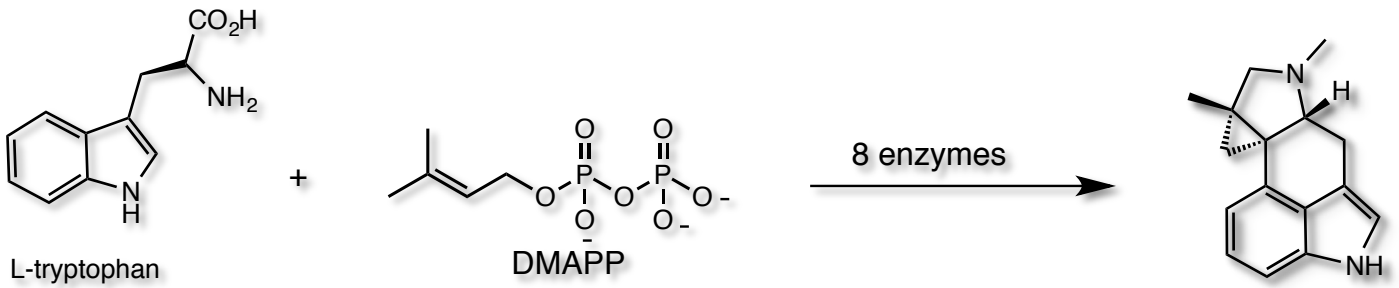
Discovery and Reconstitution of the Cycloclavine Biosynthetic Pathway
- Enzymatic Formation of a Cyclopropyl Group

Dorota Jakubczyk, Lorenzo Caputi, Anaëlle Hatsch, Curt A. F. Nielsen, Melanie Diefenbacher, Jens Klein, Andrea Molt, Hartwig Schröder, Johnathan Z. Cheng, Michael Naesby, and Sarah E. O'Connor

ACIE, **2015**, *54*, 5117-5121

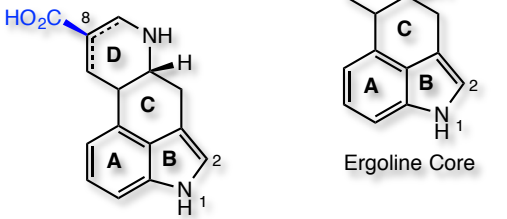


Steph McCabe
Wipf Group- Current Literature
27th June 2015

Ergot Alkaloids

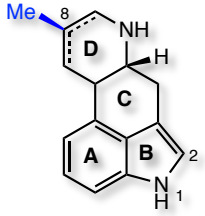
Ergot on wheat stalks

- Ergot alkaloids are produced by fungi of the *Clavicipitaceae* and *Trichocomaceae* families, which infect the seeds of grass or grains
- Consumption of ergot contaminated grains leads to ergotism (St. Anthony's fire);
  - ergotismus convulsivus characterized by hallucinations and paranoia
  - ergotismus gangrenous characterized by gangrene of the feet, legs, hands and arms
- *Ergot* alkaloids are characterized by the presence of a tetracyclic Ergoline core and can be further classified into Lysergic acid or Clavine subclasses based on the oxidation state of the substituent at C8

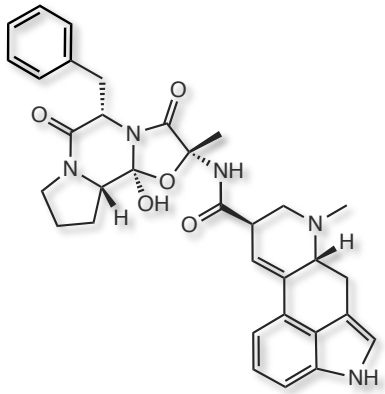


Lysergic acid subclass

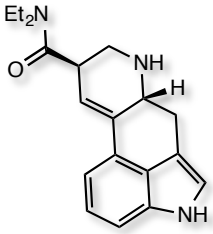
Ergoline Core



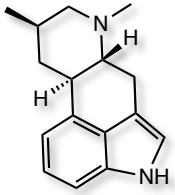
Clavine subclass



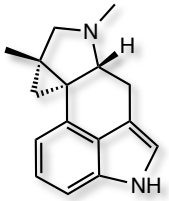
ergotamine



Lysergic acid diethylamide (LSD)



