# ALKALOIDS AND THEIR BIOSYNTHESIS

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**Keywords:** alkaloids, biosynthesis, biogenesis, ornithine, tyrosine, tryptophan, porphyrine, xanthine, lysine, anthranilic acid, histidine, terpenes

## Contents



## Summary

Alkaloids, first discovered in the beginning of the 19$^{th}$ century based on the examination of plants used in traditional medicine, have evolved to become a class of natural products of exceptional taxonomic and structural diversity, and of substantial chemical, biological, and therapeutic significance. Their biogenesis and biosynthesis have challenged chemists since the beginning of the 20$^{th}$ century, and only very few pathways are known in detail. Although recent efforts at the enzymatic level have evoked significant improvements in elucidating how alkaloids are produced in nature, even for many, pharmaceutically significant, alkaloids much remains unknown. Selective examples of the mechanistic pathways of formation of alkaloids, either experimentally determined or hypothesized, are discussed with an emphasis on those alkaloids of clinical relevance.

## 1. Introduction

Since their first isolation in the beginning of the 19$^{th}$ century, alkaloids have stirred the imagination, the creativity, and the very souls of chemists. They remain passionately pursued, for their beautiful and seemingly endless structural variation, for the challenges their synthesis provides to even the most sophisticated and erudite organic chemists, for the diverse biological responses which they provide, and for the abundant novelty and

acrobatics in the pathways of biosynthetic formation. No other group of natural products has provided such stimulation for chemists and biologists in the past 200 years.

Probably the first, semi-purified alkaloid isolated was the "*principium somniferum*" from opium obtained by Serturner, and published in 1805 in the *Journal der Pharmazie*. Alkaloids really came of age though as a result of the efforts of French chemists Pelletier and Caventou. Between 1819 and 1821 they succeeded in isolating brucine, quinine, and strychnine, following their successful isolation of emetine in 1817. Other chemists took up the challenge of investigating the constituents of biologically significant plants, and piperine, atropine, caffeine, solanine, chelidonine, coniine, nicotine, aconitine, and colchicine were all isolated before 1833. By 1837, when the Swedish chemist Berzelius wrote his *Lehrbuch der Chemie*, he was able to list thirteen "Pflanzenbasen". Sparteine was isolated in 1851, and cocaine in 1860. Although Meissner first coined the term "alkaloid" in 1819, it was not until 1882 that it was brought into common usage by Jacobsen in a review. What an alkaloid is, in terms of a definition, however, has remained elusive, and no attempt will be made here to fill that perceived gap. As has been said…."You know one when you see one." In previous discussions, one of us (GAC) has avoided any definition, choosing instead to suggest that they can be classified by their origin from nature and that they contain nitrogen, and yet are not polypeptides, proteins, or nucleic acids. Dewick has provided a very useful brief introduction to alkaloids and their significance, biologically and pharmaceutically.

Initial alkaloid isolations were from higher plants, particularly those used as medicines or known to be highly toxic. As the natural world was investigated chemically in the late 20$^{th}$ century, "alkaloids" were isolated from many different terrestrial and marine sources, including amphibians, arthropods, mammals, insects, sponges, fishes, fungi and bacteria, and, of course, *Homo sapiens*. From higher plants alone there are now at least 22,000 alkaloids known, so at this point, the total from all sources is probably in excess of 30,000.

The early alkaloid isolations were achieved before there was a notion of the complexity of molecular structure, and before the concepts of stereochemistry and the three-dimensional nature of compounds were developed. One of the dominant challenges for the ensuing 160 years was to develop the techniques, first chemical and then spectral, for determining the detailed structures of these alkaloids.

The first alkaloid structure to be determined was that of xanthine in 1882, and in 1886 that the first synthesis of an alkaloid, that of (+)-coniine, was reported by Ladenburg. Structure determination frequently involved chemical degradation under harsh conditions. As a result, the core heterocyclic nucleus was sometimes all that survived, and this became the foundation of a new branch of organic chemistry involving heterocyclic nuclei; for example, the distillation of quinine with KOH led to the isolation of quinoline, and indigo afforded indole.

The structural and stereochemical complexity of many of the alkaloids defied both structure determination and synthesis. Thus, while the indole alkaloid strychnine was first isolated in 1818, it was not fully characterized until 1947, and its synthesis was first described by Woodward in 1954. On the other hand, the important tropane nucleus was

synthesized in brilliant fashion, and along what proved to be biogenetic lines, by Robinson in 1917. It was many years however, before this philosophical concept led to the biogenetically-patterned synthesis of alkaloids of many different types.

In this context, synthesis and biogenesis progressed hand-in-hand, bolstered at crucial junctures with biosynthetic experimentation, initially with radio-isotopes and subsequently with stable isotopes. Following these studies, attempts were made to isolate and characterize the enzymes and the genes involved in alkaloid biosynthesis, and locate them within the organism and its cellular structure. Even today though, experimentation still significantly lags behind idealized concepts of natural formation. Consequently, the clear distinction is made that "biogenesis" refers to the theoretical concepts regarding the pathway of formation of a natural product, whereas "biosynthesis" refers to the verification of that pathway through experimentation (feeding experiments with precursors, enzyme isolations and characterizations, etc). As will be demonstrated in this chapter, experimentation has yet to surpass theory for all but a few alkaloid groups.

