Sponge's Molecular Diversity Through the Ambivalent Reactivity of 2-Aminoimidazole: A Universal Chemical Pathway to the Oroidin-Based Pyrrole-Imidazole Alkaloids and Their Palau'amine Congeners

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Dedicated to Professor Ekkehard Winterfeldt

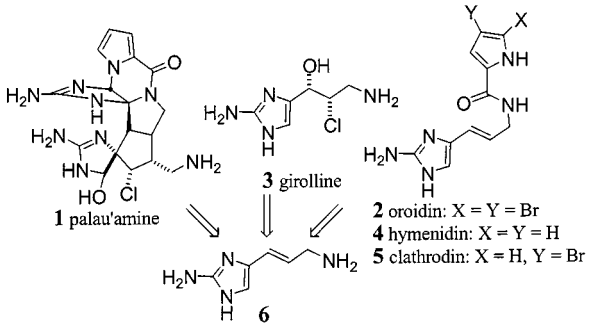
Keywords: Alkaloids / Marine natural products / Metabolism / 2-Aminoimidazole / Biogenetic pathway / Palau'amine

The plausible biogenetic mechanism proposed in this communication clarifies the chemical pathway leading to over 60 polycyclic pyrrole-imidazole marine alkaloids isolated from more than 20 different species of various genera (*Agelas*, *Hymeniacidon*, *Axinella*, *Acanthella*, *Cymbastella*, *Phakellia*...) of sponges. The tautomerism and ambivalent reactivity of 2-aminoimidazole precursors provide a consistent chemical pathway explaining the intriguing formation of all the compounds of this class. The mechanistic proposal proposed here for the first time is unique in the sense that the chemical pathway is universal and therefore provides fertile intellectual ground for the study of the enzymatic mechanism involved in this system.

Introduction

In current practice, combinatorial synthesis is used to produce libraries of focused classes of compounds by varying the starting building blocks. This technique was inspired by Nature which has developed such chemistry progressively over 4.5 billion years and improved it to enzymatic-catalysed primary and secondary metabolisms. In addition to this aspect of combinatorial chemistry, Nature has also selected a more subtle chemistry, which is based on the reactivity variation of a single precursor or a restricted number of precursors. Related to the latter concept, one of the fascinating large variety of secondary metabolites exhibited by marine sponges (Phylum Porifera) is the unprecedented pyrrole-imidazole alkaloid family. The most intriguing molecule of this group is the polycycle palau'amine **(1)** isolated by Scheuer et al.[1] from the Sponge *Stylotella aurantium*. The first member of this growing group to be isolated was oroidin **2**, initially from *Agelas oroides* in 1971,[2,3] and then from various other sponges.[4−12] Compounds **1**−**5** (Scheme 1) are closely related to their probable precursor 3-amino-1-(2-aminoimidazolyl)-prop-1-ene **(6)**.

Over the last thirty years, numerous similar alkaloids with various structures and interesting biological activities have been isolated essentially (but not exclusively)[13] from various species of *Agelasidae*, *Hymeniacidonidae and Axinellidae*.[13] Not only have these studies improved our understanding of their structures and arrangement, but they also supply new information on the biogenetic chemical reactions. This may lead to new ideas for biomimetic total synthesis of this group of natural molecules even in advance of their identification and characterisation. A recent success of this approach is the biogenetic hypothesis postulated by Baldwin and Whitehead and modified by Marazano and co-workers[14] for manzamine alkaloids. This is an extraordinary example, which proves that the combination of the isolation of natural products and their chemical analysis could provide valuable approaches not only for synthetic models but for biogenesis experiments as well.



