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Minireview

Oxidative cascades: A facile biosynthetic strategy for the assembly of complex molecules

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Abstract

Electron-rich aromatic compounds undergo a facile tandem reaction sequence involving an iterative two-electron oxidation/aromatization. This review will describe the application of this motif to the synthesis of dimethylbenzimidazole, pyoverdine, actinomycin, cystodytin, pyrroloquinoline quinone, and the cataract pigment.

Keywords: Oxidative cascades

1. Introduction

Organic synthesis is challenging. Each step in a synthetic route requires a careful search for suitable reagents and reaction conditions, and each successful reaction is likely to have required many unsuccessful attempts. Because of this, synthetic chemists are fascinated by tandem reactions in which several steps occur, one after the other, in a single reaction vessel [17]. The difficulties of running successful reactions is even greater in living systems where most biosynthetic reactions require enzymatic catalysis and the evolution of a successful catalyst may require millions of years. It is

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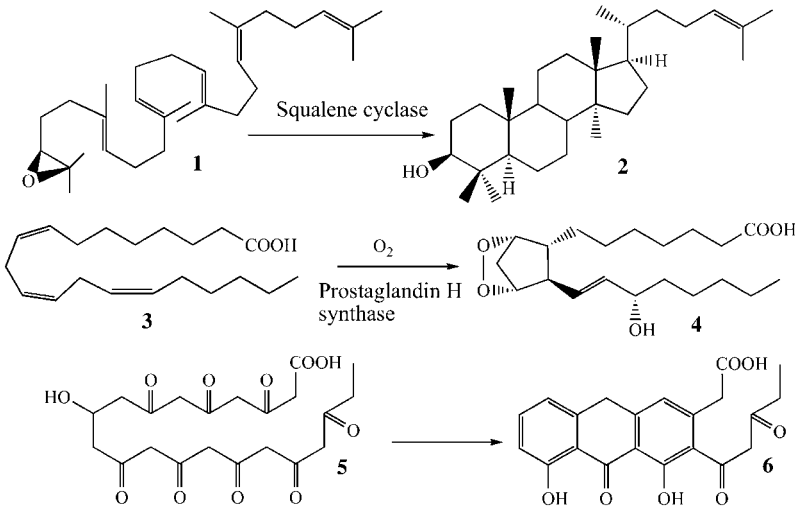

Fig. 1. Three examples of tandem reactions in biosynthesis.

therefore not surprising that living systems, just like the synthetic chemist, have also exploited tandem reactions to assemble complex molecules. Three well-studied examples involving a steroid (**2**), a prostaglandin (**4**), and a polyketide (**6**) are shown in Fig. 1. In this mini-review, a new family of tandem reactions, oxidative cascades, will be described.

2. Actinomycin biosynthesis

Phenoxazinone synthase is a copper-containing oxidase that catalyzes the six-electron oxidation of aminophenols to generate the phenoxazinone chromophore of actinomycin (**8,** Fig. 2).

This reaction has been studied in some detail and the current mechanistic proposal is outlined in Fig. 3 [3,4,9]. We will initially focus on the aminophenol

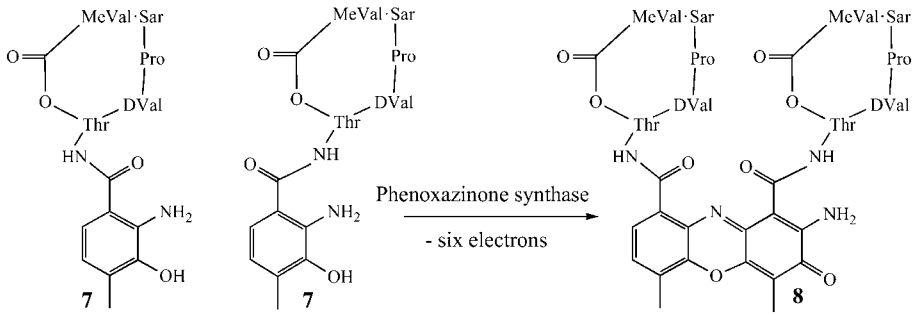

Fig. 2. The phenoxazinone synthase catalyzed formation of the chromophore of actinomycin (**8**).