As increasing numbers of alkaloid structures were elucidated, the need arose to begin to classify "alkaloids" into various sub-groups, and to review these groups individually. In 1950, "*The Alkaloids, Chemistry and Pharmacology*" series of volumes began publication under the editorship of R.H.F. Manske, and the series continues today. Classification of alkaloids was made on a structural basis, and groups of alkaloids were named based on their parent heterocyclic nucleus, such as the tropanes, the indole alkaloids, the isoquinoline alkaloids, the benzylisoquinoline alkaloids, the acridone alkaloids, the steroidal alkaloids, etc. As it transpired, in many cases, although not all, these classifications also reflected a common biosynthetic origin. For example, an indole alkaloid would be derived from the amino acid containing an indole nucleus, tryptophan. These alkaloid group names typically continue to reflect both a core structural element and a common biosynthetic origin. Care is warranted however, because some heterocyclic nuclei, such as the piperidine nucleus and the quinoline nucleus, are known to have several different biosynthetic origins, and thus a classification of "piperidine" or "quinoline" alkaloids is not appropriate.

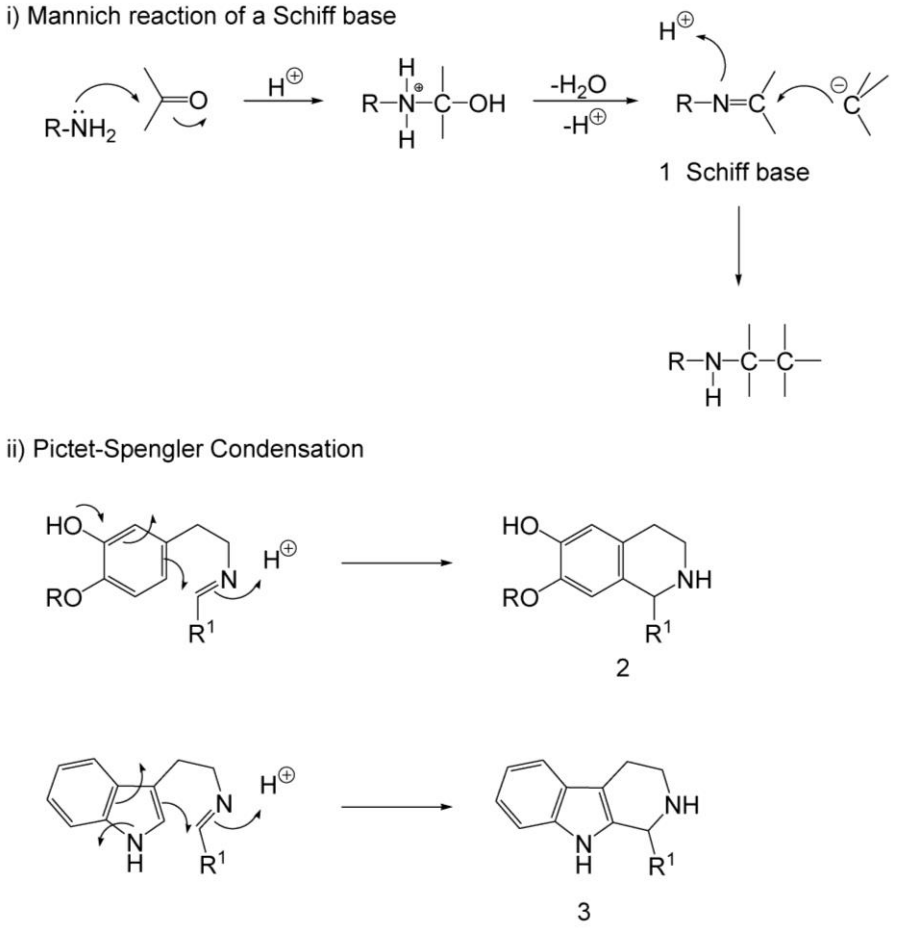
An analysis by Cordell, Quinn, and Farnsworth in 2001 found that of the 83 higher plant orders according to Cronquist, 16 do not contain alkaloids, whereas based on plant genera, about 14.2% of higher plants contain alkaloids (1730 of 7231). Approximately 35 higher plant families have had alkaloids detected in them, and no alkaloids have yet been isolated. In addition, there are 153 plant families which have never been examined for alkaloids. At that time, there were over 1870 alkaloid skeleta known and over 21,120 alkaloids structurally determined. The twenty most important plant families for the production of alkaloids are the Amaryllidaceae, Annonaceae, Apocynaceae, Asteraceae, Berberidaceae, Boraginaceae, Buxaceae, Celastraceae, Fabaceae, Lauraceae, Liliaceae, Loganiaceae, Menispermaceae, Papaveraceae, Piperaceae, Poaceae, Ranunculaceae, Rubiaceae, Rutaceae, and Solanaceae.

