18033585]
18. Loris EA, Panjikar S, Ruppert M, Barleben L, Unger M, Schubel H, Stockigt J. Structure-based engineering of strictosidine synthase: Auxiliary of alkaloid libraries. *Chem Biol* 2007;14:979–985. [PubMed: 17884630]
19. Bernhardt P, McCoy E, O'Connor SE. Rapid identification of enzyme variants for reengineered alkaloid biosynthesis in periwinkle. *Chem Biol* 2007;14:888–897. [PubMed: 17719488]
20. Maresh JJ, Giddings L, Friedrich A, Loris EA, Panjikar S, Trout BL, Stockigt J, Peters B, O'Connor SE. Strictosidine synthase: mechanism of a Pictet-Spengler catalyzing enzyme. *J Am Chem Soc* 2008;130:710–723.723 [PubMed: 18081287]*A detailed acid-catalyzed mechanism based on isotope effects and structural analysis is proposed by the authors. The findings suggest that a spiroindolenine intermediate, often invoked in the Pictet-Spengler mechanism, does not occur and that the rate-controlling step in the rearomatization of the positively charged intermediate.
21. Hampp N, Zenk MH. Homogeneous strictosidine synthase from cell suspension cultures of Rauwolfia serpentina. *Phytochemistry* 1988;27:3811–3815.
22. McCoy E, Galan MC, O'Connor SE. Substrate specificity of strictosidine synthase. *Bioorg Med Chem Lett* 2006;16:2475–2478. [PubMed: 16481164]
23. Chen S, Galan MC, Coltharp C, O'Connor SE. Redesign of a central enzyme in alkaloid biosynthesis. *Chem Biol* 2006;13:1137–1141. [PubMed: 17113995]
24. Runguphan W, O'Connor SE. Metabolic reprogramming of periwinkle plant culture. *Nat Chem Bio* 2009;5:151–153. [PubMed: 19151732]
25. Stadler R, Zenk MH. A revision of the generally accepted pathway for the biosynthesis of the benzyloquinoline alkaloid reticuline. *Liebigs Ann Chem* 1990:555–562.
26. Stadler R, Kutchan TM, Zenk MH. Norcoclaurine is the central intermediate in benzyloquinoline alkaloid biosynthesis. *Phytochemistry* 1989;28:1083–1086.
27. Stadler R, Kutchan TM, Loeffler S, Nagakura N, Cassels B, Zenk MH. Revision of the early steps of reticuline biosynthesis. *Tet Lett* 1987;28:1251–1254.
28. Samanani N, Facchini PJ. Purification and characterization of norcoclaurine synthase. The first committed enzyme in benzyloquinoline alkaloid biosynthesis in plants. *J Biol Chem* 2002;277:33878–33883. [PubMed: 12107162]
29. Samanani N, Liscombe DK, Facchini PJ. Molecular cloning and characterization of norcoclaurine synthase, an enzyme catalyzing the first committed step in benzyloquinoline alkaloid biosynthesis. *Plant J* 2004;40:302–313.313 [PubMed: 15447655]*The authors isolated a molecular clone encoding norcoclaurine synthase from *T. flavum* cell suspension culture cDNA library and produced a recombinant protein with enzymatic activity. The catalytic activity of norcoclaurine synthase defined a new class of plant secondary metabolic enzymes within the Bet v 1 and PR10 protein families. Kinetic analysis suggests a regulatory role for this enzyme.
30. Minami H, Dubouzet E, Iwasa K, Sato F. Functional analysis of norcoclaurine synthase in *Coptis japonica*. *J Biol Chem* 2007;282:6274–6282.6282 [PubMed: 17204481]*The authors report two distinct enzymes isolated from *C. japonica* which each catalyze the norcoclaurine synthase reaction. The differences between the two highly divergent proteins are discussed.
31. Ilari A, Franceschini S, Bonamore A, Arengi F, Botta B, Macone A, Pasquo A, Bellucci L, Boffi A. Structural basis of enzymatic (S)-norcoclaurine biosynthesis. *J Biol Chem* 2009;284:897–904.904 [PubMed: 19004827]*The crystallographic structure of norcoclaurine synthase from *T. flavum* in

complex with its natural substrate, dopamine, and a non-reactive aldehyde partner substrate is solved. This study revealed the residues and geometry of the active site and will provide the basis for more detailed mechanistic studies.