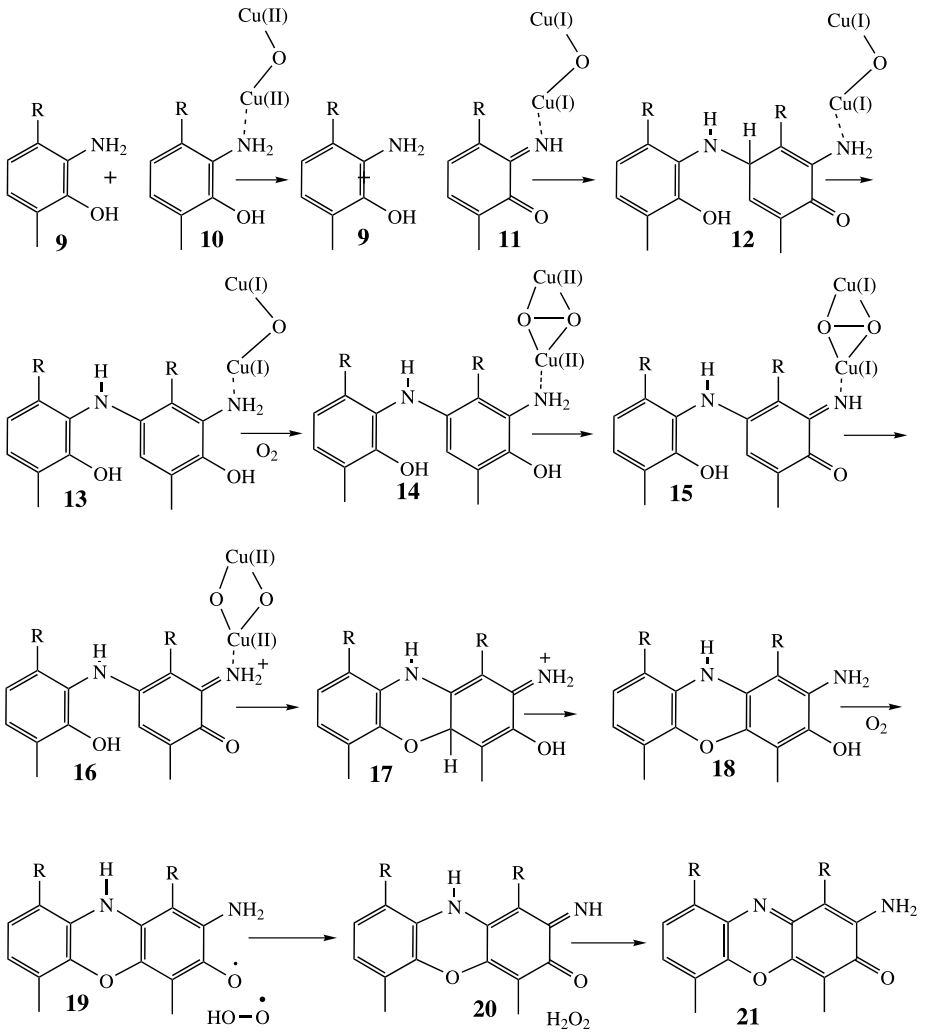

Fig. 3. Mechanism for the formation of the phenoxazinone chromophore of actinomycin.

chemistry and return to the role of the metal ions later. In the first step, the aminophenol is oxidized to the highly electrophilic quinone imine **11**. The amino group from a second aminophenol then adds to **11**, in a conjugate addition reaction, to give **12**. Tautomerization regenerates the aromatic ring in **13**. This oxidation/addition/tautomerization sequence is repeated to form the C–O bond of the phenoxazinone. Thus, oxidation of **14** gives the quinone imine **15**, conjugate addition of the phenol gives **17** and tautomerization regenerates the aminophenol moiety in **18**. A final two-electron oxidation gives quinone imine **20** which then tautomerizes to give the phenoxazinone chromophore **21**. As the reaction proceeds, through three rounds of oxidation, the redox active aminophenols **9**, **14**, and **18** become progressively more

electron rich and therefore easier to oxidize. For example, while **9** is relatively air stable, **18** is immediately oxidized in air. It is therefore, not surprising that the later steps in phenoxazinone formation (**16**–**21**) do not require enzymatic catalysis.

Phenoxazinone synthase shows wide substrate tolerance. It was therefore, possible to trap several of the intermediates shown in Fig. 3 using suitably substituted aminophenols. These trapping reactions are shown in Fig. 4. With aminophenol **22**, the first conjugate addition reaction was blocked allowing the isolation of the quinoneimine hydrolysis product **23**. Aminophenol **24** blocked the second conjugate addition (**16**–**17**) allowing the trapping of **16** as **25**. With the methyl substituted aminophenol **26**, the tautomerization of **17**–**18** was prevented allowing the trapping of **17** as **27**. The newly formed chiral center in **27** was racemic demonstrating that the cyclization **16**–**17** does not occur at the active site of the enzyme. This suggests that the final five steps (**16**–**21**) in phenoxazinone formation are non-enzymatic.

While the mechanism of aminophenol oxidation has not yet been worked out, it is likely to proceed at the type III copper center of the enzyme by a mechanism that is analogous to the mechanism of tyrosinase [21] or catechol oxidase [10]. In this mechanism, the aminophenol is coordinated to one of the copper ions as in **10**, sequential electron transfer to the two electronically coupled copper ions gives the quinone imine **11**. Before the next aminophenol oxidation, the cuprous ions must be oxidized. This is accomplished by the transfer of two electrons to oxygen. The resulting peroxide forms a bridged complex with the two copper ions as in **14**. Aminophenol oxidation gives **15** and the cuprous ions in **15** then reduce the peroxide to give **16**.