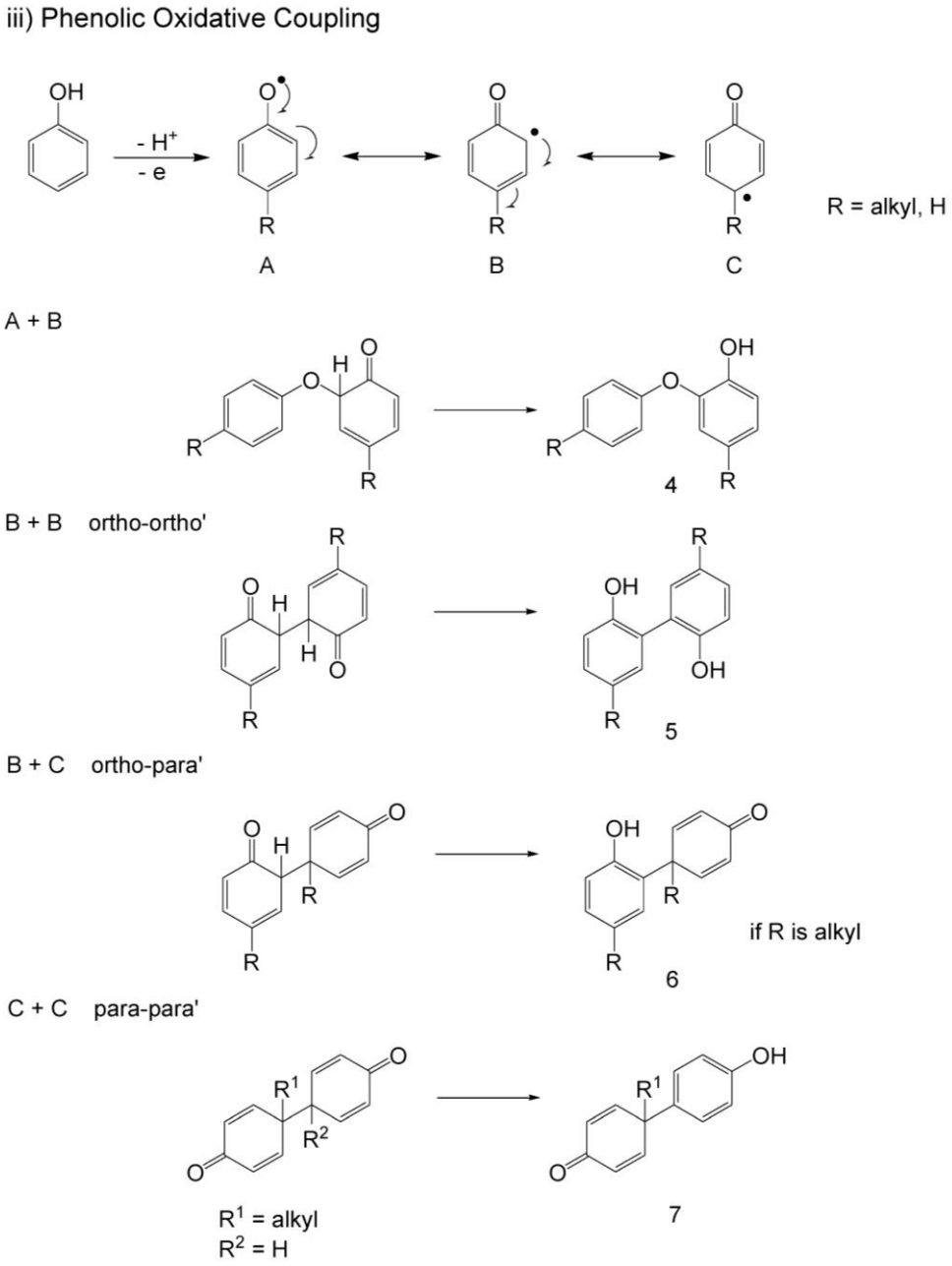
Before there was any experimental proof of the derivation of alkaloids from amino acids, there was substantial speculation regarding their origin and the interrelationships between alkaloids and alkaloid groups. When biosynthetic experimentation began in the

early 1950's after the introduction of radio-labeled precursors, it was organic chemists who took the initiative, with groups led by Birch, Barton, Battersby, Arigoni, Scott, Spencer, and Leete who clarified many important fundamental aspects of alkaloid biosynthetic pathways. When it became apparent about 20 years ago that studies were needed at the cellular and then enzyme levels, and from these to the cloning and expression of systems which could produce alkaloids *ex situ*, it was the groups of Zenk, Stöckigt, Kutchan, Robins, Yamada, and Verpoorte which led the way. Now, a new phase has evolved in which alkaloid biosynthesis is being studied from a regulatory and from a metabolic engineering perspective. Here the leaders have been Kutchan, Facchini, and Yamada.