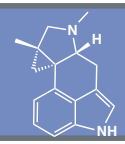
festuclavine



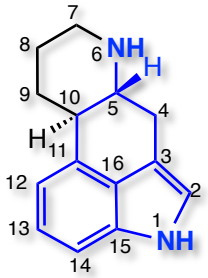
cycloclavine

Wallwey, C. and Li, S.,*Nat. Prod. Rep.*, **2011**, *28*, 496
Jakubczyk, D., Cheng, J. Z. and O'Connor, S., *Nat. Prod. Rep.*, **2014**, *31*, 1328

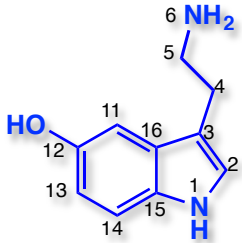
Biological Activity



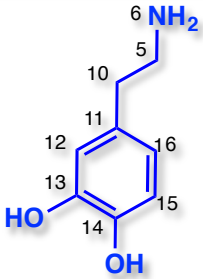
- The broad spectrum of bioactivity exhibited by ergot alkaloids is related to their ability to act as an agonist or antagonist toward neuroreceptors for dopamine, serotonin and adrenaline.



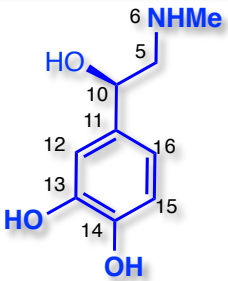
Ergoline



serotonin (5-HT)



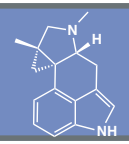
dopamine (DA)

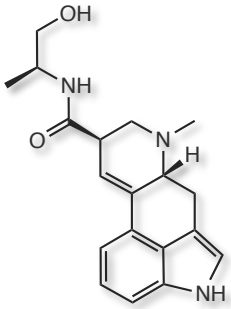


epinephrine (adrenalin)

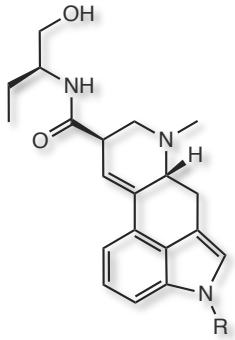
Jakubczyk, D., Cheng, J. Z. and O'Connor, S., *Nat. Prod. Rep.*, **2014**, *31*, 1328

Examples of FDA Approved Drugs with an Ergoline Core



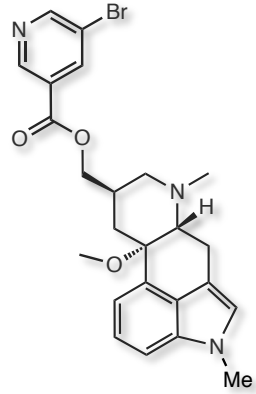


Ergometrine
Use: induces contractions, prevents post partum haemorrhage
Mechanism of Action (MOA): acts on alpha-adrenergic and serotonergic receptors

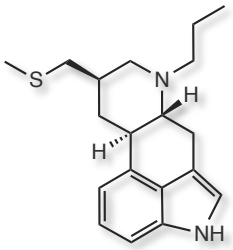


R = Me
Methysergide
Use migraine treatment
MOA: antagonist of serotonin 5-HT_{2B} receptors

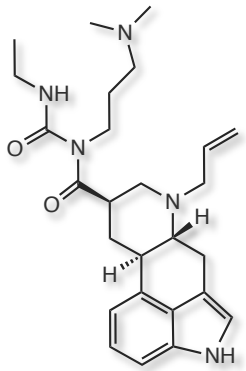
R = H
Methylergometrine
Use: prevents post partum haemorrhage, migraine treatment
MOA: partial agonist/antagonist of serotonergic, dopaminergic and alpha adrenergic receptors



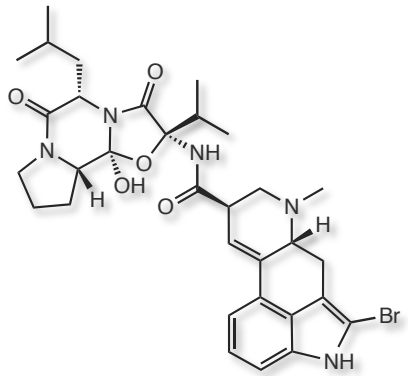
Nicergoline
Use: treatment of senile dementia
MOA: alpha-1A adrenergic receptor antagonist



Pergolide
Use: treatment of Parkinson's Disease
MOA: agonist of dopaminergic receptors (*withdrawn from the US market in 2007 due to increased risk of cardiac valvulopathy)



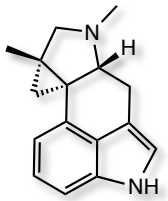
Cabergoline
Use: treatment of hyperprolactinaemia and Parkinson's disease
MOA: agonist of dopamine D2 receptors.