The mechanistic analysis of phenoxazinone formation revealed a facile strategy for assembling complex molecules from simpler electron-rich aromatic compounds. In this strategy, a benzene ring, substituted with electron donating groups in a 1,2 orientation undergoes a two-electron oxidation to generate a highly electrophilic intermediate, which then reacts with a nucleophile. The resulting adduct undergoes a tautomerization reaction, regenerating the original electron rich benzene ring, which can undergo additional cycles of oxidation/chemistry/tautomerization to build

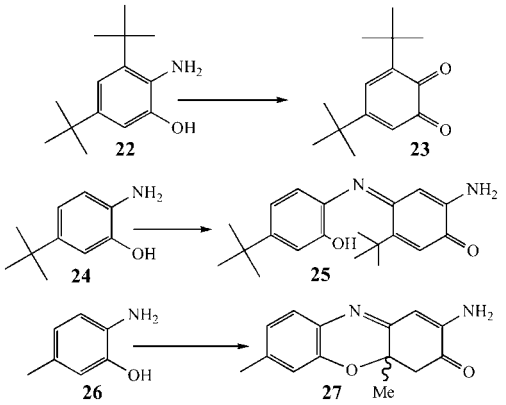

Fig. 4. Reactions used to trap intermediates for the phenoxazinone synthase catalyzed reaction.

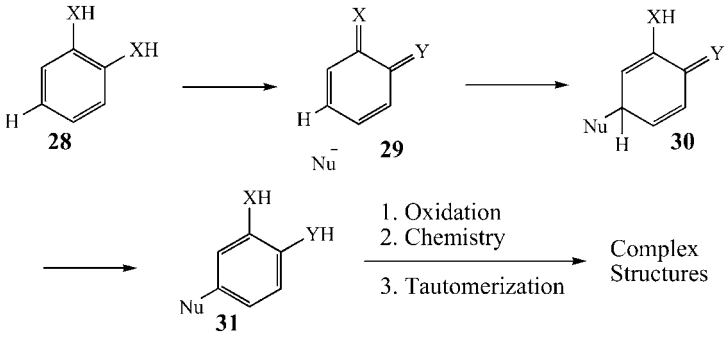

Fig. 5. General features of an oxidative cascade involving tandem repetition of an oxidation/addition/tautomerization sequence. The nucleophilic addition can occur at any position on the benzene ring and the electron-donating substituents can be *ortho* or *para*.

up more complex structures (Fig. 5). If the nucleophile is an electron-donating group, subsequent cycles of oxidation become progressively more facile and in the case of phenoxazinone formation, the final two-electron oxidation is non-enzymatic. This reaction motif, which we will refer to as an oxidative cascade, is also possible for 1,4 disubstituted benzene rings. Several additional examples of possible oxidative cascades are shown in Fig. 6, and mechanistic proposals for each of these will be described in the following sections.

3. The pyrovedine chromophore—a four-electron oxidative cascade

Various *Pseudomonads* secrete pyoverdine siderophores (Fig. 7) under iron starvation conditions as a strategy for the efficient uptake of iron from the growth medium [6,18].

The presence of the dihydroxybenzene ring in **45** suggests the possibility that the pyroverine chromophore is derived from an oxidative cascade of a peptide precursor (Fig. 8). In this mechanistic proposal an oxidation/addition/tautomerization sequence generates catechol **49**, which is converted to the pyoverdine chromophore by an oxidation/tautomerization/tautomerization sequence.

In support of this mechanism, catechol **53** was oxidized with polyphenol oxidase, an enzyme known to oxidize catechols to the corresponding *o*-quinones, to a mixture of **54** and **55** (Fig. 9) [8]. This oxidative cascade could also be achieved using manganese dioxide. In addition, a cell free extract from *Pseudomonas aeruginosa*, grown under iron limiting conditions to induce the pyoverdin biosynthetic genes, also catalyzed the oxidation of **53**. None of the required enzymes have yet been isolated.

4. Dimethylbenzimidazole biosynthesis—a four-electron oxidative cascade

Dimethylbenzimidazole is an axial ligand in adenosyl cobalamin, one of the biologically active forms of vitamin B_{12} (Fig. 10).