Scheme 1. Important isolated pyrrole-imidazole alkaloids

Results and Discussion

A hypothetical biogenetic pathway is an important step to the selection of precursors for biosynthesis studies by labelled compounds in vitro or in vivo. In the group of pyrrole-imidazole alkaloids, it is easy to see that all derivatives are closely related, but a logical chemical pathway to corroborate the presumption of a common biogenesis is still lacking. Different molecular mechanisms have been separately postulated by Büchi for dibromophakellin,[15] Maximov for dibromoagelaspongin,[16] Pietra for Agelastatin A,[17] Fusetani for mauritiamine[18] and Scheuer for pa-

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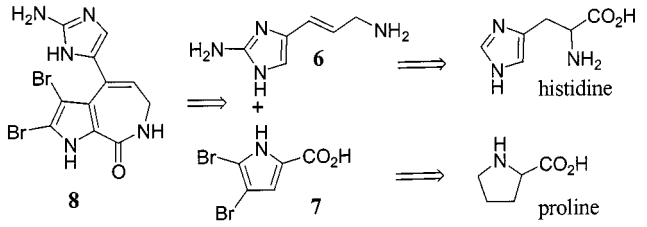
lau'amine.[1a] Recently, we introduced the controlled regioselective pyrrolic N_1 or C_3 intramolecular cyclization and the possible role of oroidin as a central precursor in similar cyclic compounds ($C_{11}N_5$).[19] Kitagawa et al.[20] and Braekman et al.[21] have reported chemotaxonomic considerations.

The investigation of metabolic pathways using labelled compounds in marine sponges is still difficult: only a few organisms are amenable to culture. A recent and first biosynthetic study using a cell culture of the sponge *Teichaxinella morchella* (Axinellidae) was reported by Kerr.[22] These authors demonstrated by feeding studies using labelled proline and histidine that these amino acids are precursors of odiline (syn: stevensine) **8** via 3-amino-1-(2-aminoimidazolyl)-prop-1-ene **(6)** and 4,5-dibromopyrrole-2-carboxylic acid **(7)** (Scheme 2).



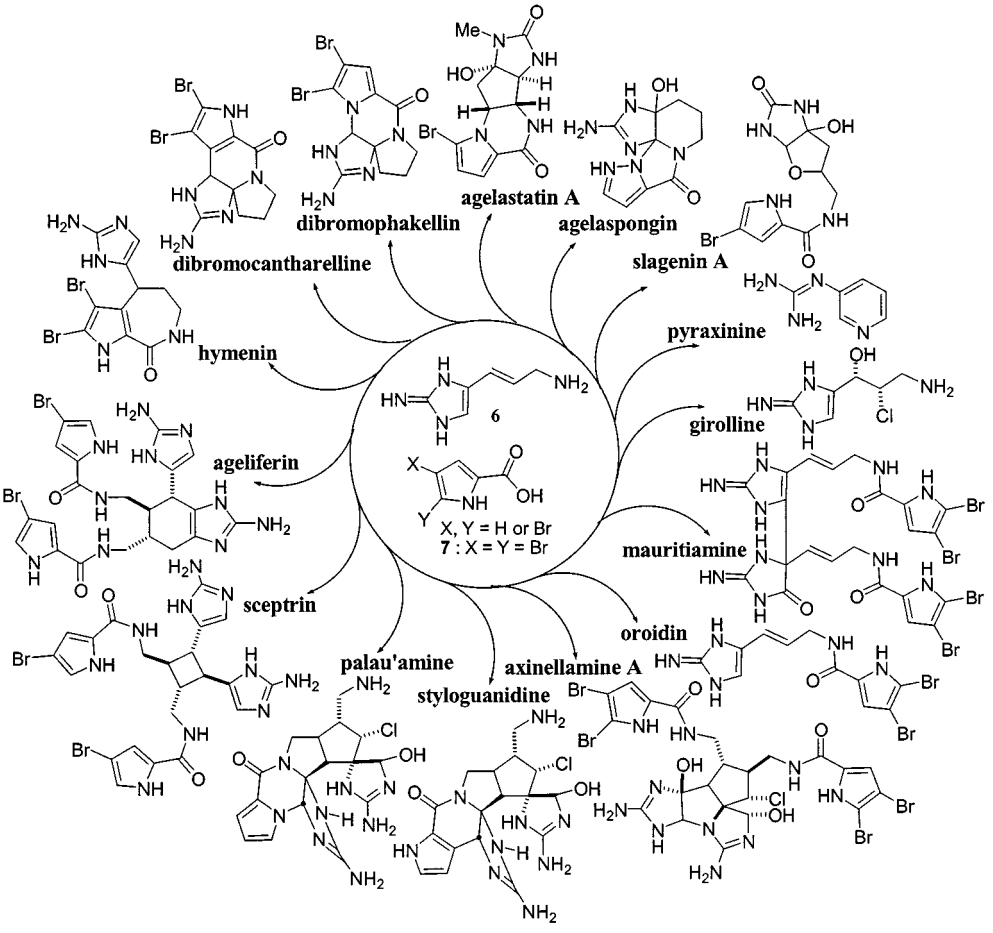
Scheme 2. The biogenetic precursors of stevensine/odiline from the sponge *Teichaxinella morchella*