32. Berkner H, Schweimer K, Matecko I, Rosch P. Conformation, catalytic site, and enzymatic mechanism of the PR10 allergen-related enzyme norcoclaurine synthase. *Biochem J* 2008;413:281–290. [PubMed: 18384289]
33. Pasquo A, Bonamore A, Franceschini S, Macone A, Boffi A, Ilari A. Cloning, expression, crystallization, and preliminary X-ray data analysis of norcoclaurine synthase from *Thalictrum flavum*. *Acta Cryst* 2008;64:281–283.
34. Luk LYP, Bunn S, Liscombe DK, Facchini PJ, Tanner ME. Mechanistic studies on norcoclaurine synthase of benzylisoquinoline alkaloid biosynthesis: an enzymatic Pictet-Spengler reaction. *Biochemistry* 2007;46:10153–10161.10161 [PubMed: 17696451]*The mechanism of norcoclaurine synthase is examined in detail. Using substrate analogs and isotope labels, the authors suggest that the two-step cyclization reaction does not proceed through a spirocyclic intermediate.
35. Steffens P, Nagakura N, Zenk MH. Purification and characterization of the berberine bridge enzyme from *Berberis beaniana* cell cultures. *Phytochemistry* 1985;24:2577–2583.
36. Winkler A, Lyskowski A, Riedl S, Puhl M, Kutchan TM, Macheroux P, Gruber K. A concerted mechanism for berberine bridge enzyme. *Nat Chem Bio* 2008;4:739–741.741 [PubMed: 18953357] **The structure of berberine bridge enzyme is elucidated from *Eschscholzia californica* and the mechanism of reaction is determined based on kinetic studies and site directed mutagenesis. The evidence strongly suggests a concerted enzymatic reaction mechanism.
37. Winkler A, Hartner F, Kutchan TM, Glieder A, Macheroux P. Biochemical evidence that berberine bridge enzyme belongs to a novel family of flavoproteins containing a bi-covalently attached FAD cofactor. *J Biol Chem* 2006;281:21276–21285. [PubMed: 16728404]
38. Winkler A, Kutchan TM, Macheroux P. 6-S-cysteinylation of bi-covalently attached FAD in berberine bridge enzyme tunes the redox potential for optimal activity. *J Biol Chem* 2007;282:24437–24443. [PubMed: 17573342]
39. Kutchan TM, Dittreich H. Characterization and mechanism of the berberine bridge enzyme, a covalently flavinylated oxidase of benzophenanthridine alkaloid biosynthesis in plants. *J Biol Chem* 1995;270:24475–24481. [PubMed: 7592663]
40. Burow M, Wittstock U. Regulation and function of specifier proteins in plants. *Phytochem Rev* 2009;8:87–99.
41. Matile P. The mustard oil bomb-compartmentation of the myrosinase system. *Biochem Physiol Pflanz* 1980;175:722–731.
42. For review seeBauer L, Exner O. The chemistry of hydroxamic acids and n-hydroxyimides. *Angew Chem Int Ed* 1974;13:376–384.384
43. Wittstock U, Burow M. Tipping the scales- Specifier proteins in glucosinolate hydrolysis. *Life* 2007;59:744–751. [PubMed: 18085474]
44. Ettlinger M, Dateo GP, Harrison B, Mabry TJ, Thompson CP. Vitamin C as a coenzyme: the hydrolysis of mustard oil glucosides. *Proc Natl Acad Sci USA* 1961;47:1875–1880. [PubMed: 13890918]
45. Bednarek P, Pislewska-Bednarek M, Svatos A, Schneider B, Doubek J, Mansurova M, Humphry M, Consonni C, Panstruga R, Sanchez-Vallet A, Molina A, Schulze-Lefert P. A glucosinolate metabolism pathway in living plant cells mediates broad-spectrum antifungal defense. *Science* 2009;323:101–106. [PubMed: 19095900]
46. Rask L, Andreasson E, Ekblom B, Eriksson S, Pontoppidan B, Meijer J. Myrosinase: gene family evolution and herbivore defense in Brassicaceae. *Plant Mol Biol* 2000;42:93–113. [PubMed: 10688132]
47. Clay NK, Adio AM, Denoux C, Jander G, Ausubel FM. Glucosinolate metabolites required for an *Arabidopsis* innate immune response. *Science* 2009;323:95–101. [PubMed: 19095898]
48. Morant AV, Jorgensen K, Jorgensen C, Paquetts SM, Sanchez-Perez R, Moller BL, Bak S. β -Glucosidases as detonators of plant chemical defense. *Phytochemistry* 2008;69:1795–1813. [PubMed: 18472115]

