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Biosynthetic Pathways to Glycosidase Inhibitors

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ABSTRACT

Glycosidase inhibitors are important compounds that can interfere with several biosynthetic processes including *N*-linked glycosylation and the biosynthesis of several glycoproteins. Understanding the biogenesis of naturally occurring glycosidase inhibitors would be a crucial step towards the chemical synthesis of analogues of choice. This review focuses on the current knowledge regarding the biosynthesis of a series of polyhydroxylated saturated nitrogen heterocycles including nojirimycin and swainsonine among others, with a potent biological activity as inhibitors of glycosidases and transglycosidases.

KEYWORDS

Biosynthesis / Glycosidase Inhibitors / Nojirimycin / Deoxynojirimycin / Swainsonine

1. Introduction

Glycosydase inhibitors are a rapidly growing family of molecules mostly consisting of polyhydroxylated mono- and bicyclic saturated nitrogen heterocycles commonly referred to as iminosugars (Figure 1).[1-3] Compounds like nojirimycin **1**, 1-deoxynojirimycin **2**, DMDP **3**, castanospermine **4** or swainsonine **5** and their derivatives play crucial roles in the biological activities of some pharmaceutically important compounds. [4-6]

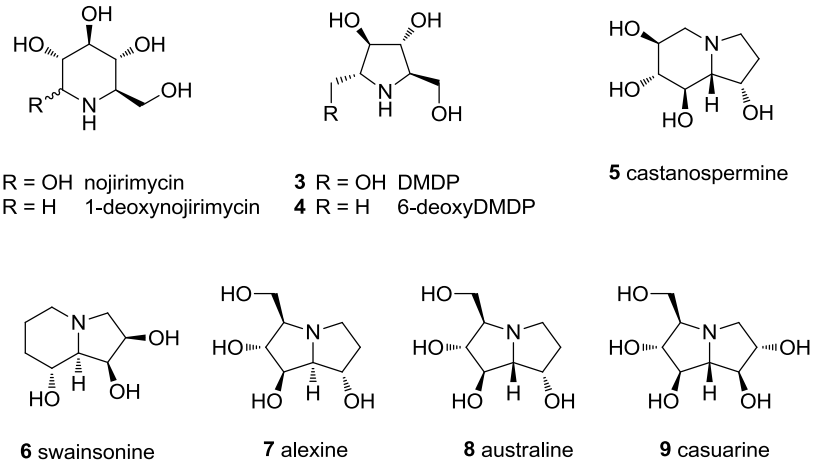
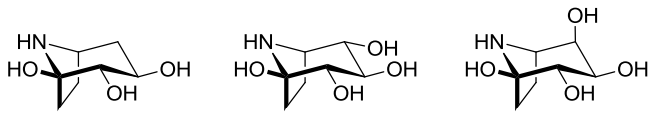


Figure 1. Glycosidase inhibitors

Nojirimycin **1** was originally isolated from cultures of several strands of *Streptomyce*s [7,8] and *Bacillus*[9] and 1-deoxynojirimycin was isolated from plants of genus *Morus*.[10] Compounds **1** and **2** inhibit various glycosidases having important effects on the biosynthesis of membrane and secretory glycoproteins.[11] DMDP **3** can be isolated from the cyanobacterial genus *Cylindrospermum* and it is capable of effectively inhibiting digestive glycosidases.[12] The 6-deoxyderivative **4** has been isolated from *Angylocalyx pynaertii* and in contrast to other polyhydroxylated pyrrolidines it was found to be unique in inhibiting β–mannosidase.[13] Castanospermine **5** was first isolated from the seeds of *Castanosperma australe*[14] and it has demonstrated antiviral activity.[15,16] It is also known that castanospermine interfere with the metabolism of glycogen[17] and it inhibits several glycosidases.[18] Swainsonine **6** was first isolated from *swainsona* in Australia but it is also present in numerous plants and fungi. Compound **6** has antitumoral activity[19,20] although some clinical trials were discouraging.[21] Other natural pyrrolidine alkaloids like alexine **7**, australine **8** and casuarine **9** have also been isolated from plants and microorganisms.[22]

Calystegines were found in the medicinal plant *Atropa belladonna* and consist of a nortropane skeleton with three or four hydroxyl groups (Figure 2). There are up to 14 different structures of natural occurring calystegines isolated from a variety of vascular plants. They cannot be found in fungi or microorganisms. The chemistry and biology of calystegines including chemotaxonomy, biological activity and some insights on the biogenesis in the context of co-occurrence with tropane alkaloids have been compiled by Dräger in an excellent review[23] and a chapter book,[24] covering literature from 1998 to middle 2003 and up to 2007, respectively. Since then there has not been relevant communications in the topic; so, in this review calystegines will not be treated and for previous work the reader is directed to the above mentioned reviews.