Bromocriptine
Use: treatment of pituitary tumors, Parkinson's disease, hyperprolactinaemia,
MOA: agonist of dopamine D2 receptors

DrugBank 4.0: Nucleic Acids Res., **2014**, 42(1),1091-7.

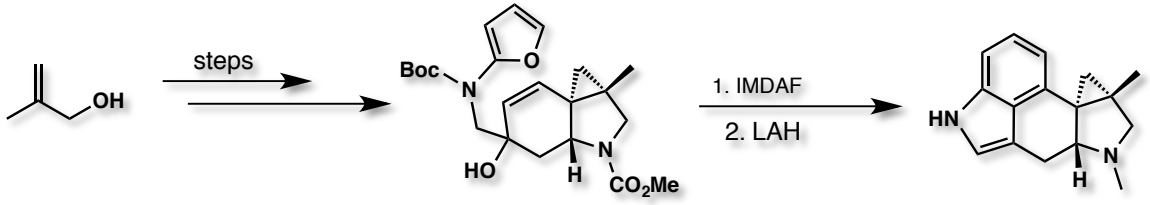
Cycloclavine



Cycloclavine

Ipomea hildebrandtii

- First isolated in 1969 from the seeds of the African morning glory (*Ipomea hildebrandtii*) by Hoffman and co-workers
- Subsequently isolated *Aspergillus japonicus,* a species of in filamentous fungus
- Absolute configuration assigned from X-ray crystallographic analysis of the methobromide salt
- No significant biological activities reported thus far
- 3 total syntheses, including an earlier synthesis from our group in 14 linear steps and 1.2% overall yield