49. Tookey HL. Crambe thioglucoside glucohydrolase: separation of a protein required for epithiobutane formation. *Can J Biochem* 1973;51:1654–1660. [PubMed: 4130022]
50. Bernardi R, Negri A, Ronchi S, Palmieri S. Isolation of the epithiospecifier protein from oil-rape (*Brassica napus* ssp. *Oleifera*) seed and its characterization. *FEBS Lett* 2000;467:296–298. [PubMed: 10675557]
51. Lambrix V, Reichelt M, Mitchell-Olds T, Kliebenstein DJ, Gershenzon J. The Arabidopsis epithiospecifier protein promotes hydrolysis of glucosinolates to nitriles and influences *Trichoplusia ni* herbivory. *Plant Cell* 2001;13:2793–2807. [PubMed: 11752388]
52. Matusheski NV, Swarup R, Juvik JA, Mithen R, Bennett M, Jeffrey EH. Epithiospecifier protein from broccoli (*Brassica oleracea* L.ssp. *italica*) inhibits formation of the anticancer agent sulforaphane. *J Agric Food Chem* 2006;54:2069–2807. [PubMed: 16536577]
53. Burow M, Bergner A, Gershenzon J, Wittstock U. Glucosinolate hydrolysis in *Lepidium sativum*- Identification of the thiocyanate forming protein. *Plant Mol Biol* 2007;63:49–61.61 [PubMed: 17139450]**The authors identify one of the few plant specifier protein known to date, thiocyanate-forming protein, from *Lepidium sativum*. The identification of this protein defines a new family of proteins that control glucosinolate hydrolysis.
54. Hasapis X, MacLeod AJ. Effects of metal ions on benzylglucosinolate degradation in *Lepidium sativum* seed autolysates. *Phytochemistry* 1982;21:559–563.
55. Burow M, Markert J, Gershenzon J, Wittstock U. Comparative biochemical characterization of nitrile-forming proteins from plants and insects that alter myrosinase-catalyzed hydrolysis of glucosinolates. *FEBS J* 2006;273:2432–2446. [PubMed: 16704417]
56. Adams J, Kelso R, Cooley L. The Kelch repeat superfamily of proteins: propellers of cell function. *Trends Cell Biol* 2000;10:17–24. [PubMed: 10603472]

Figure 1.

Enzymes from natural product biosynthetic pathways convert simple starting materials from primary metabolism into complex natural products. Representative pharmaceutically important compounds derived from plants are shown.

Figure 2.

Enzymes that catalyze the Pictet-Spengler reaction are shown. **A.** Strictosidine synthase catalyzes the condensation of secologanin and tryptamine to form an iminium species. The nucleophilic indole then attacks the iminium, and deprotonation leads to the strictosidine product. Computational studies suggest that pathway A is favored. Glu309 is believed to be responsible for all acid-base catalysis. **B.** Norcoclaurine synthase catalyzes iminium formation between dopamine and 4-HPAA (4-hydroxyphenylacetaldehyde). Lys122 is proposed to enhance the electrophilicity of the aldehyde so that iminium formation is facilitated. The C-2 hydroxyl group may be deprotonated prior to cyclization of the aromatic group onto the iminium. However, it is not clear which protein residues assist in this step. Glu110 may be involved in deprotonation of the final reaction intermediate to lead to formation of the product, (S)-norcoclaurine.

Figure 3.

The berberine bridge enzyme catalyzes carbon-carbon bond formation between the N-methyl group and the C-2' carbon of (S)-reticuline. Deprotonation of the C-3' hydroxyl group is critical for enhancing the nucleophilicity of the C-2' carbon for attack on the N-methyl group. As the carbon-carbon bond is formed, a hydride from the N-methyl group is transferred to the flavin. A base, not yet definitively identified, deprotonates the reaction intermediate to yield (S)-scoulerine.

Figure 4.

Glucosinolates are deglycosylated by myrosinase, and the resulting aglycone product rearranges to form isothiocyanates. However, when specifier proteins such as epithiospecifier protein (ESP) and thiocyanate-forming protein (TFP) the reaction pathway changes to yield epithionitriles, nitriles and thiocyanates. The mechanistic details of these specifier proteins remain to be elucidated.