Linked to our previous work in the field of identification and synthesis[23] of pyrrole-imidazole alkaloids, we report here the further analysis of compounds biogenetically related to oroidin **2** and girolline **3.** We propose a very likely universal chemical pathway through simple precursors such as **6** and **7**. For this purpose, all of the known oroidin alkaloids have been arranged in structural groups (Scheme 3).

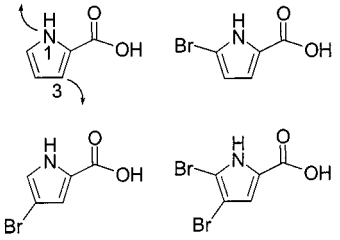
We have considered that each of the compounds depicted in Scheme 3 represents one structural group. Examples of different transformations leading to diverse groups are given and their mechanisms are proposed. It is extremely stimulating to find that simple chemistry can explain, in a logical way, the biogenetic syntheses of all compounds which share the reactive 3-amino-1-(2-aminoimidazolyl)-prop-1-ene and four pyrrole-2-carboxylic acid building blocks as a common origin. It is clear that enzymatic oxidoreduction, hydrolysis, hydration and alkylation, enhance the number of derivatives formed by this process. Being unwilling to speculate too much, no attempt has been made to discuss these additional transformations leading to various functionalised natural derivatives.

Variation of Building Blocks by Pyrrole Bromination

Pyrrole-2-carboxylic acid and its 4- or 5-brominated derivatives combined with N1 and C3 nucleophilicity allow for eight entries (Scheme 4).



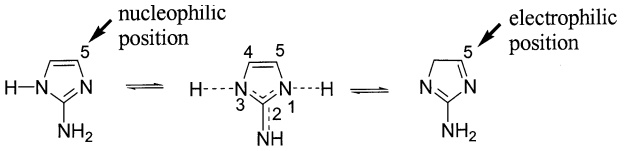
Scheme 3. Representative structural groups



Scheme 4. Four pyrrole building blocks and two nucleophilic positions (N1 and C3)

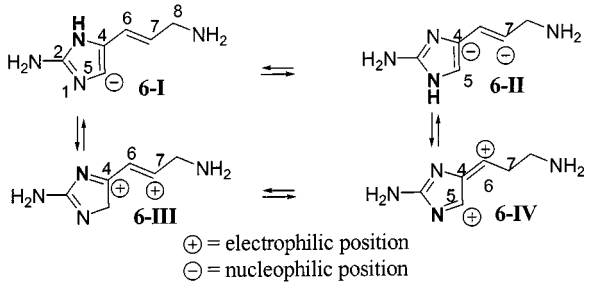
Reactivity Variation of 2-Aminoimidazolic Building Blocks by Tautomeric Equilibrium

The ambivalent reactivity of the key structural feature 2-aminoimidazole (Scheme 5) is responsible for the molecular diversity observed in this group of alkaloids. The electrophilic or nucleophilic reactivity at the same position C-4(5) is dependent on the tautomeric isomer involved.



