Curiosities in Biosynthesis: Lessons & Revelations

Christine T. Chong

Historically, how has the study of biosynthesis benefitted synthetic chemistry?

1. Driving innovation in chemical synthesis

Two-phase biosynthesis of terpenoids
- **Cyclase phase** (via cascade polyene cyclizations)
- **Oxidase phase** (late-stage, selective oxidations via oxidases)
- mimicked by C-H activation

2. Overturning chemical “rules”

- Baldwin rules: *exo* epoxide-opening products are kinetically favored
- The first biomimetic, contra-Baldwin cascade without the use of directing groups was developed by Jamison (template THF & neutral water promote *endo*-selectivity)

Retrosynthetic pyramid of the eudesmane family:
*Nature* 2009, 459, 824-828

Lessons learned:
- minimization of protecting groups
- employment of divergent synthesis

Relevant Baran GMs:
Biocatalysis (Gulder, 2009)

Useful resources:
Natural Product Biosynthesis: Chemical Logic and Enzymatic Machinery, 2017

Nature Chem. Biol. 2011, 7, 865-875
Bond formation: Sulfur insertion in biotin

Sulfur-insertion often arises from amino acids

Yersiniabactin

Biotin synthase is a radical S-adenosylmethionine (SAM) enzyme that performs sulfur insertion via iron-sulfur clusters

Two modes of cleavage of SAM:

- Methyltransferase heterolytic cleavage
- Radical SAM enzyme homolytic cleavage

Biotin biosynthesis via biotin synthase:

- Pimeloyl CoA + L-Ala
- Dethiobiotin
- 2Fe-2S cluster
- 4Fe-4S cluster source of sulfur
- Radical initiator
- 5'-deoxyadenosyl radical (5'-dA·)
- 5'-dAH Met + adenine
- 2Fe-2S HAT
- [4Fe-4S]^{2+}
- [4Fe-4S]^*
- e⁻ donor

Mechanism of thiazoline biosynthesis (via NRPS assembly line):

[4Fe-4S]^{2+}

Iron-sulfur clusters are cofactors that commonly mediate electron transfer

Curiosities in Biosynthesis: Lessons & Revelations

Christine T. Chong
Baran Group Meeting
4/29/23

Curiosities in Biosynthesis: Lessons & Revelations

Bond Formation: Nature’s Strategies Towards N-N Bonds

**ACS Chem. Biol. 2021, 16, 559-570**

Strategies:
1. Comproportionation
2. Rearrangement
3. Radical Recombination

1. **Comproportionation**

Kutzneride 1 biosynthesis
- nonribosomal peptide synthase (NRPS) assembly line
- piperazic acid (non-proteinogenic amino acid) as a building block

\[
\begin{align*}
\text{HO}_2\text{C} - \text{NH}_2 & \xrightarrow{\text{KtzI}} \text{HO}_2\text{C} - \text{NH}_2 \\
\text{L-ornithine} & \xrightarrow{\text{KtzT}} \text{CO}_2\text{H} \\
\text{NH}_3 & \xrightarrow{\text{KtzT}} \text{piperazic acid}
\end{align*}
\]

*increased N electrophilicity*

2. **Rearrangement**

\[
\begin{align*}
\text{HO}_2\text{C} - \text{NH}_2 & \xrightarrow{\text{KtzI}} \text{NH}_3 \\
\text{HO}_2\text{C} - \text{NH}_2 & \xrightarrow{\text{Sbp40 metRS domain}} \text{HO}_2\text{C} - \text{NH}_2 \\
\text{HO}_2\text{C} - \text{NH}_2 & \xrightarrow{\text{Sbp40 cupin domain}} \text{HO}_2\text{C} - \text{NH}_2 \\
\end{align*}
\]

3. **Radical Recombination**

\[
\begin{align*}
\text{NADPH/FMNH}_2/\text{FADH}_2 & \xrightarrow{\text{non-enzymatic}} \text{NADP}^+/	ext{FMN}/\text{FAD} \\
\text{ArNH}_2 & \xrightarrow{\text{activation}} \text{HO}_2\text{N} - \text{N} - \text{OH} \\
\text{R} = & \text{HO}_2\text{C} - \text{NH}_2 \\
\text{HO}_2\text{C} - \text{NH}_2 & \xrightarrow{\text{Sbp40 metRS domain}} \text{HO}_2\text{C} - \text{NH}_2 \\
\end{align*}
\]