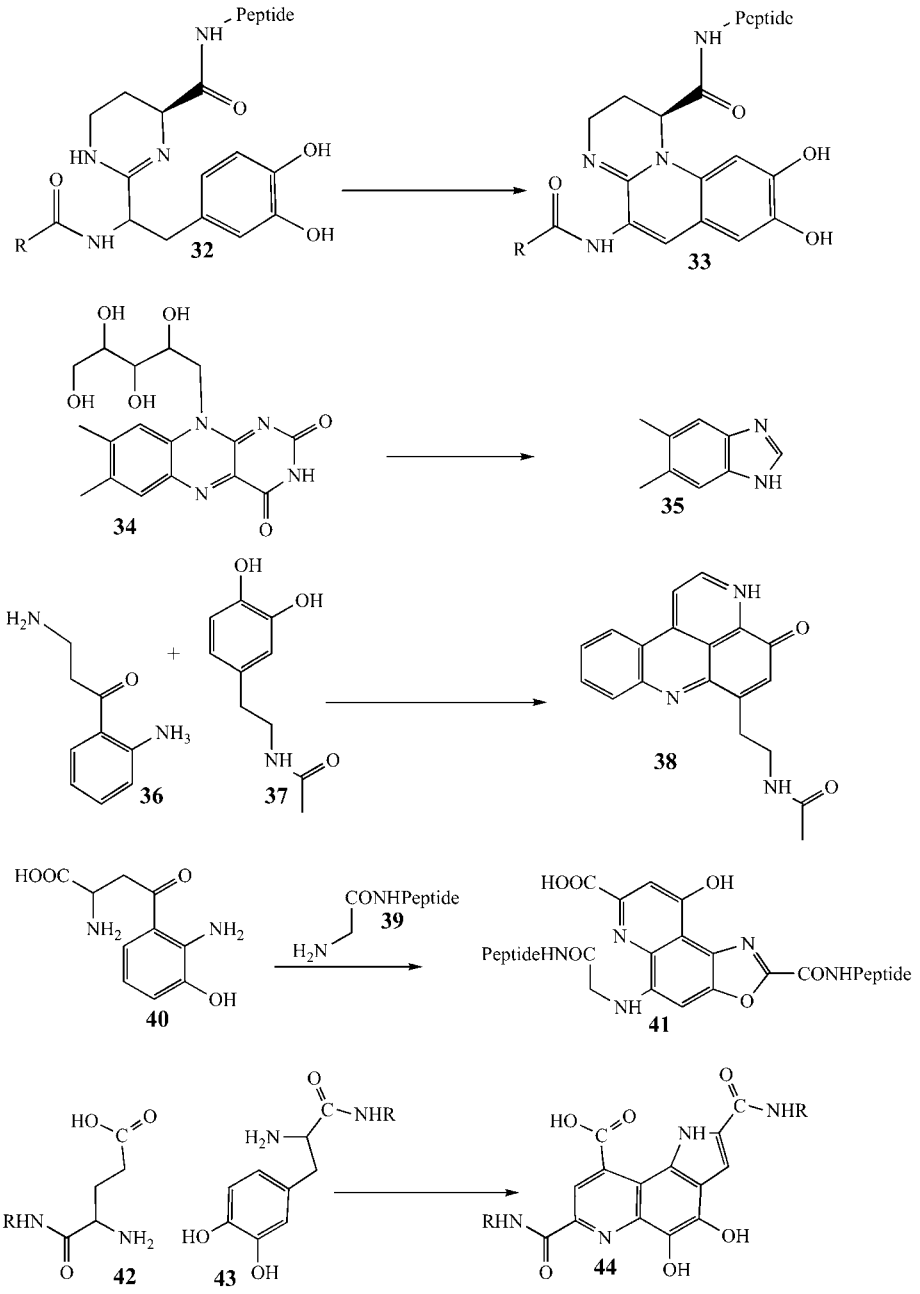

Fig. 6. Selected examples of putative oxidative cascades.

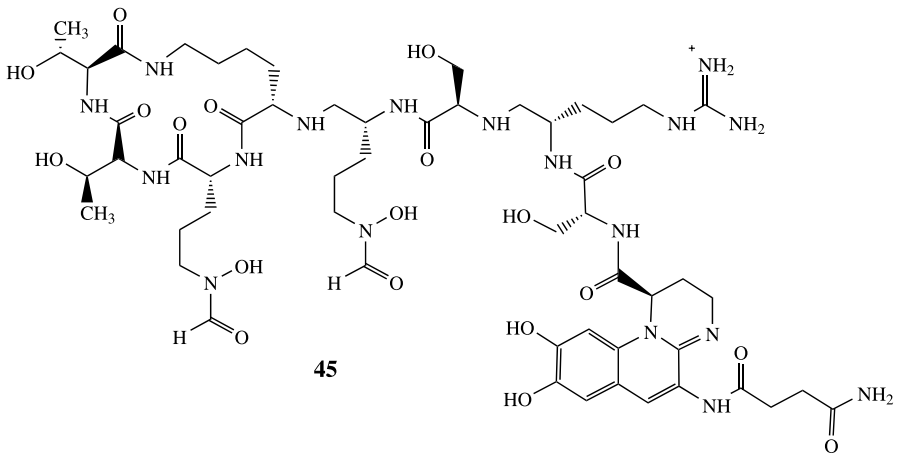

Fig. 7. The structure of a pyoverdine. Labeling studies demonstrate that the chromophore is derived from tyrosine and diaminobutyric acid [5].