10 calystegin A_3 **10** calystegin B_2 **10** calystegin B_4

Figure 2. Calystegines

The interest in glycosidase inhibitors has increased enormously during the past two decades and the number of synthetic approaches to their preparation is extremely extensive and growing at a rapid rate.[25,26] One reason for this huge synthetic activity is the great variety of biological activity against different enzymes that can be found depending on the absolute configuration of the stereogenic centers bearing the hydroxyl groups.

The mechanism of the various existing glycosidases is known to proceed through oxocarbenium-like transition structures[27,28] and it is well-accepted that inhibition of typical glycosidase inhibitors occur because such sugar mimics resemble the structural features of the transition state.[29] Different configurations as well as conformational restrictions in inhibitors help to a better recognition by the enzyme contributing to a higher inhibition activity.[30]

In the large group of glycosidase inhibitors, synthetic studies have already been highlighted in several classes,[31-33] e.g. for pyrrolidines,[34,35] piperidines,[36,37] bicyclic compounds such as indolizidines[38] and pyrrolizidines[39,40] and imino disaccharides.[41,42] However, only a little is known about biosynthetic routes towards that sort of compounds. It can be expected that the knowledge of the biosynthetic

pathways can be used to manipulate the metabolite pattern of involved microorganisms, directing the fermentation process to produce desired metabolites.

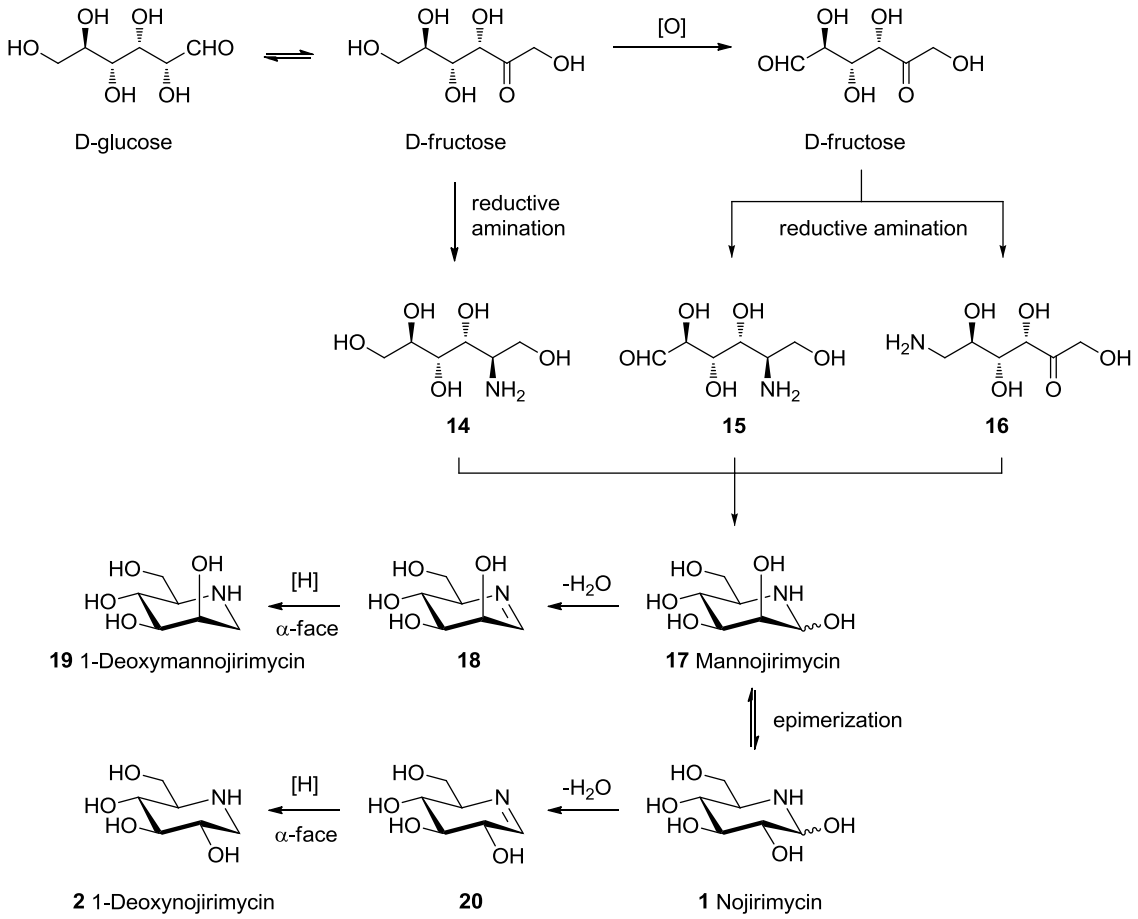
In this review, we summarize the current knowledge regarding the biosynthesis of naturally occurring glycosidase inhibitors. Semisynthetic studies on the structural and pharmaceutical properties of these compounds have been extensively reported and will not be covered here. Similarly, for detailed information of natural occurrence of discussed molecules and precise synthetic approaches the reader is referred to previous reviews. On the other hand, experimental evidences supporting the existence of various routes in microorganisms will be discussed.