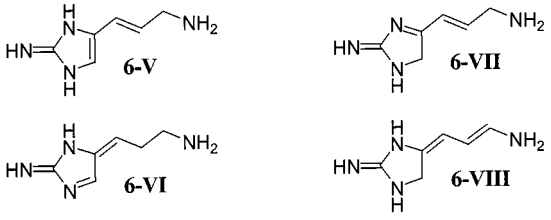
Scheme 5. The tautomerism and ambivalent reactivity of 2-aminoimidazole

A similar "umpolung" is also exhibited by the vinylogous natural building block **6** (Scheme 6). The generation of selected tautomers **6-I**, **6-II**, **6-III** and **6-IV** by proton migration seems to be the key step of this metabolic route. The tautomer forms and their behaviour are probably controlled by the catalytic ability of the host enzyme to exchange protons with its substrate. The protonation-deprotonation property of the 2-aminoimidazole ring is undoubtedly crucial for this proton mediating transfer.



Scheme 6. The tautomerism and ambivalent reactivity of vinylogous 2-aminoimidazole

The tautomers **6-V**, **6-VI**, **6-VII** and **6-VIII** (Scheme 7) are also possible. This makes the intermediate **6** a very good candidate as source of molecular diversity.



Scheme 7

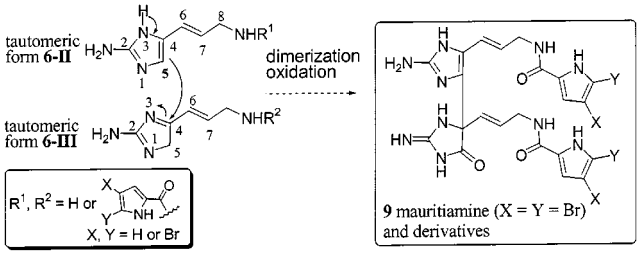
It is noteworthy to emphasise that the reactivity of the different ethylenic positions depends on the considered tautomer form. For example, the positions 4 and 7 are nucleophilic if the tautomer form **6-II** (Scheme 6) is considered but they are electrophilic in **6-III.** The flexibility and versatility of this system should be controlled by the coexistence of all these tautomers in a pH-dependent equilibrium. Because of their simple structures and reactivity, we believe that such a tautomeric equilibrium existed under prebiotic conditions at an early stage of the bioorganic synthesis of this class of compounds.

We suggest that these tautomers (Scheme 6), which can exist simultaneously, give rise to polycyclic metabolites through various combinations with pyrrolic building blocks and diverse modes of cyclization dimerization. Each tautomer engaged in this process may act as an initiator of controlled chain reactions leading to various and complex compounds.

The classification of these alkaloids can be made according to different points of view. With respect to their chemical arrangement, they can be divided into five groups: linear monomers, polycyclic dimers, polycyclic monomers, the agelastatin group and the girolline group. In order to rationalise the formation of these constituents, only strategic bond formation leading to new structural elements are taken into account.