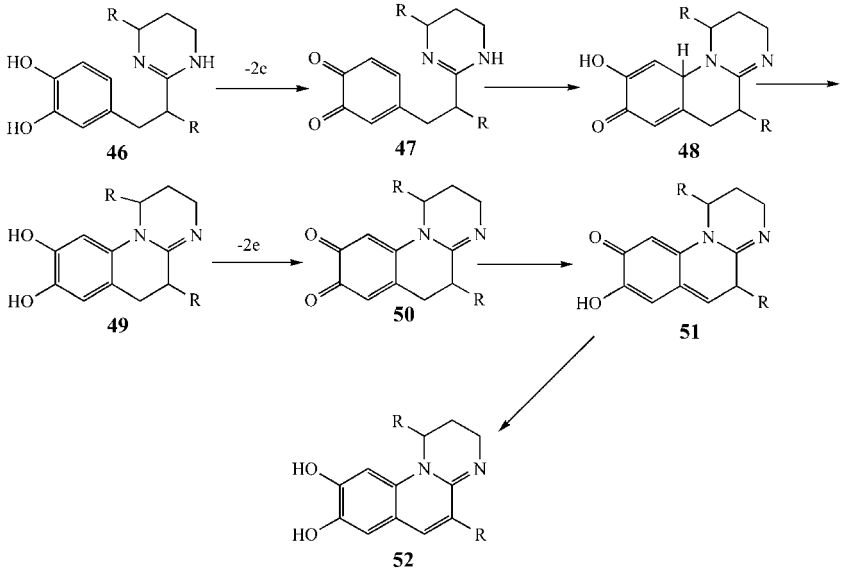

Fig. 8. Mechanistic proposal for the formation of the pyoverdine chromophore by an oxidative cascade involving an oxidation/addition/tautomerization–oxidation/tautomerization/tautomerization sequence. The peptide substituents are represented as R groups.

Dimethylbenzimidazole (**35**) is formed from riboflavin (**34**) in some bacteria (Fig. 6) [19]. This suggested the possibility that riboflavin might first be hydrolyzed to the diamino benzene **57** which could then be converted to dimethylbenzimidazole by an oxidative cascade (Fig. 11). In this proposal, an oxidation/tautomerization/cycliza-

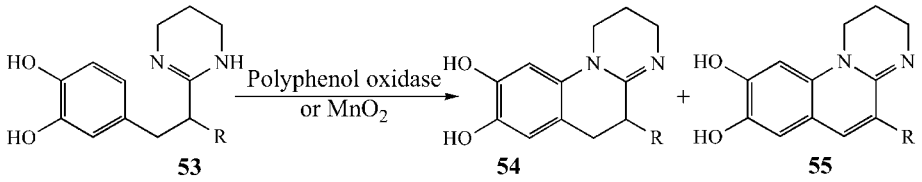

Fig. 9. A biomimetic synthesis of the pyoverdine chromophore.

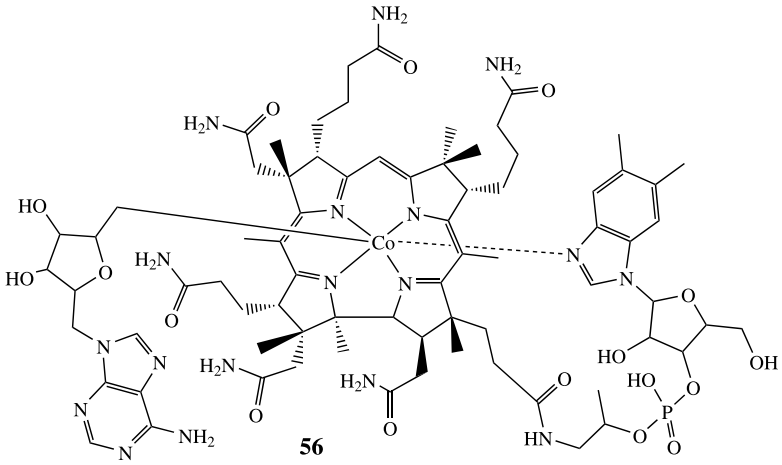

Fig. 10. The structure of adenosyl cobalamin showing the dimethylbenzimidazole ligand.

tion sequence would generate aminophenol **60**. A second oxidation followed by a retroaldol reaction would complete the formation of dimethylbenzimidazole.

In support of this hypothesis, aerobic non-enzymatic oxidation of **57** resulted in the formation of **35** [14]. In addition, imine **59** is air-oxidized to **35**. Intermediate **61** aromatizes by a retroaldol reaction to give **35** or by deprotonation to give **62**. While the non-enzymatic formation of **35** occurs readily, nothing is yet known about the enzymology of dimethylbenzimidazole formation from riboflavin.