2. Biosynthesis of monocyclic compounds. Piperidines and pyrrolidines

Nojririmycin **1** has been isolated from a variety of microorganisms including *S. roseochromogenes, S. lavendulae, S. nojiriensis* and *S. subrutilus*. The last one, when grown on a glucose-containing soyabean medium produces both 1-deoxymannojirimycin and 1-deoxynojirimycin. Experiments with deuterated glucose showed incorporation of deuterium at C-6 in both alkaloids indicating that the first step in the biosynthesis of both iminosugars is the isomerization of glucose to fructose. Accordingly, it is proposed mannojirimycin **17** as the first iminosugar to be formed.[43]

In fact, when 6,6-[$^2H_2$]-glucose was employed, NMR analysis of deuterium labellled 1-deoxynojirimycin **2** showed that only the equatorial proton at C-1 had been replaced by deuterium, in agreement with the oxidation of the primary hydroxyl group at C-6 in fructose to give **13** with the loss of one hydrogen atom. The introduction of the amino group is certainly unknown and three ways are possible through derivatives **14**-**16**, all of them being possible precursors of mannojirimycin **17**.[44] Elimination of water from **17** and further reduction afforded the observed 1-deoxymannojirimycin **19**. This route was confirmed by using 5-[^{2}H]-glucose in the fermentation. Under such conditions deuterium was only incorporated at C-2 in mannojirimycin **17**. The 1-deoxynojirimycin **2** obtained in this experiment did not show incorporation of deuterium. This is in agreement with the hypothesis that either mannojirimycin **17** or its 1-deoxy derivative **19** are precursors in the biosynthetic scheme, since loss of hydrogen isotope from C-2 would be expected upon epimerization of **17** or **19**. Further experiments with deuterated substrates deomonstrated that epimerization occurs predominantly at nojirimycin level, i.e. between **17** and **1**. It had been reported that 1-deoxynojirimycin **2** could be epimerized to 1-deoxymannojirimycin **19** by a strain of

Agrobacterium sp. trough an oxidation to a cyclic ketocompound followed by reduction to give the epimeric derivative. However, by considering this hypothesis it is difficult to justify the presence of nojirimycin **1**.



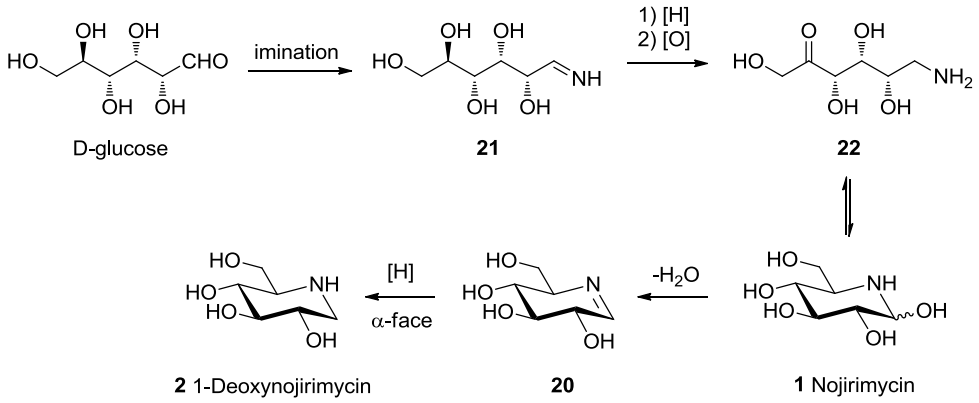
Scheme 1. Biosynthesis of mannojirimycin **17**, nojirimycin **1** and their deoxy derivatives **19** and **2** from D-glucose in *Streptomyces subrutilus*