Until recently, the review journal *Natural Products Reports*, published by The Royal Society of Chemistry, and through the magnificent efforts of Richard Herbert, provided excellent coverage of this area of natural product chemistry and biology.

Prior to discussing a few of the amazing pathways to the diversity of alkaloids, it is pertinent to review three key reactions which form the cornerstone of the biosynthesis of alkaloids: i) the Mannich reaction of a Schiff base with a nucleophile, ii) the Pictet-Spengler condensation, and iii) the phenolic coupling reaction (Scheme 1).






Scheme 1. Important Reactions in Alkaloid Biosynthesis

i) When an aldehyde or a ketone condenses with an amine and elimination of water occurs, the product is a Schiff base (e.g. **1**). This species is a very powerful electrophile, and can attract a nucleophilic cation from any one of a number of sources. Frequently, the product represents a new, enhanced carbon skeleton with a heterocyclic nucleus.

ii) When the Schiff base is attacked intermolecularly by an aromatic nucleus, the product is often either a tetrahydroisoquinoline (e.g. **2**) or a tetrahydro-beta-carboline (e.g. **3**). This process is known as the Pictet-Spengler reaction.

iii) When a phenolic hydroxyl group is oxidized by the loss of a hydrogen radical, a highly reactive radical intermediate is produced which can be trapped either internally, or by another radical-containing unit, to form several different products, including those derived from carbon-oxygen bond formation (e.g. **4**) and carbon-carbon bond formation at the positions *ortho*- (e.g. **5**) or *para*- (e.g. **6** and **7**) to the phenolic radical. In this way, new linkages and a variety of new basic alkaloid skeleta can be produced. Some examples of these processes will be given in the section on alkaloids derived from phenylalanine and tyrosine.

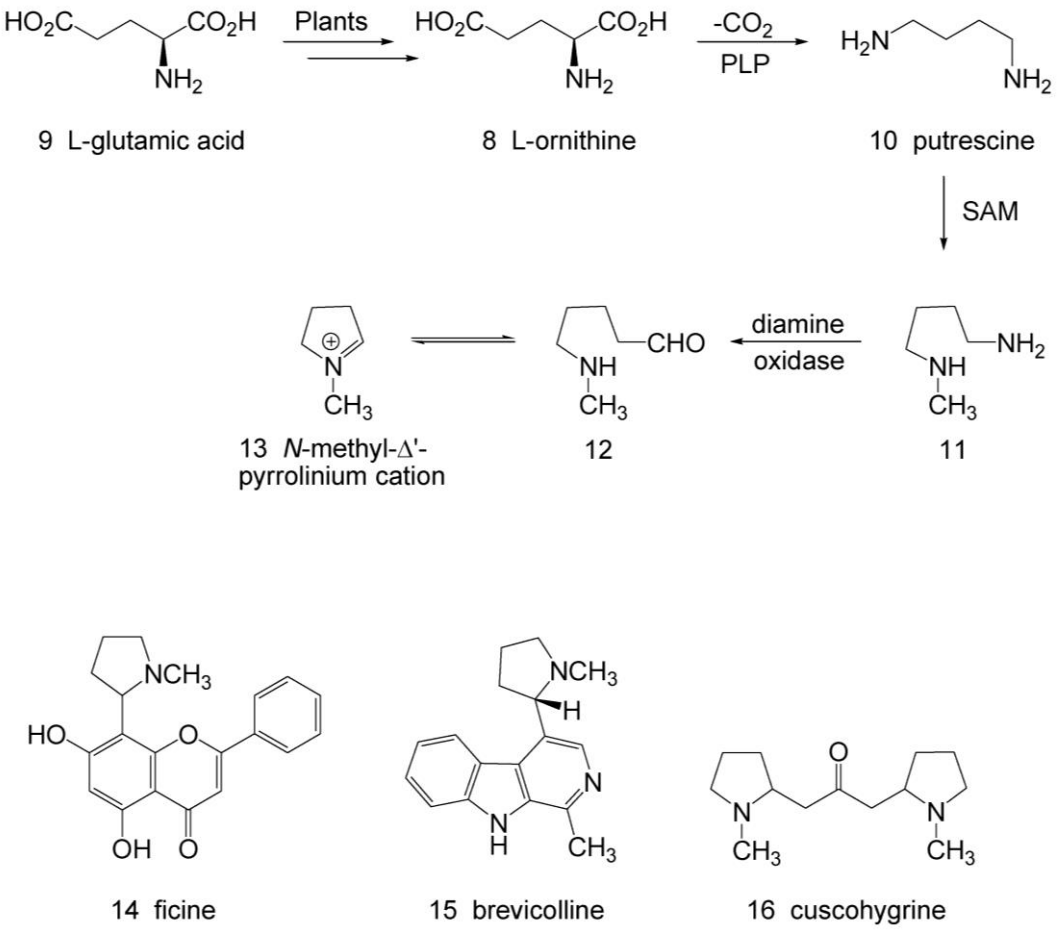
Metabolic engineering has become a major driving force for the reinvestigation of alkaloid biosynthesis. Through the overexpression of an enzyme that is rate-limiting in the pathway an important avenue to improve the availability of crucial intermediates or of pharmaceutically important products may be provided. Introducing new aspects to the pathway may produce metabolites that have not been observed previously. Or a "knock-out" strategy of deleting a key enzyme may allow a crucial intermediate in the pathway to accumulate. These techniques can evolve into significant approaches to increase the number of available metabolites which an organism can produce, and thus is of interest as an approach to increase the molecular diversity available for biological screening in pharmaceutical companies. Metabolic engineering can also be used to develop strains of plants which no longer produce undesired metabolites, such as caffeine in tea and coffee. Placing alkaloid-producing enzymes in a heterologous system, such as a microbial or insect system has been successful in enhancing the yield of the enzymic product more rapidly than in plant tissue culture systems, and in making the enzyme more available for crystallographic study. The future potential is very exciting as there is much to learn regarding the utilization of alkaloid biosynthetic pathways to produce medicinal agents more effectively for enhanced health care.