5. Cystodytin J—a six-electron oxidative cascade

Cystodytin J **38** (Fig. 6) is a toxic alkaloid isolated from marine invertebrates. A biomimetic synthesis of this compound has been reported and is outlined in Fig. 12 [20]. The first oxidation/addition/tautomerization sequence gives **65**. The second oxidation triggers an aldol condensation to give **68**. Imine formation is followed by tautomerization, first to **70** and then to **71**. The third two-electron oxidation gives Cystodytin J **38**.

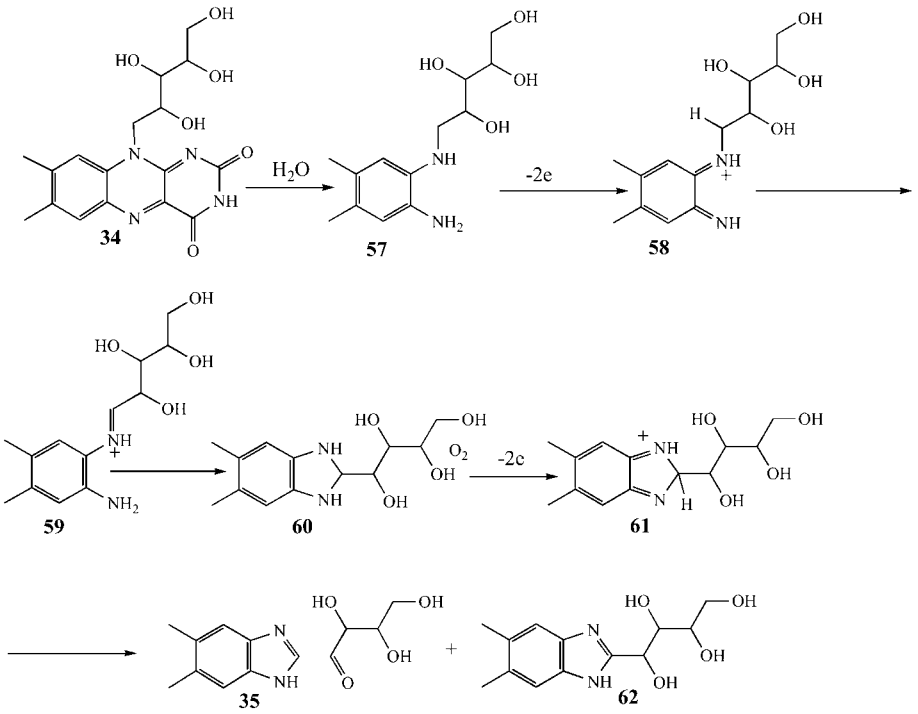

Fig. 11. Mechanistic proposal for the formation of dimethylbenzimidazole (**35**) by an oxidative cascade.

6. Formation of the cataract pigment—a 10-electron oxidative cascade

As the human eye ages, the lens protein become opaque and colored due to covalent modification by chromophoric compounds. One such modification arises from the oxidation of 3-hydroxykynurenine **40**, a compound that normally functions as a UV filter in the eye. In a model reaction, it has been demonstrated that **40** will form a colored adduct **41** with peptide amines [2]. A mechanistic proposal for the non-enzymatic formation of this adduct is shown in Fig. 13. The first stage involves an oxidation/addition/tautomerization sequence to give **74**. An oxidation/addition/addition/tautomerization sequence gives **78**. Then an oxidation/tautomerization/tautomerization/tautomerization sequence gives **81**, The fourth stage involves an oxidation/tautomerization/cyclization sequence to give **84** and the final oxidation/tautomerization sequence generates the product **41**. All of this chemistry is non-enzymatic!

7. Formation of Pyrroloquinoline quinone—a 14-electron oxidative cascade

Pyrroloquinoline quinone **44** is a redox cofactor found in several alcohol and aldehyde dehydrogenases [1,7,11]. It is biosynthesized from tyrosine and glutamic