Similar experiments carried out with *Bacillus subtilis var niger* only produced 1-deoxynojirimycin **2** and no traces of 1-deoximannojirimycin **19** were found.[45] Also in this case, labeling studies demonstrated that glucose is the precursor of 1-deoxynojirimycin **2**. Additional enzyme assays and labeling studies supported that both mannojirimycin **17** and nojirimycin**1** are intermediates in the biosynthesis of 1-deoxynojirimycin **2**.

Recently, the complete genome sequence of *Bacillus amiloliquefaciens* has been determined[46] and a gene cluster that initiates the biosynthesis of **2** in such microorganism has been identified and provided further evidence for the pathway illustrated in Scheme 1.[47] Additionally, three enzymes involved in the first steps of

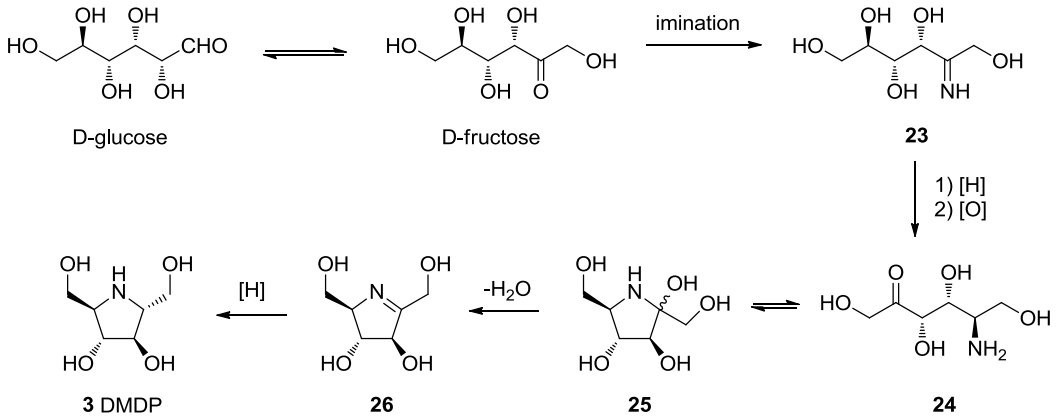
the biosynthesis have also been identified. Noteworthy, the same gene cluster has been found in *Bacillus atrophaeus*[48] as well as in *Bacillus subtilis*,[49] known producers of 1-deoxynojirimycin **1**.

The biosynthetic route to 1-deoxynojirimycin **2** is, however, different for higher plants as demonstrated by Shibano and co-workers.[50] These authors studied the biosynthesis of **2** by using 1-[^{13}C]-glucose in the higher plant *Commelia communis*. While a significant ^{13}C enrichment was observed at C-6 for compound **2** obtained in microorganisms, in the case of that being produced in plants the ^{13}C enrichment was located at C-1. These experiments resulted in the proposal outlined in Scheme 2. According to this hypothesis C-1/C-5 cyclization is produced in the original glucose molecule without any type of inversion. Additional support was provided by the fact that the same ^{13}C enrichment was observed in fructose obtained from administration of 1-[^{13}C]-glucose.



Scheme 2. Biosynthesis of nojirimycin **1** and1-deoxynojirimycin **2** from D-glucose in *Commelia communis*.

When imination is produced on fructose, formed by isomerization of glucose, the same process led to DMDP **3** (Scheme 3). Indeed, compound **3** is obtained from 1-[^{13}C]-glucose under the same conditions employed for the preparation of 1-deoxynojirimycin **2**.[50]



Scheme 3. Biosynthesis of DMDP **3** from D-glucose in *Commelia communis*.