Other examples:
- dixiamycin A
- azoxymycin C
Oxidative Cleavage
*J. Biol. Chem. 2014, 289, 19, 13661-13666*

- β-carotene

```
Me  Me  Me  Me  Me  Me  Me  Me

O2

“carotene monooxygenase”?
```

- carotene dioxygenase

```
Me  Me  Me  Me  Me  Me

O2

O2

Me  Me  Me  Me

proposed dioxetane
```

- oxidative cleavage via dioxygenase logic

```
Me  Me  Me  Me  Me  Me

H2O

Me  Me  Me  Me

Me  Me  Me  Me

Me  Me  Me  Me

Me  Me  Me  Me

Me  Me  Me  Me

Me  Me  Me  Me

OH

OH

```

- all-trans retinal

- carotene oxygenase was previously falsely named as a monooxygenase
- ¹⁸O labelling studies: aldehydic oxygens in the retinal product pair derive from the same molecule of substrate O₂
- oxidative cleavage via dioxygenase logic
- methods in synthesis: OsO₄/NaIO₄, O₃, photoexcited nitroarenes

*Nature 2022, 610, 81-86*
Curiosities in Biosynthesis: Lessons & Revelations

Oxidative Dearomatization/Ring Contraction

**JACS 2023, 145, 12, 6643–6647**

Synthesis of the all-cis cyclopentanetetraol moiety

Biosynthesis of AS2077715

- PKS-NRPS hybrid
- all-cis cyclopentanetetraol

Examples in natural product biosynthesis

**Nature Comm. 2018, 9, 1963**

**Chem. Eur. J. 2021, 27, 11895-11903**

Examples in synthesis: Cu promoted oxidation of phenol with dioxygen

**J. Mol. Cat. A: Chem. 1995, 101, 1, 75-80**

Oxidative Rearrangements: Ring Expansion in Cephalosporin C

Synthesis of Cephalosporin skeleton (Lilly)
- Hypothesis: cephalosporin C is a metabolic transformation product of penicillin N

\[
\text{penicillin skeleton} \xrightarrow{\text{H}^+ \text{ reflux}} \text{cephalosporin skeleton}
\]

Biosynthetic pathway

- **Isopenicillin N synthase (IPNS)**
  - aminoadipoyl-cysteinyl-D-valine (ACV) peptide
  - \( R^1 = H, R^2 = \text{NH}_3^+ \)

- **Isopenicillin N, epimerase**
  - \( R^1 = \text{NH}_3^+, R^2 = H \)

Typical iron-based oxygenase reactivity:

\[
\text{DAOCS-Fe(IV)=O} \xrightarrow{\text{C-H bond homolysis}} \text{DAOCS-Fe(III)-OH} \xrightarrow{\text{oxygen rebound}} \text{DAOCS-Fe(III)-OH}
\]

*intramolecular reactions can outcompete intermolecular hydroxylation*

\[
\text{DAOCS-Fe(III)-OH} \xrightarrow{\text{desacetoxy-cephalosporin synthase (DAOCS)}} \text{“expandase”}
\]

**DAOCS-Fe(IV)=O**

**DAOCS-Fe(III)-OH**

**DAOCS-Fe(III)-OH**

**DAOCS-Fe(III)-OH**

\[
\text{DAOCS-Fe(III)-OH} \xrightarrow{\text{DAOCs-Fe(III)-OH}} \text{DAOCS-Fe(IV)=O}
\]

\[
\text{DAOCS-Fe(III)-OH} \xrightarrow{\text{DAOCS-Fe(III)-OH}} \text{DAOCS-Fe(IV)=O}
\]

\[
\text{DAOCS-Fe(III)-OH} \xrightarrow{\text{DAOCS-Fe(III)-OH}} \text{DAOCS-Fe(IV)=O}
\]

\[
\text{DAOCS-Fe(III)-OH} \xrightarrow{\text{DAOCS-Fe(III)-OH}} \text{DAOCS-Fe(IV)=O}
\]

\[
\text{DAOCS-Fe(III)-OH} \xrightarrow{\text{DAOCS-Fe(III)-OH}} \text{DAOCS-Fe(IV)=O}
\]

\[
\text{DAOCS-Fe(III)-OH} \xrightarrow{\text{DAOCS-Fe(III)-OH}} \text{DAOCS-Fe(IV)=O}
\]

\[
\text{DAOCS-Fe(III)-OH} \xrightarrow{\text{DAOCS-Fe(III)-OH}} \text{DAOCS-Fe(IV)=O}
\]
Curiosities in Biosynthesis: Lessons & Revelations