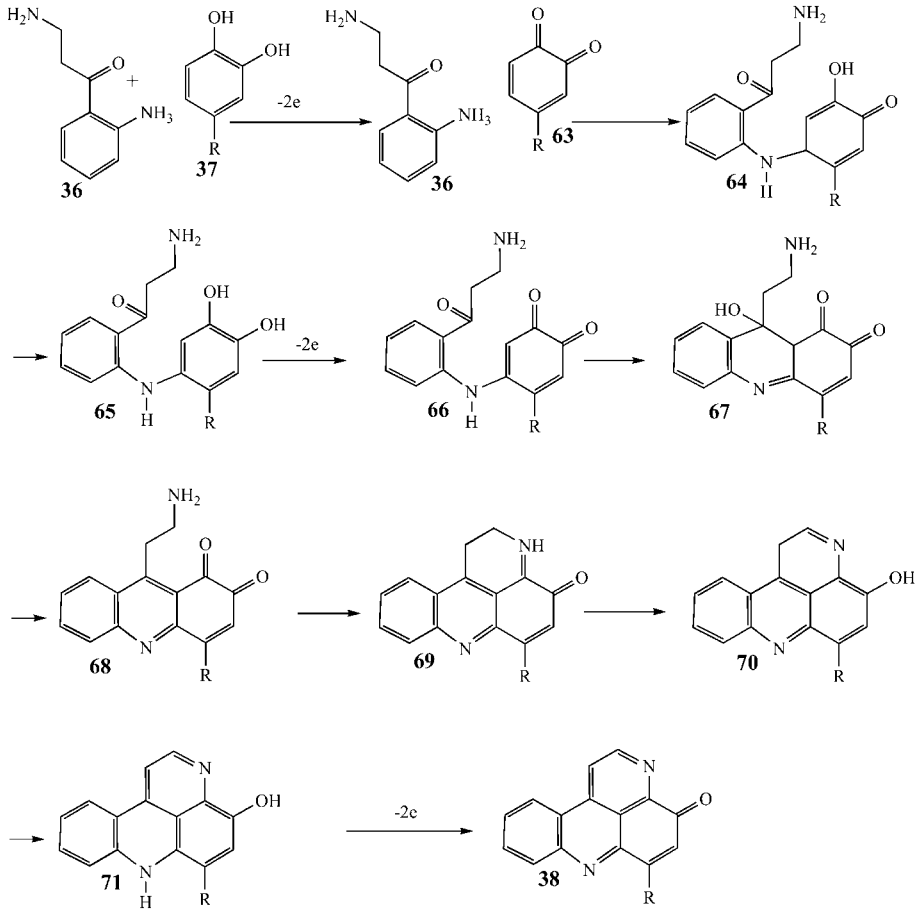

Fig. 12. A biomimetic synthesis of the Cystodytin J alkaloid **38**.

acid residues contained within a precursor peptide [13]. A mechanistic proposal for its formation by an oxidative cascade, is outlined in Fig. 14 and involves seven sequential oxidation steps. The first step involves an oxidation/addition/addition/tautomerization sequence to give **89**. This is followed by an oxidation/addition/tautomerization sequence to give **92**, an oxidation/tautomerization sequence to give **94** and an oxidation/tautomerization/tautomerization sequence to give **97**. The fifth step involves an oxidation/addition/tautomerization sequence to give **100**. The pyrrole ring is formed by an oxidation/tautomerization sequence to give **103** and a final oxidation in the seventh step generates the pyrroloquinoline quinone cofactor.

The pyrroloquinoline quinone biosynthetic gene cluster has been cloned [12,16,22,23] and **92** has been identified as an intermediate [15]. However, the mechanistic enzymology of this pathway is still at an early stage of development. This