In this brief chapter, the selected alkaloid groups are organized by their experimentally demonstrated, or biogenetically postulated and extrapolated, precursor molecular unit, whether that is an amino acid or other precursor entity. Only four groups of alkaloids are discussed (the tropanes, some of the alkaloids derived from phenylalanine and tryptophan, some alkaloids from miscellaneous amino acids, the purines, and some selected other alkaloid groups, such as the terpenoid alkaloids). Some discussion is included with respect to the origin of the alkaloid group and very brief mention is made of the biological properties and pharmaceutical uses. The synthesis of alkaloids is not discussed, and readers are directed to either "*The Alkaloids: Chemistry and Biology*" series edited by G.A. Cordell or the "*Alkaloids: Chemical and Biological Perspectives*" series edited by the late S.W. Pelletier, for more details of the chemistry of the various alkaloid groups.

## 2. Alkaloids Derived from Ornithine

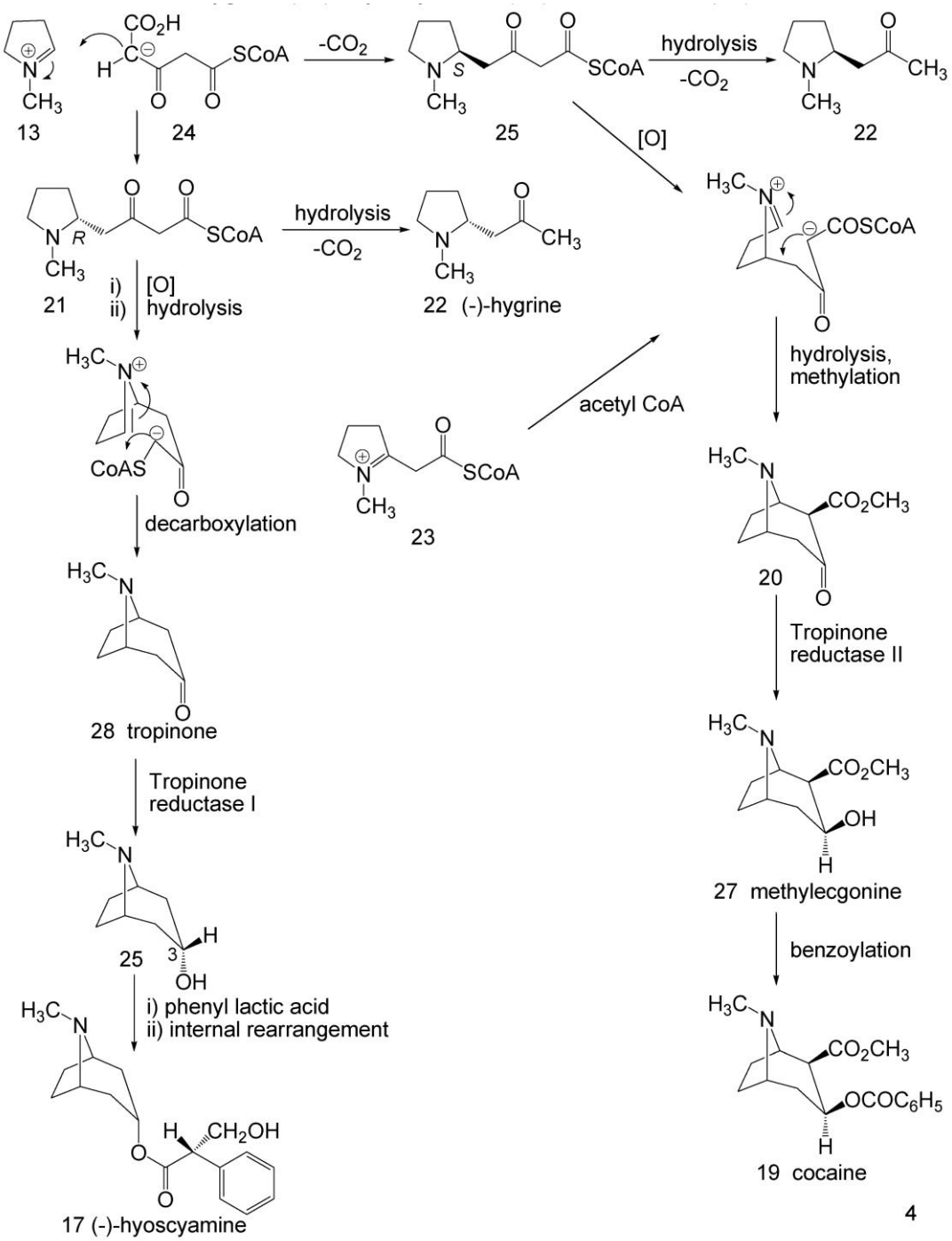
Ornithine (**8**) is a simple amino acid comprised of five carbons and two nitrogen atoms. In animals it is produced from arginine, through the action of the enzyme arginase; in plants it is derived from glutamic acid (**9**). In plants, not all of these atoms are incorporated into the secondary metabolic product. Typically, one of the nitrogen atoms, and often the carboxylic acid carbon, is lost, and the symmetrical intermediate putrescine (**10**) is often critical in the pathway. *N*-Methylation of putrescine with SAM