Broussonetines are polyhydroxylated pyrrolidines bearing a long carbon chain at C-2. They have been isolated from *Broussonetia kazinoki*.[51] ^{13}C NMR spectroscopy studies after feeding experiments using 1-[^{13}C]-glucose demonstrated that broussonetine J **27** is synthesized through routes similar to those of sphingosine and phytosphingosines.[52] Similar results were obtained for broussonetines C **28** and E **29**.

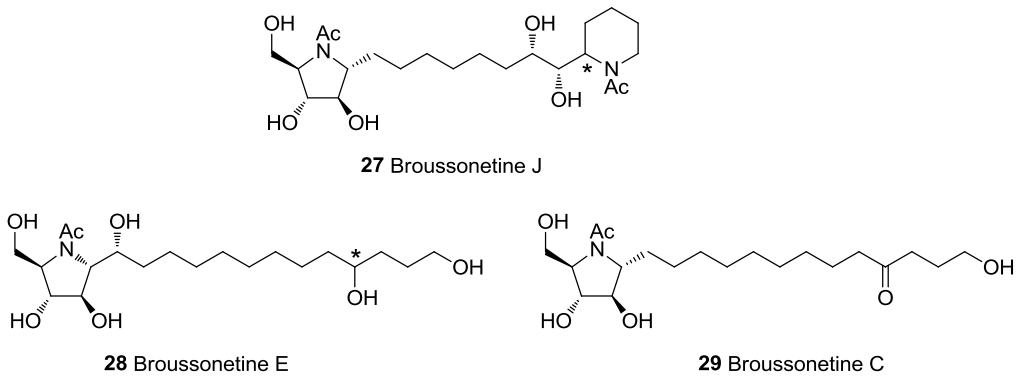
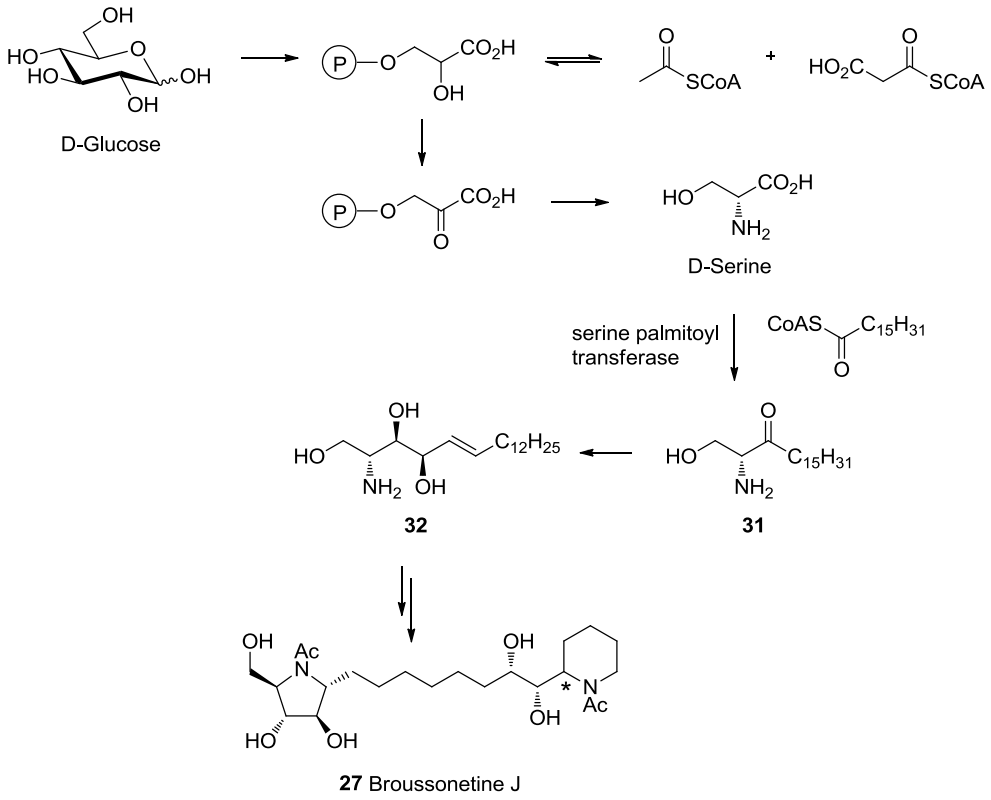