Simple Dimers

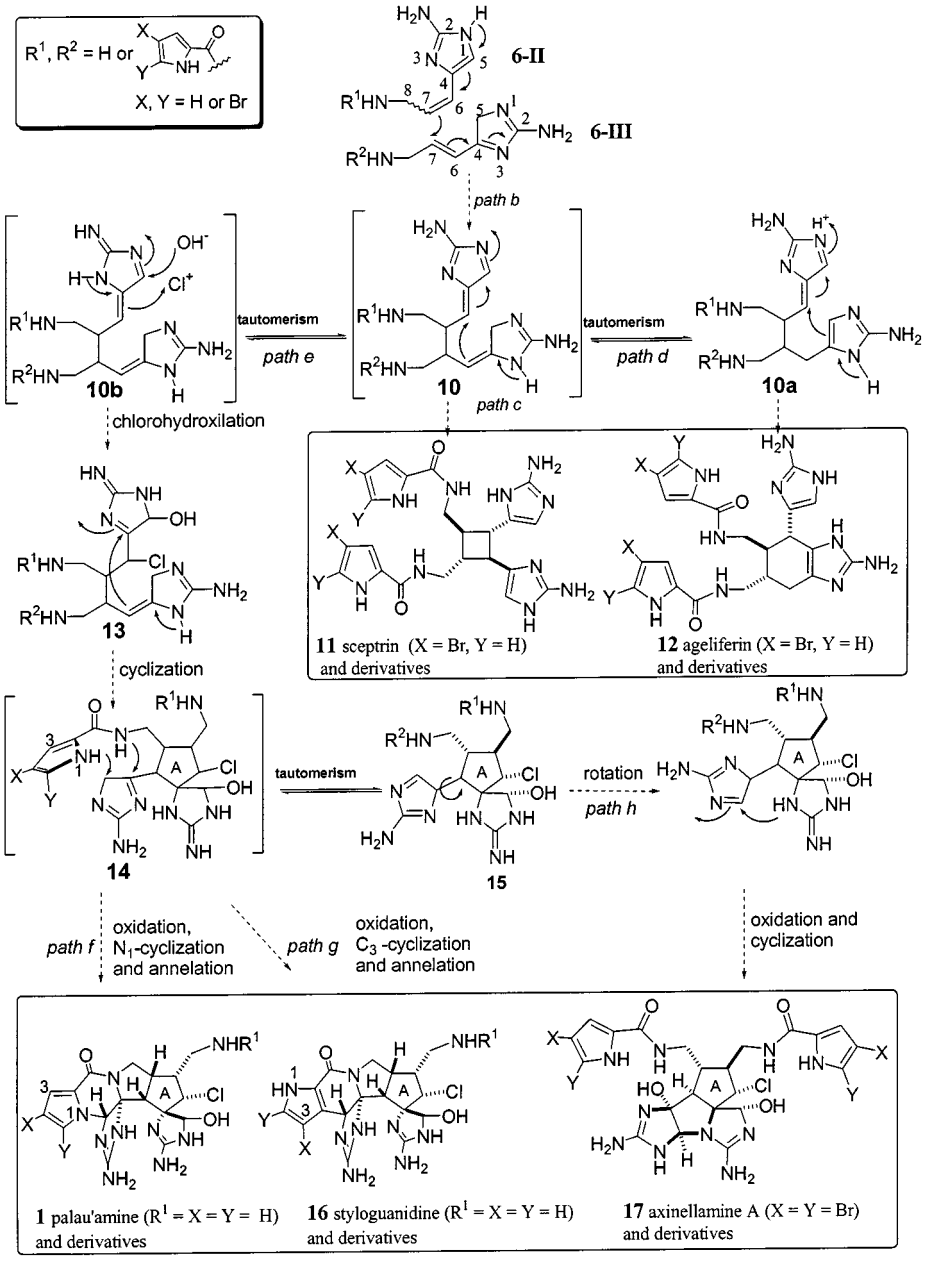
A simple reaction between nucleophilic C_5 of **6-II** and electrophilic C_4 of its tautomer **6-III** (Scheme 8) affords the mauritiamine skeleton **9**.



Scheme 8. C4−C5 dimerization leading to mauritiamine derivatives

The Palau'amine Congeners

The first step involving tautomers **6-II** and **6-III** (Scheme 9) seems to be the key step of the head-to-head dimerization. The intriguing connection between the nucleophilic C7 (**6-II**) and the electrophilic C7 (**6-III**) results in the central intermediate **10**, which displays the appropriate arrangement for cyclization to the sceptrine skeleton **11** (*path c*). A simple tautomerism through an imine/enamine equilibrium to **10a** would lead to a second mode of cyclization and furnish the ageliferine skeleton **12** (*path d*). The very reactive precursor **10** (tautomer **10b**) can also undergo a chlorohydroxylation (*path e*) to give the second multireactive intermediate **13**, which cyclizes to **14**. The diastereselection of the latter cyclization is probably the key to the orientation of the following reaction pathway. Ring A shows a different stereochemistry in *path f*/*path g* leading to pa-



Scheme 9. A plausible universal chemical pathway leading to complex palau′amine congeners.