is the first step in tropane alkaloid biosynthesis, and the cDNAs encoding for this transferase have been isolated from *Atropa belladonna* L. and *Hyoscyamus niger* L. Reaction of *N*-methyl putrescine (**11**) with diamine oxidase yields an equilibrium mixture of the amino-aldehyde **12** and the important Schiff base, the *N*-methyl-$\Delta^1$-pyrrolinium cation (**13**) (Scheme 2). A methylputrescine oxidase from *Nicotiana tabacum* has been cloned and characterized. The *N*-methyl-$\Delta^1$-pyrrolinium cation (**13**) is readily attacked by nucleophilic centers and appears as a unit in alkaloids such as ficine (**14**), brevicolline (**15**), and cuscohygrine (**16**).




Scheme 2. Formation of *N*-Methyl-$\Delta^{'}$-pyrrolinium Cation (13)

The tropane and ecgonine alkaloids are a biologically powerful group of alkaloids. They include (-)-hyoscyamine (**17**) (whose racemate is atropine) and scopolamine (**18**) in the tropane group, and cocaine (**19**) in the ecgonine group, each with a long history of traditional use over the millennia. Atropine (**17**) is used as a mydriatic agent and scopolamine (**18**) for motion sickness. Cocaine (**19**), besides its widespread illicit use, remains as a powerful anesthetic, and served as the model for several important synthetic anesthetic agents (benzocaine, lidocaine, etc.). The tropane alkaloids occur in several genera in the family Solanaceae (*Atropa*, *Datura*, *Hyoscyamus*, and *Duboisia*), while the ecgonine alkaloids are restricted to the genus *Erythroxylum* in the family Erythroxylaceae. They differ in structure by the presence in the ecgonine alkaloids of a carbomethoxy group at the C-2 position and an inversion of stereochemistry at C-3.



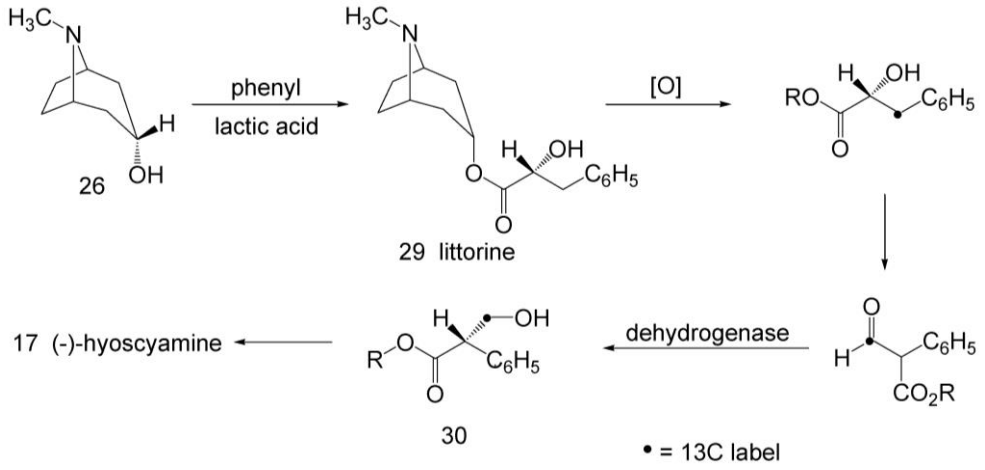

Scheme 3. Formation of the Tropane Alkaloids Hygrine (22), Hyoscyamine (17) and Cocaine (19)

The pathway towards (-)-hyoscyamine (**17**) involves acetoacetate or the sequential addition of acetate to the *N*-methyl-$\Delta^1$-pyrrolinium species **13** to afford the ecgonine nucleus (**20**) (Scheme 3). The acid **21** (as its CoA ester) is viewed as the next intermediate, after it was found that hygrine (**22**) and 2-(1-methylpyrrolidin-2-yl)acetate (**23**) were not precursors of hyoscyamine (**17**) in root cultures of *D. stramonium*. These results indicate that a four-carbon acetoacetate unit **24** is incorporated as a single unit.