Divergent Biosynthesis: Oxonium Ions as Key Intermediates in Laurencia Natural Products

\[
\begin{align*}
\text{(E)-enyne: laurefurenyne F} & \quad \text{(E)-enyne: laurefurenyne D} \\
\text{(Z)-enyne: laurefurenyne B} & \quad \text{(Z)-enyne: laurefurenyne A} \\
\text{(reassigned structures)} & \quad \text{(reassigned structures)}
\end{align*}
\]

\[
\begin{align*}
\text{C}_{15} \text{ halogenated ether acetogenins} & \quad \text{polyketide origin, isolated from red algae of the genus Laurencia} \\
\text{triaklyloxonium ions proposed with chemical evidence as key intermediates} & \quad \text{triaklyloxonium ions are susceptible to rapid hydrolysis and typically do not participate in biological processes, in contrast to triaklylsulfonium ions}
\end{align*}
\]

\[
\begin{align*}
\text{(E)-elatenyne} & \quad \text{(Z)-elatenyne} \\
\text{laurendecumenyne B}
\end{align*}
\]
Biosynthetic Proposals Towards Key Oxonium Ion Intermediates in Laurencia Natural Products

Snyder:

(3E, 6R, 7R)-laurediol

\[\text{Me} \quad \text{O} \quad \text{H} \quad \text{Br} \quad \text{H} \quad \text{Me}\]

\[\text{Me} \quad \text{O} \quad \text{H} \quad \text{Br} \quad \text{H} \quad \text{Me}\]

5-endo bromoetherification

Murai & Suzuki:

(3E/Z, 6S, 7S)-laurediols via fatty acid metabolism

\[\text{Me} \quad \text{O} \quad \text{H} \quad \text{Br} \quad \text{H} \quad \text{Me}\]

\[\text{Me} \quad \text{O} \quad \text{H} \quad \text{Br} \quad \text{H} \quad \text{Me}\]

enzymatic bromocyclization

\[\text{Me} \quad \text{O} \quad \text{H} \quad \text{Br} \quad \text{H} \quad \text{Me}\]

\[\text{Me} \quad \text{O} \quad \text{H} \quad \text{Br} \quad \text{H} \quad \text{Me}\]

enzymatic bromocyclization

\[\text{Me} \quad \text{O} \quad \text{H} \quad \text{Br} \quad \text{H} \quad \text{Me}\]

\[\text{Me} \quad \text{O} \quad \text{H} \quad \text{Br} \quad \text{H} \quad \text{Me}\]

(E)-bromofucin (Z)-bromofucin
**Curiosities in Biosynthesis: Lessons & Revelations**

**Metal-containing Antibiotics**
*Science 2021*, 374, 1005-1009

**Fluopsin C**
- Cu(II) complex containing 2 N-methylthiohydroxamate ligands
- Discovered in bacterium *Pseudomonas aeruginosa*
- Found in soil, water & on human skin
- Responsible for many dangerous infections for the immunocompromised
- Antimicrobial activity & cytotoxicity against mammalian cells & whole animals

**Biological background**
- Cu is utilized by bacterial immune cells as a bactericide to poison invading microbes
- Copper resistance and detoxification is essential for the bacteria
- Cu-chelating compounds facilitate Cu import or detoxification in the cytoplasm

**Biosynthesis of Fluopsin C:**

**Extracellular space**

**Periplasm**

**Cytoplasm**

**CopZ1**

**CueR**

*CueR: copper-response regulator*

**Adenylosuccinate lyase**

**Efflux pump protein**

**Methyltransferase**

**Cu(I) chaperone**

**Heme oxygenase protein**

**CueR binding site**

**Limited precedence in biosynthesis**
Unresolved Biosyntheses

- 1,2,3-oxadiazine (yet to be accessed synthetically)
- 1,3,4- and 1,2,4-oxadiazines have been prepared by chemical synthesis

ACS Chem. Biol. 2022, 17, 2528-2537

J. Nat. Prod. 2013, 76, 2, 142–149

This section is intentionally left blank.