Can biosynthesis of natural products involve spontaneous, non-enzymatic self-assembly? What biomimetic synthesis can tell us.
Biosynthesis and biomimetic synthesis

Biosynthesis of Steroids

\[
\text{Squalene} \xrightarrow{\text{O}_2, \text{NADPH}, \text{Squalene Monooxygenase}} \text{2,3(S)-Epoxysqualene}
\]

Exploration of Chemical Space by Nature

Nature relies on different enzymes for making different natural products even if the precursor to all these natural products is the same.

Challenges in mimicking nature

- It is often difficult to control side reactions.
- It is difficult to access molecules which are formed from more energetic transition states.

Biomimetic Synthesis

• Biomimetic Chemistry is novel chemistry inspired by that done in living systems – Ronald Breslow

• Biomimetic synthesis aims to identify biosynthetic patterns that can be exploited in the laboratory without the sophisticated catalysis, pre-organization and compartmentalization used in nature. – Dirk Trauner

Breslow, R.; Chem.Soc.Rev., 1972, 1,553
Nature’s natural product assembly line: Role of enzymes

- Enzymes play a pivotal role in accelerating reaction rates and pre-organizing molecules in appropriate orientations before bond formation.

- Bond formation may be very facile even in what may be perceived as energetically unfavorable conformations.

- **In almost all** cases, the structural complexity of natural products is attributable to enzymes.

- But synthesis of enzymes has biogenetic roots and is quite energy consuming.
Are enzymes the solitary source of natural products’ complexity and diversity?

Photoisomerization of 7-Dehydrocholesterol to Vitamin D₃

7-dehydrocholesterol

6 electron conrotatory ring opening

hν, skin

Precholecalciferol/Pre-Vitamin D₃

Photoisomerization of 7-Dehydrocholesterol to Vitamin D$_3$

What is non-enzymatic self-assembly important for any organism?

- It is an additional pathway available for exploration of chemical space by nature.

- Biosynthesis of an enzyme is a highly energy consuming process; hence avoiding the use of enzymes will be a very economical way of accessing natural products.
Enzyme biosynthesis

http://en.wikipedia.org/wiki/Central_dogma
Unique structural features of some natural products – Pointers to a non-enzymatic mechanism?

Endiandric Acid A
8 contiguous stereocenters
Exists as a racemate in nature

Stephacidin B

Avrainvillamide

Stephacidin A
Occurrence of motifs easily obtained without enzyme assistance

\[
\text{Dienal} \quad \xrightleftharpoons{6\pi} \quad 2\text{-H Pyran}
\]

\[
\text{2-H Pyran} \quad \xrightarrow{[4+2]} \quad \text{O}
\]
Occurrence of Motifs easily obtained without enzyme assistance

Epoxyquinol C

Torreyanic Acid
How does one prove that spontaneous assembly of natural products is possible?

- If one is to invoke a non-enzymatic mechanism for the formation of natural products, it must be possible to make them using conventional chemical synthesis.

- If synthetic mimics of reactive biosynthetic precursors can be made, they should spontaneously react without enzyme mediation to give natural products.

- *Biomimetic synthesis aims to identify biosynthetic patterns that can be exploited in the laboratory without the sophisticated catalysis, pre-organization and compartmentalization used in nature.* – Dirk Trauner

Few of the possible biosynthetic routes of spontaneous assembly

1. Electrocyclizations.

2. Dimerization.

3. Cascade Reactions
Electrocyclizations

- The activation barriers for $8\pi$ electrocyclization reactions are generally low (typically $\sim 20$ kCal/mol).

- There is very little literature precedence for catalysis of any sorts in electrocyclization reactions.

- Asymmetric/enzyme mediated electrocyclizations are extremely rare.

Thermal $6\pi$ and $8\pi$ electrocyclizations

SNF4435 C and D were isolated from the culture broth of *Streptomyces spectabilis* in 2001.

They are optically active polyketides that have been isolated as 3:1 mixture.

Spectinabillin had already been isolated from *S. spectabilis* in 1976.

It was proposed that Spectinabillin could be the common biosynthetic precursor for both SNF4435 C and SNF4435 D.