Similar results were observed for scopolamine (**18**) thereby precluding the stepwise addition of acetate. However, studies in *Erythroxylum coca* Lam. on cocaine (**19**) biosynthesis did suggest a sequential build up of the four carbon chain from the ester **23**, which as the labeled methyl ester was well incorporated into **19**. Cuscohygrine (**16**) from *Erythroxylum coca* is derived from an acetoacetate **24** precursor and two *N*-methyl-$\Delta^1$-pyrrolinium (**13**) species.

The two series of tropane alkaloids differ in their stereochemistry at the C-3 position, an α-orientation in the formation of tropine (**26**) on the pathway to **17**, and a β-orientation in the formation of methylecgonine (**27**) on the pathway to **19**. The separate reductases for these processes, TRI and TRII, have been isolated and characterized. They selectively reduce the tropinone system of **28** or **20** to produce an α- or a β-hydroxy group, respectively for the tropane or ecgonine alkaloids. They co-occur in all Solanaceae plants producing tropane alkaloids.

A very unusual intramolecular rearrangement occurs at the alkaloid level in the formation of (-)-hyoscyamine (**17**) from tropine (**26**) through the intermediate littorine (**29**) (Scheme 4). The lactic acid portion of this alkaloid rearranges through a mutase reaction involving radical intermediates generated by P450 enzymes, followed by the action of a dehydrogenase to afford the tropic acid derivative **30**, probably through a concerted carbonation process.




Scheme 4. Formation of (-)- Hyoscyamine (17) from Littorine (29)

Other modifications to the tropane nucleus can involve hydroxylations at the 6β- and 7β- positions, followed by esterification, or oxidation to form a 6,7-oxido derivative, (-)-hyoscine (scopolamine) (**18**) (Figure 1). The gene for hyoscyamine 6β-hydroxylase has been expressed in *Hyoscyamus niger* and *Atropa belladonna*. Thus, 6-$^{18}O$-6β-hydroxyhyoscyamine (**31**) is incorporated intact into hyoscine (**18**) in *Duboisia myoporoides* R.Br. establishing that 6,7-dehydrohyoscyamine is not an intermediate; the dioxygenase for this process has been isolated. Evidence suggests that ornithine, as the *N*-methyl-$\Delta^1$-pyrrolinium cation (**13**), is incorporated in an unsymmetrical manner into hyoscyamine (**17**) in *Datura*, but through a symmetrical intermediate in *Nicotiana*, *Hyoscyamus*, and *Erythroxylum*. Detailed reviews of tropane alkaloid biosynthesis are available, including aspects of the architecture of the enzymes involved.

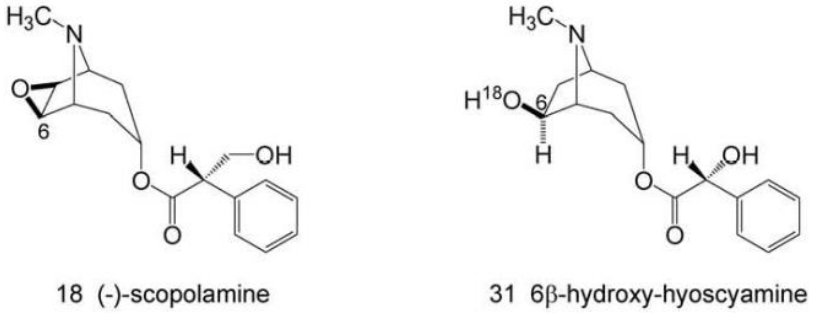



Figure 1. Scopolamine (18) and 6β-hydroxy-hyoscyamine (31)

## 3. Alkaloids Derived from Phenylalanine/Tyrosine

Phenylalanine (**32**) is a precursor of a very substantial number of alkaloids with a high level of structural diversity. 4'-Hydroxyphenylalanine (**33**), tyrosine, is produced from **32** through an oxidation reaction involving the NIH-shift of the 4'-proton. The alkaloids derived from **32** and **33** range in structure from simple derivatives, such as mescaline (**34**), to tetrahydroisoquinolines [pellotine (**35**)], the morphinan alkaloids [morphine (**36**)], and the complex bisbenzylisoquinoline alkaloids, such as tetrandrine (**37**). The aromatic nucleus can also be cleaved, yielding a system such as betanidin (**38**), or reduced and cyclized, as found in securinine (**39**) (Figure 2).