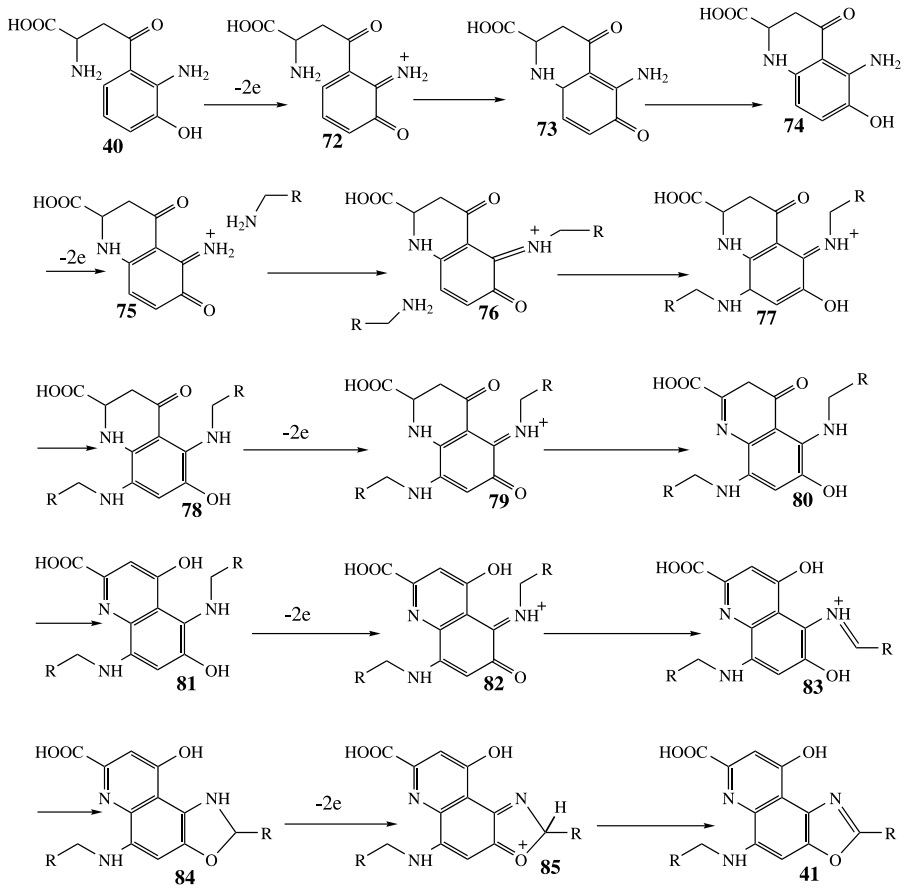

Fig. 13. Mechanistic proposal for the formation of the cataract pigment **41**.

remarkable 14-electron oxidation clearly demonstrates the versatility of oxidative cascades for the assembly of complex molecules.

8. Conclusions

Electron-rich aromatic compounds can undergo a facile tandem reaction sequence involving a two-electron oxidation followed by chemistry to regenerate the electron-rich aromatic ring. Such chemistry typically involves a nucleophilic addition followed by a tautomerization to form a new covalent bond with the aromatic ring or two tautomerization reactions to generate a new double bond. The application of this motif to the synthesis of a set of six natural products, of progressively increasing complexity, was described: dimethylbenzimidazole and the pyoverdine chromophore are formed by four-electron oxidative cascades, phenoxazinone and cystodytin

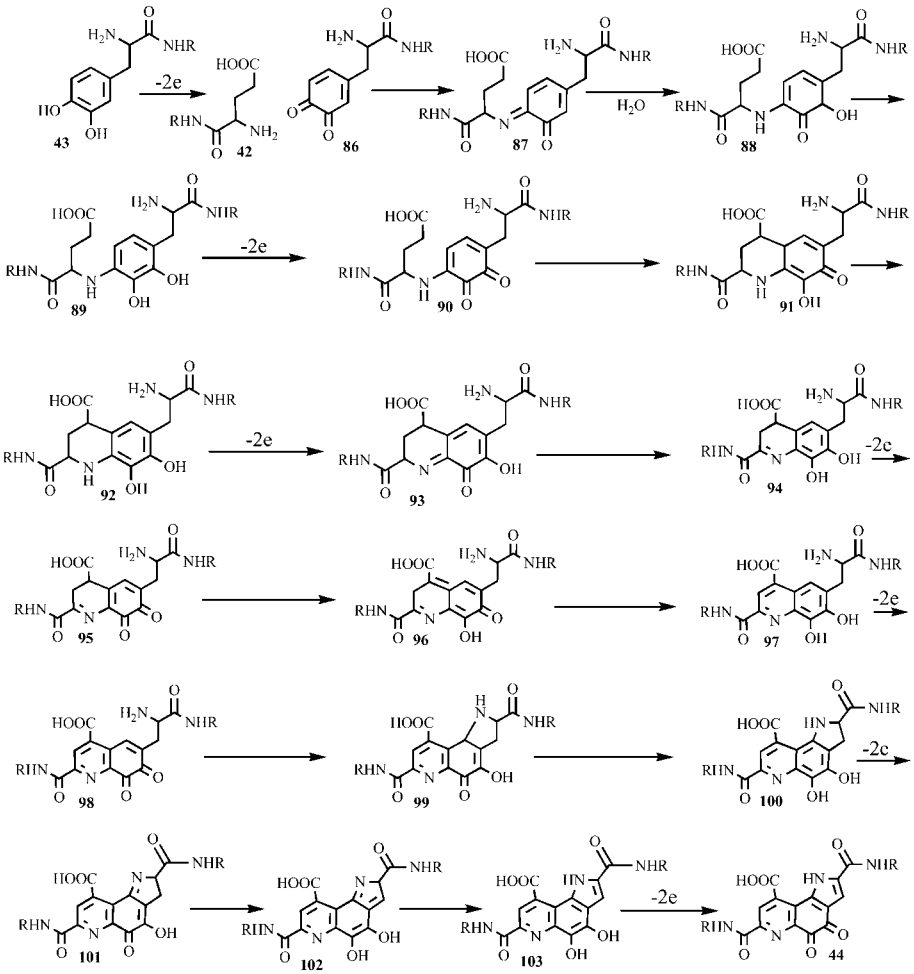

Fig. 14. Mechanistic proposal for the formation of pyrroloquinoline quinone (**44**).

are formed by a six-electron oxidative cascades, the cataract pigment is formed by a 10-electron oxidative cascade and pyrroloquinoline quinone is formed by a 14-electron oxidative cascade. Overall the mechanistic enzymology of oxidative cascades is still at an early stage of development and it is our hope that this brief review will stimulate the reader to identify and study additional examples of this catalytic strategy.

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