Proposed Biosynthesis of SNF4435 C and D

Moses, J. E.; Baldwin, J. E.; Marquez, R.; Adlington, R. M.; Cowley, A. R. 
Org. Lett. 2002, 4, 3731
Proposed Biosynthesis of SNF4435 C and D

\[ \text{H \rightarrow CON} \]

\[ \begin{align*}
\text{Ar} & \quad \text{Pyr} \\
\text{O} & \quad \text{O} \\
\text{O} & \quad \text{O} \\
\text{Ome} & \quad \text{Ome}
\end{align*} \]

\[ \begin{align*}
\text{O}_2\text{N} & \quad \text{O}_2\text{N} \\
\text{7} & \quad \text{8}
\end{align*} \]

\[ \text{8}\pi \text{ CON} \]

\[ \begin{align*}
\text{O}_2\text{N} & \quad \text{O}_2\text{N} \\
\text{7} & \quad \text{8}
\end{align*} \]

\[ \text{8}\pi \text{ CON} \]

\[ \begin{align*}
\text{A} & \quad \text{B} \\
\text{A} & \quad \text{B} \\
\text{A} & \quad \text{B}
\end{align*} \]
Proposed Biosynthesis of SNF4435 C and D

Biomimetic Synthesis of SNF4435C and D

Biomimetic Synthesis of SNF4435C and D

Reason for 3:1 selectivity

6π Disrotatory electrocyclization

Pyr:  

Ar: pNO₂-Ph

Reason for 3:1 selectivity

![Image of chemical structures](image)

Highlights of this biomimetic synthesis

- The ratio of SNF4435 C and SNF4435 D obtained from the chemical synthesis agrees with the ratio of the natural abundances of these compounds.

- This indicates that the 8π-6π electrocyclization cascade may be completely *substrate controlled even in nature* and that enzymes may play an insignificant role – if any – in the biosynthesis of these compounds from Spectinabilllin.

Few of the Possible Biosynthetic Routes of Spontaneous Assembly

1. Electrocyclizations.

2. Dimerizations.

3. Cascade Reactions
Dimerizations:
The Biomimetic Synthesis of (-)-Stephacidin B

- Stephacidin B is an optically active, fungal alkaloid isolated from the fermentation broth of *Aspergillus ochraceus* in 2001.
- Avrainvillamide had already been isolated from the same fungal species in 2000.

Proposed Mechanism of Dimerization

Avrainvillamide

Two Consecutive Michael Additions

Proposed biosynthesis of Stephacidin B

Dimerization of Avrainvillamide to Stephacidin B

<table>
<thead>
<tr>
<th>Condition</th>
<th>Yield of Stephacidin B</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Et$_3$N (50eq), MeCN 20°C, 1-3.5h</td>
<td>&gt;95% conversion by NMR</td>
<td>a, b, c</td>
</tr>
<tr>
<td>PTLC (SiO$_2$, EtOAc)</td>
<td>15-20% (70-80% recovered 17)</td>
<td>b</td>
</tr>
<tr>
<td>DMSO then dried in vacuo</td>
<td>2:1 mixture of 18 and 17</td>
<td>b</td>
</tr>
</tbody>
</table>

Retrodimerization of Stephacidin B

**Condition** | **Products**
---|---
Concentration of a MeCN-H$_2$O solution 38 °C | 2:1 mixture of 17:18
3 Å molecular sieves 1:1 DMSO d$_6$:CD$_3$CN 1h, 33°C | 1:2 mixture of 17:18
SiO$_2$, PTLC | Partial retrodimerization No Yield Reported

Take Home Messages

• The dimerization of the fairly complex nitrone – Avrainvillamide – seems to occur with great ease under a variety of conditions – basic, acidic or neutral.

• Retrodimerization of Stephacidin B is also quite facile suggesting that the ready interconversion of the monomer and dimer may be feasible.

• This suggests that biosynthesis of Stephacidin B may result due to the inherent reactivity of Avrainvillamide to dimerize.
Electrocyclization-Dimerization cascade: Synthesis of Torreyan acid

- Torreyan acid is a complex polycyclic molecule isolated by Lee and coworkers from various species of the fungus *Pestalotiopsis*.

- It was proposed that a spontaneous dimerization of a tricyclic precursor can account for the formation of this heptacyclic compound.

Why was a dimerization proposed in the Biosynthesis of Torreyanic Acid?

Why was a dimerization proposed in the Biosynthesis of Torreyanic Acid?

But the key dimerization step can give 8 different stereoisomers.