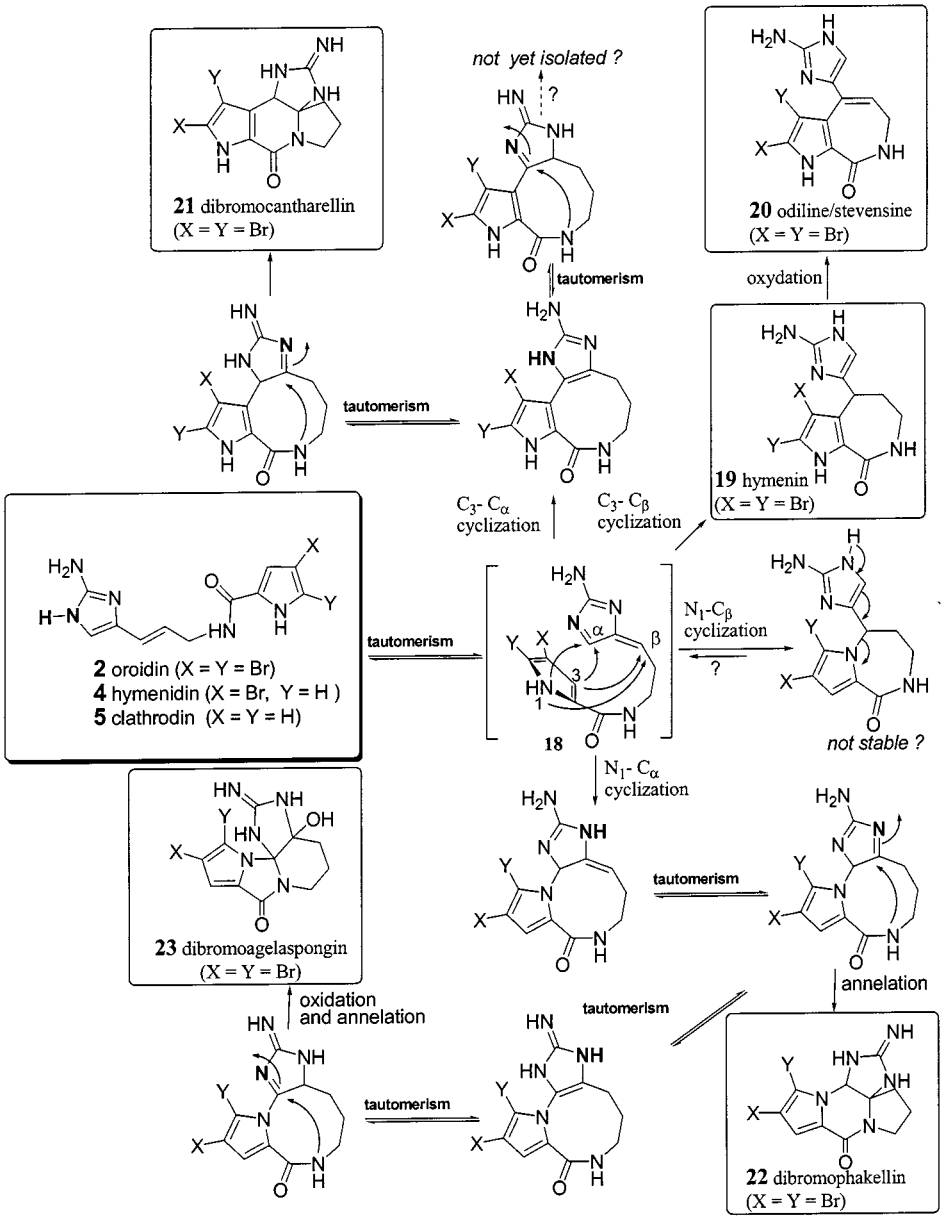
lau'amine/styloguanidine and *path h* leading to axinellamine derivatives. Oxidation of **14**, pyrrolic N1 (*path f*) or C3 (*path g*) cyclization and annelation would lead to palau'amine **1** and styloguanidine **16**, respectively. The tautomeric form **15** of the intermediate **14** provides the appropriate disposition for one additional cyclization (*path h*) to form the axinellamine skeleton **17** and derivatives.