Figure 3. Broussonetines

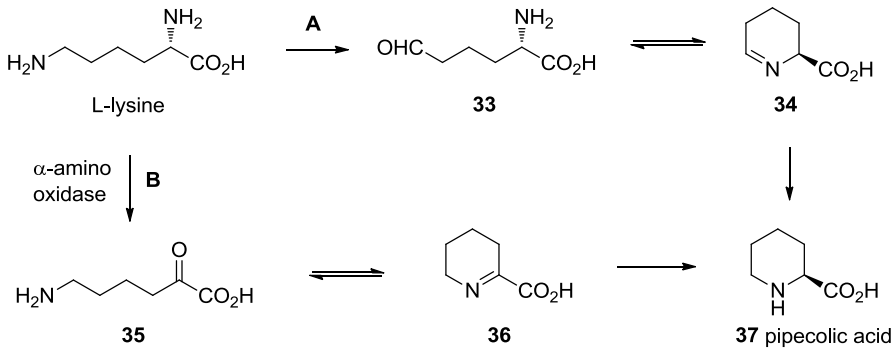
Accordingly, in the case of broussonetines, it is assumed that after typical transformation of D-glucose into serine by well-known metabolic routes[53,54] the introduction of the side chain is achieved by condensation of serine with palmitoyl-CoA. The labeling pattern found in the side chain also indicated that the palmitoyl fragment was formed through the acetate-malonate pathway (Scheme 4). The absolute configuration of the pyrrolidine moieties in most broussonetines is related to D-serine except in the case of broussonetine U **30** in which is related to L-serine.



Scheme 4. Biosynthesis of broussonetine J from D-glucose in *Broussonetia kazinoki*.

3. Biosynthesis of bicyclic compounds. Indolizidines.

The biosynthesis of the piperidine nucleus of bicyclic iminosugars starts with the production of pipecolic acid, which is the precursor of several compounds such as swainsonine or slaframine. Pipecolic acid was found to be a product of lysine catabolism in animals, microorganisms and plants. Grobbelaar and Steward established the transformation oflysine into pipecolic acid through route A (Scheme 5) by using labeled lysine in bean plants *Phaseolus vulgaris*.[55] According to their findings the nitrogen of the pipecolic acid should be supplied by the α-amino group of the lysine.

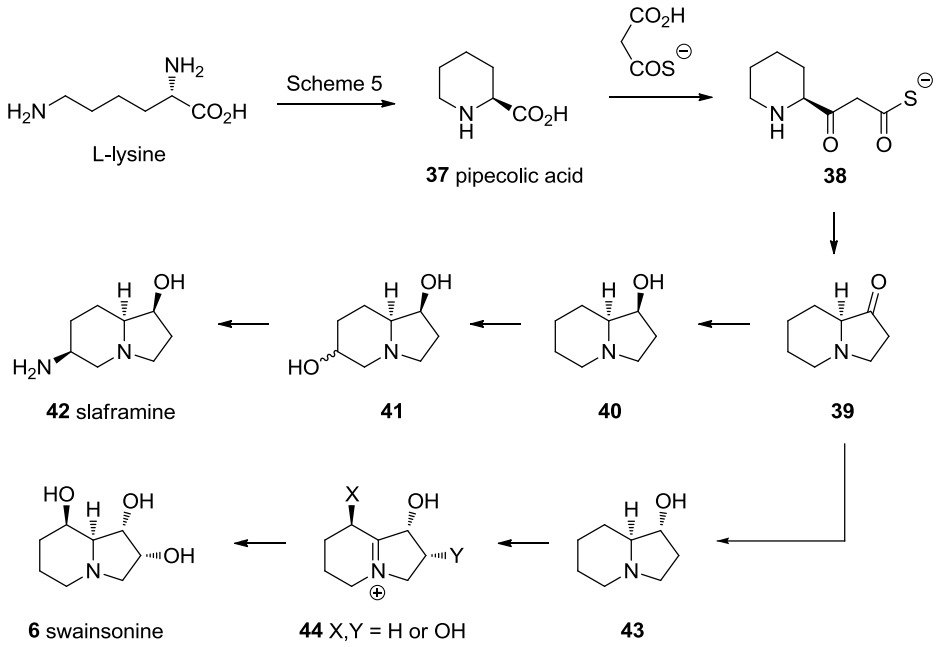


Scheme 5. Biosynthesis of pipecolic acid from lysine.