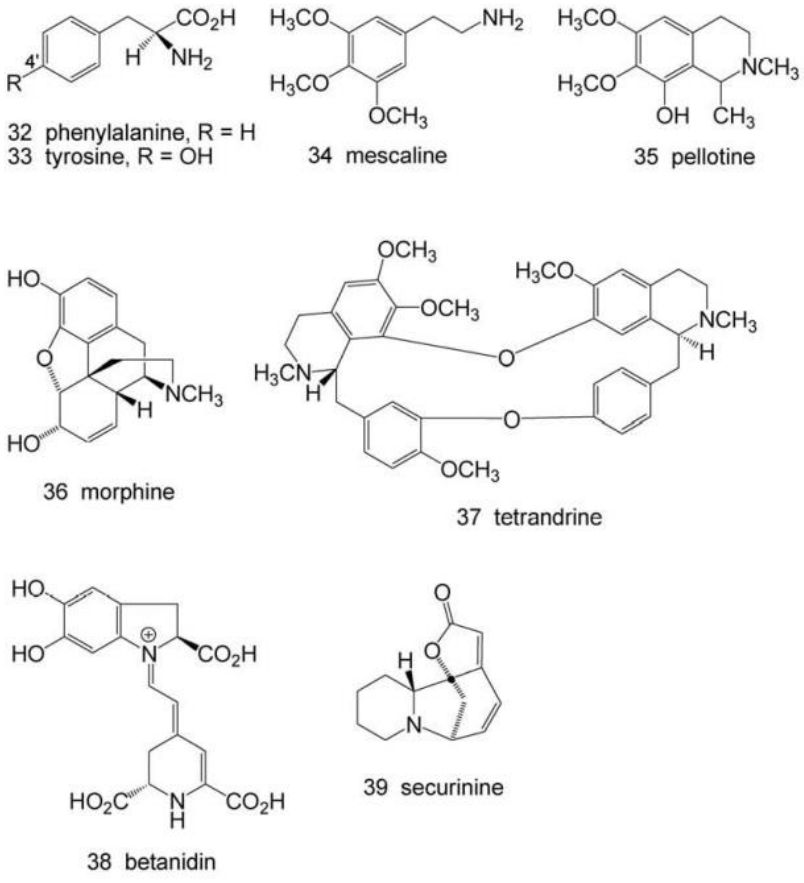



Figure 2. Representative Phenylalanine (32)/Tyrosine (33) Derivatives.

There is a wide range of plant families associated with alkaloids derived from phenylalanine (**32**) and tyrosine (**33**), although some individual structural types do have a very limited distribution reflecting taxonomically-limited biosynthetic pathways. This section will focus only on selected members of the benzylisoquinoline and phenethylisoquinoline alkaloids.

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**Biographical Sketches**

**Professor Geoffrey A. Cordell** obtained his Ph.D. on the isolation and synthesis of secodine alkaloids at the University of Manchester in 1970 with Drs. George F. Smith and George N. Smith. After two years as

a NATO postdoctoral fellow with Professor George Büchi at the Department of Chemistry, M.I.T. he joined the research group of Professor Norman R. Farnsworth at the College of Pharmacy, University of Illinois at Chicago (UIC). A Professor since 1980, he served as Head of the Department of Medicinal Chemistry and Pharmacognosy for 12 years and as Interim Dean of the College of Pharmacy for almost three years, as well as holding several other senior administrative positions at the Department, College, and Campus levels. Most recently he was Director of the Center for Advanced Design, Research and Exploration in the Office of the Vice Chancellor for Research.

He is the author of over 560 research publications, book chapters, and comprehensive reviews, and the editor of 35 books, including 29 volumes in the series "*The Alkaloids: Chemistry and Biology*". He was the author of "*Introduction to Alkaloids: A Biosynthetic Approach*". He is a member of the Editorial Advisory Board of twenty-one international scientific journals and has served as a consultant for numerous local, national, and international granting agencies, universities, and corporations. He is a former President of the American Society of Pharmacognosy, and in 2007 was named as one of 14 Honorary Members world-wide. He is an elected Fellow of the Royal Chemical Society, the Linnean Society of London, and the American Association of Pharmaceutical Scientists, an Honorary Professor at Sichuan University, the Foreign Director of the Sichuan Academy of Chinese Medical Sciences in Chengdu, China, and an International Collaborative Partner at Universiti Tunku Abdul Rahman, Kuala Lumpur, Malaysia. He has been invited to give lectures at over 140 international meetings. His research is focused on the sustainability of medicinal agents, new methods for the analysis of biologically active natural products, and the use of vegetables as chemical reagents. He is presently assisting institutions and organizations in several countries around the world develop their natural product research programs.

**Dr. Taylor Choi** recently completed her Ph.D. in pharmacognosy at the University of Illinois at Chicago, of which a portion was completed at the Technische Universität Dresden, Germany with Professor Hans-Joachim Knölker. She worked as a post-doctoral fellow at the Institute for Tuberculosis Research, specializing in drug metabolism and pharmacokinetic studies. She then worked as a post-doctoral fellow at the University of Medicine and Dentistry of New Jersey, focusing her research on select agents and select agent toxins.