The Polycyclic Monomers Group of Oroidin

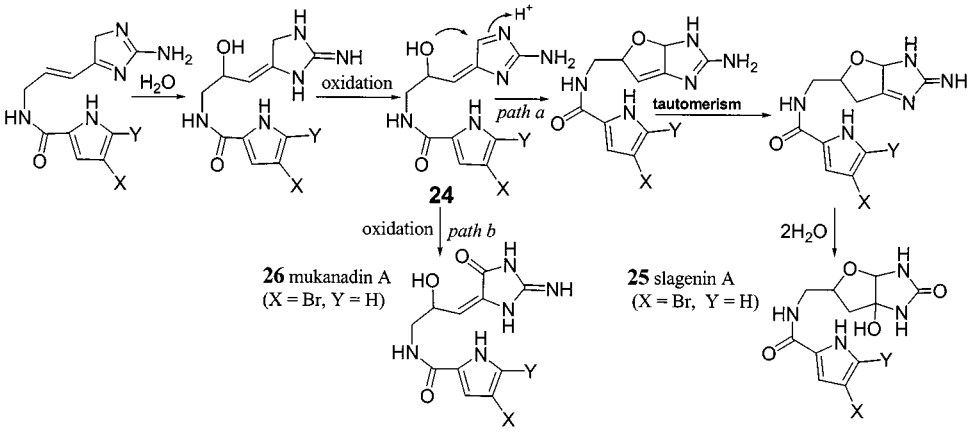
The variety of modes of intramolecular cyclization exhibited by the linear compounds **2**, **4** and **5** is sufficient to overcome various assemblies of the intricate polycyclic natural derivatives (Scheme 10). If we consider the intermediate **18** (tautomer form **6-IV**), four cyclization pathways are possible: N_1-C_α, N_1-C_β, C_3-C_α and C_3-C_β. The chemistry involving imine/enamine tautomerism summarised in Scheme 8 would provide the polycyclic natural metabolites **19**−**23** and other isolated derivatives exhibiting the same framework.

The Slagenin A and Mukanadin A Group

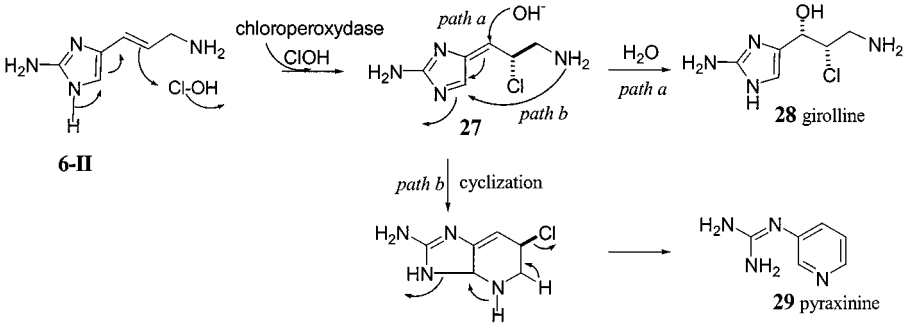
The tautomeric form corresponding to **6-III** could be hydrated and oxidised to the common intermediate **24** (Scheme 11) which can undergo *O*-cyclization and dihydration to slagenin A **25** (*path a*), or further oxidation leading to mukanadin A **26** (*path b*).



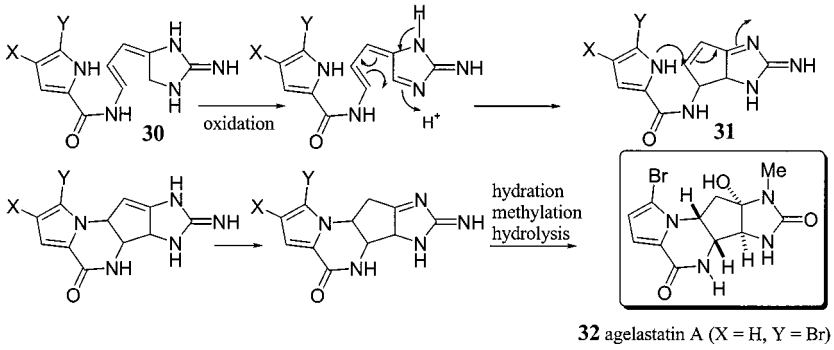
Scheme 10. A universal chemical pathway leading to polycyclized oroidin derivatives