On the other hand, Gupta and Spenser demonstrated that in rats the conversion of lysine into pipecolic acid proceeds via ε-amino-α-ketocapric acid **35**.[56] This mechanism indicated that the nitrogen atom of the pipecolic acid should be supplied by the ε-amino group of lysine (Scheme 5, route B). Similar experiments carried out with *Neurospora crassa* and *Phaseolus vulgaris* afforded identical results being in conflict with previous findings. Further studies in animals and plants demonstrated that both routes A and B, illustrated in Scheme 5, distinguishable at the loss of a particular amino group of the lysine, are possible. In fact, there are several experimental evidences supporting the existence of various routes in microorganisms, as well as specific enzymes involved in some steps. This topic has been reviewed elsewhere[57] and the reader is referred to that publication for more details concerning the biosynthesis of pipecolic acid.

The biosynthesis of slaframine **42** has been studied in *Rhizoctonia leguminicola* a fungus that causes black spot disease of red clover.[58] By using radiolabelled 1-[^{14}C]-lysine and 6-[^{14}C]-lysine it has been demonstrated their incorporation into slaframine.[59] Moreover, that incorporation was efficiently blocked by adding pipecolic acid, thus indicating that pipecolic acid is an intermediate in the prcess of biogenesis. The same authors also studied the origin of pipecolic acid in *Rhizoctonia leguminicola* and verified the biosynthetic pathway illustrated in Scheme 5.[60] Further experiments with radiolabelled pipecolic acid showed incorporation of radioactivity to slaframine in the expected positions.

The origin of the pyrrolidine ring of slaframine was also investigated[61] and it was found to be formed from malonic acid and acetic acid. Spectrometric analysis of radiolabelled/deuterated compounds indicated that the methyl carbon of acetate is joined to the carboxyl carbon of the pipecolate. These results suggest the formation of intermediate **38** by acylation of malonate with pipecolic acid (Scheme 6). Furthermore, preparation of deuterated **40** allowed to identify this compound as an advanced intermediate in the biogenesis of slaframine[62] and to propose 1-oxooctahydroindolizine **39** as the intermediate precursors of **40**. In addition to slframine **42**, swainsonine **6** was also isolated from *Rhizoctonia leguminicola*.[63] By employing perdeutero pipecolic acid it was demonstrated that both slaframine and swainsonine have common precursors in their biogenesis.[64]



Scheme 6. Biogenesis of slaframine from lysine in *Rhizoctonia leguminicola*

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In the case of swainsonine, compound **39** is reduced by the other face providing **43**. Oxidation at C-8a of this intermediate should be postulated, probably through an iminium ion, with subsequent reduction by the appropriate face to provide the *R* configuration of swainsonine **6** (Scheme 6). Further experiments with deuterated compounds allowed to corroborate that hypothesis.[65]

The biosynthesis of swainsonine **6** has also been studied in plants. In particular, studies carried out with *Astragalux oxyphysus* showed that swainsonine **6** is biosynthesized in that plant by a very similar pathway (if not identical) to that in the fungus by incorporating pipecolic acid into the swainsonine skeleton.[66] On the other hand, it has been observed that the plant does not produce slaframine **42**; neither does it produce intermediates **40** and **41**.

The role of both pipecolic acid and malonic acid in the biosynthesis of swainsonine **6** has also been pointed out by stimulating production of such alkaloid by transformed root cultures of *Swainsona galegifolia*.[67]

4. Conclusions

Polyhydroxylated saturated nitrogen heterocyles provided a variety of biosynthetic challenges. Up to now, several aspects related to their biogenesis have been revealed. However, there is still much work to do. Further investigations are still required to clarify the biosynthetic enzymes involved in the catalytic processes. Such enzymes

should be of a great value for enzymatic approaches to iminosugars and their biomimetic synthesis. There are experimental evidences indicating that different biosynthetic mechanisms operate on diverse microorganisms (for instance *Bacillus subtilis vs. Streptomyces subrutilus* for 1-deoxynojirimycin) or higher plants. Very recently, a gene cluster has been identified providing evidence of the biosynthetic pathways. In this regard, the catalytic mechanism of individual domains should continue to be probed by using standard mutagenesis techniques with studies involving purified enzymes. By acquiring this knowledge it will be possible to design new analogues by developing more sophisticated and improved strains. The fields of synthetic organic chemistry and biochemistry will be united by employing new bioorganic tools for further chemical elaboration of new compounds of pharmaceutical interest.

Acknowledgments

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