The more energetic transition states

Model studies of the dimerization

Model studies of the dimerization

- The n-pentyl chains are always anti to each other
- The dienophile approaches diene from the face opposite to the epoxide
- Chiral epoxide was expected to give a single stereoisomer

Figuring out the most favorable transition state

Biomimetic Synthesis of Torreyanic Acid

Proposed Biosynthetic Precursor

Biosynthetic Precursor Mimic

Biosynthetic Precursor Mimic (29)

Dess Martin Oxidation

DCM, 23 °C

\[
\text{30} \quad \text{31} \quad \text{32}
\]

Biomimetic Synthesis of Torreyanic Acid

[4+2] Dimerization
80% Yield

Torreyanic Acid di-tButyl Ester (33)

48% HF, MeCN
1h, 70%

Torreyanic Acid

Take Home Messages

• Only 1 out of the 8 possible stereoisomers was predicted and obtained using chemical synthesis. This product also happens to be the only isolated stereoisomer of Torreyanic acid.

• This example highlights that enzymatic participation may be minimal in the biosynthesis of Torreyanic Acid from the proposed biosynthetic precursor.

Few of the possible biosynthetic routes of spontaneous assembly

1. Electrocyclizations.

2. Dimerizations.

3. Cascade Reactions.
Cascade reactions
The biomimetic synthesis of bisorbicillinoids

- Bisorbicillinoids are dodecapolyketides isolated from a variety of fungal species.
- It is postulated that they are dimeric sorbicillin-derived natural products

Cascade Reactions:
The Biomimetic Synthesis of Bisorbicillinoids

Abe’s Biosynthetic Hypothesis

\[
\begin{align*}
\text{Sorbicillin} & \xrightarrow{\text{Enatioselective Oxidation}} \text{35} \\
\text{35} & \xrightarrow{\text{Rotamer}} \text{35}' \\
\text{35}' & \xrightarrow{\text{Acyl Rearrangement}} \text{Trichodimerol} \\
\text{35} & \xrightarrow{\text{Acyl Rearrangement}} \text{Bisorbicillolinol}
\end{align*}
\]

Bisorbicillinol : A product of [4+2] dimerization

Bisorbicillinol: A product of [4+2] dimerization

Biomimetic Synthesis of Bisorbicillinol

Biomimetic Synthesis of Bisorbicillinol

In all structures:
R = \begin{align*}
&\text{Diene} \\
&\text{Dienophile} \\
&\text{Bisorbicillinol} 22%
\end{align*}

Trichodimerol: A product of consecutive Michael Addition – Ketalization reactions

Biosynthetic Hypothesis for Trichodimerol

Biosynthetic Hypothesis for Trichodimerol

Biosynthetic Hypothesis for Trichodimererol

Biomimetic Synthesis of Trichodimerol

Biomimetic synthesis of Trichodimerol

Biomimetic synthesis of Trichodimerol

In all structures

\[ R = \text{structure} \]
Biomimetic synthesis of Trichodimerol

In all structures
\( R = \text{cis-trans-1,3-dienes} \)

2nd Micheal Addition
Intramolecular

Ketalization

Trichodimerol
16%

Take home messages

• Diels-Alder dimerization and Michael addition – Ketalization cascades must be competing processes as both natural products are isolated in nature.

• Further rearrangement of these two natural products result in formation of other related natural products.

• Enzymatic participation may be limited to the activation of Sorbicillin

Biomimetic Synthesis can also raise questions about biosynthetic hypotheses.

Biomimetic synthesis of 9,10-deoxytridachione

9,10-deoxytridachione (41)

• 9,10-deoxytridachione was isolated from the mollusk Tridachiella diomeda in 1981.

• It seems to be derived from a 6\pi electrocyclization of a polyene precursor.

Biomimetic synthesis of 9,10-deoxytridachione

Rationalization for the formation of Occelapyrone A

Rational biomimetic synthesis of Occelapyrones A and B

Biomimetic Synthesis of Occelapyrones A and B

- Occelapyrones have been isolated as single enantiomers in nature.
- Biomimetic Synthesis raises several questions:
  - Can enzyme mediation be ruled out completely?
  - Is the achiral polyene the real biosynthetic precursor?
  - Is there an alternate biosynthetic pathway that does not involve electrocyclization?

Conclusions

• While the role of enzymes in biosynthesis cannot be understated, alternate modes of assembly of complex natural products may exist in nature.

• Spontaneous assembly can – to some extent – account for the highly effective exploration of chemical space by nature.

• Biomimetic synthesis can go a long way in bolstering the hypotheses of spontaneous self-assembly.

• However, the conclusions drawn by biomimetic syntheses are at times ambiguous.
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