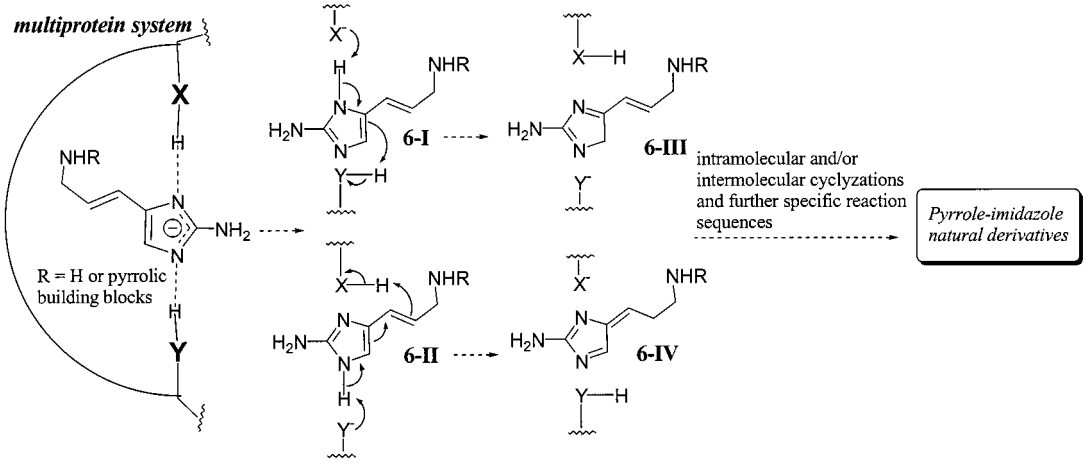
Scheme 11. A common chemical pathway leading to slagenin and mukanadin derivatives



Scheme 12. A common chemical pathway leading to girolline and pyraxinine



Scheme 13. A chemical pathway leading to agelastatin derivatives



Scheme 14. A plausible mechanistic consideration on the enzymatic production of transient tautomers; XH and YH are proton donors (CO_2H, OH, NH or SH); X^- and Y^- are proton acceptors

The Girolline and Pyraxinine Group

Girolline and pyraxinine are both isolated from the same sponge *Cymbastela cantharella.* Biogenetically, pyraxinine may be considered as being derived from the same intermediate as girolline. The chlorohydroxylation of the tautomers **6-II** (Scheme 12) provides a common chlorinated intermediate **27** which would result directly in the formation of the girolline **28** (*path a*). Cyclization and aromatization would occur to afford pyraxinine **29** (*path b*). This is presumed to be a minor process, since girolline is accompanied by only small amounts of pyraxinine. To the best of our knowledge, this has been the first isolation of a pyridine alkaloid with a guanidine unit at C-3.

The Agelastatin Group

The agelastatins are the only examples to date of pyrrole imidazole alkaloids with C8−C5 and N1−C7 bonds. In the intramolecular cyclization pattern of agelastatin, the oxidation of tautomer form **VIII** (Scheme 7) **30** seems to be the key step (Scheme 13). After the first C8−C5 cyclisation, the resulting product **31** allows the unusual pyrrolic N1−C7 connection followed by further transformation to agelasta-

tin **32** and derivatives**.** The agelastatin pathway further demonstrates the general applicability of our biogenetic proposal based on tautomeric forms.

A possible catalytic mechanism could be dependent on the participation of amino acid residues by hydrogen loss and restoration at the heteroatoms X or Y (Scheme 14). The imidazole nucleus of the key substrate seems to be involved in the proton push-pull movements.

Conclusion

In conclusion, according to our proposed biogenetic scheme, various hypothetical constituents can be predicted. It is clear that the above chemical pathway suggests that these compounds may be more widespread than presently known. The isolation and characterisation of new pyrrole-imidazole metabolites will certainly allow us to explore additional transformations which could support this hypothesis. As chemical diversity, enzymatic catalysis variation and mutagenesis are closely related, the "prebiotic chemistry" presented here can also provide strong evidence for a multifunctional enzyme-mediated biosynthesis. Such multiprotein systems would be particularly needed by the living fixed sponges for their adaptation to environmental influences, and for self-defence.

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