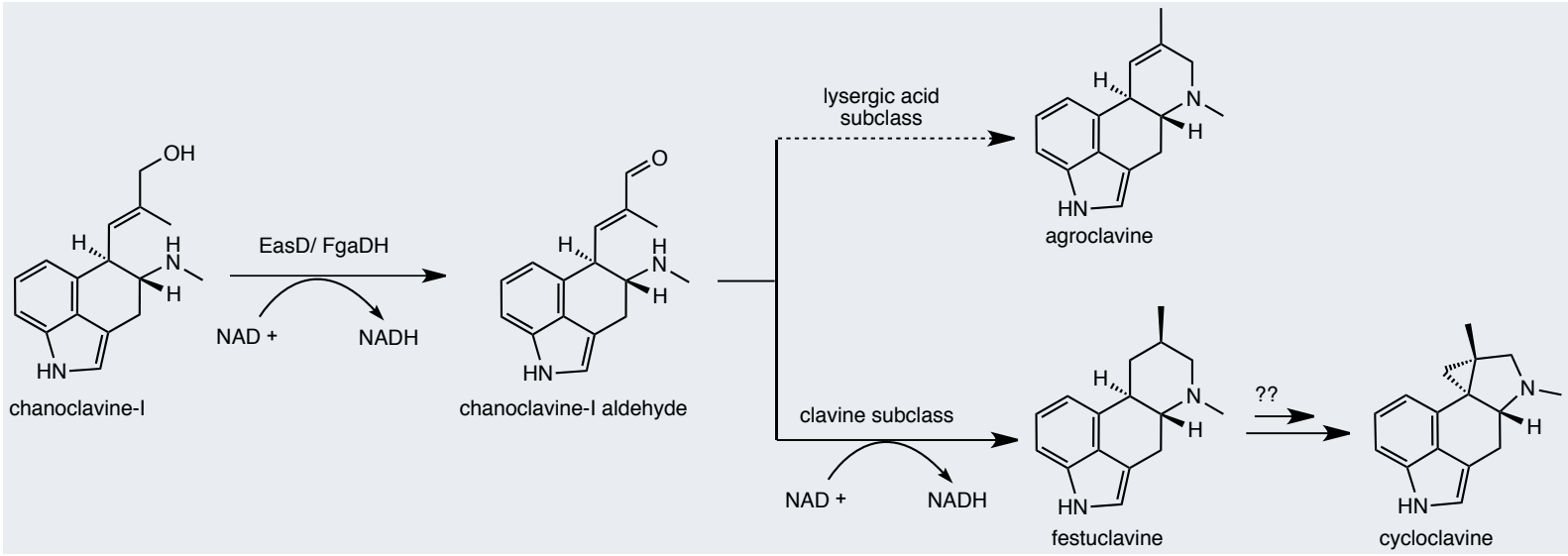


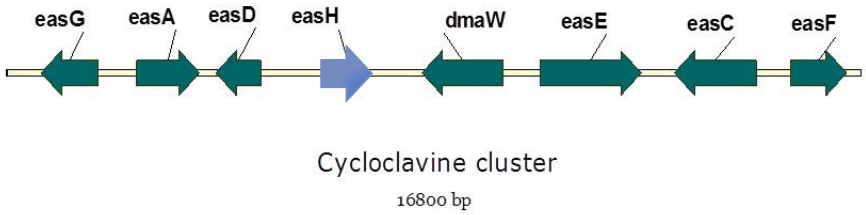
(±)-cycloclavine

Hofmann *et. al.*,*Tetrahedron*, **1969**, *25*, 5879
Furuta, T., *et al*., Agric. *Biol. Chem.,* **1982**, *46*, 1921
Incze, M., *et al*., *Tetrahedron,* **2008**, *64*, 2924
Petronijevic, F. R. and P. Wipf *JACS,* **2011**, *133*, 7704
Jabre, N. D., *et al*. ,*Tetradhedron Lett*,, **2014**, 56, 197

Biosynthetic Pathway Elucidation: Reconstitution of the Ergot Alkaloid Synthesis (EAS) Genes

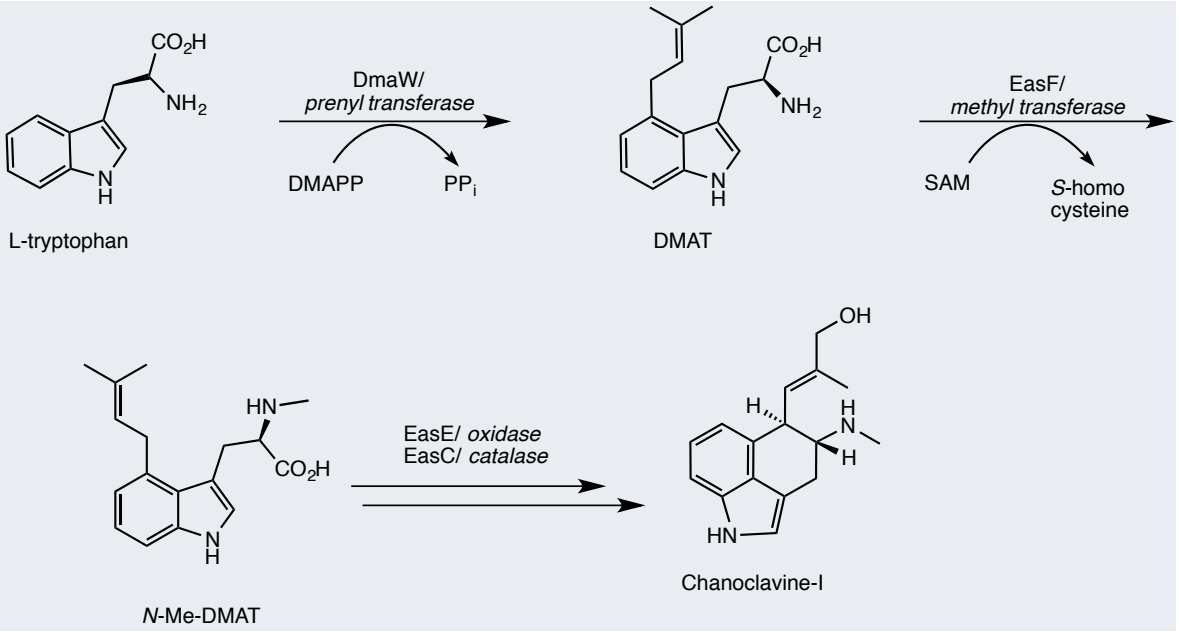
- All *ergot* alkaloids are derived from common a biosynthetic intermediate - chanoclavine-I. The mechanisms of most downstream biosynthetic transformations are unknown
- Recently a 16.8 kbp biosynthetic cluster, containing 8 genes in the genome of cycloclavine producer *Aspergillus japonicus* was identified (O'Connor *et al*)
- 7 genes are homologous to genes previously implicated in the biosynthesis of festuclavine or agroclavine.
- Role of *easH* unknown





Biosynthetic Pathway Elucidation: Reconstitution of the Ergot Alkaloid Synthesis (EAS) Genes

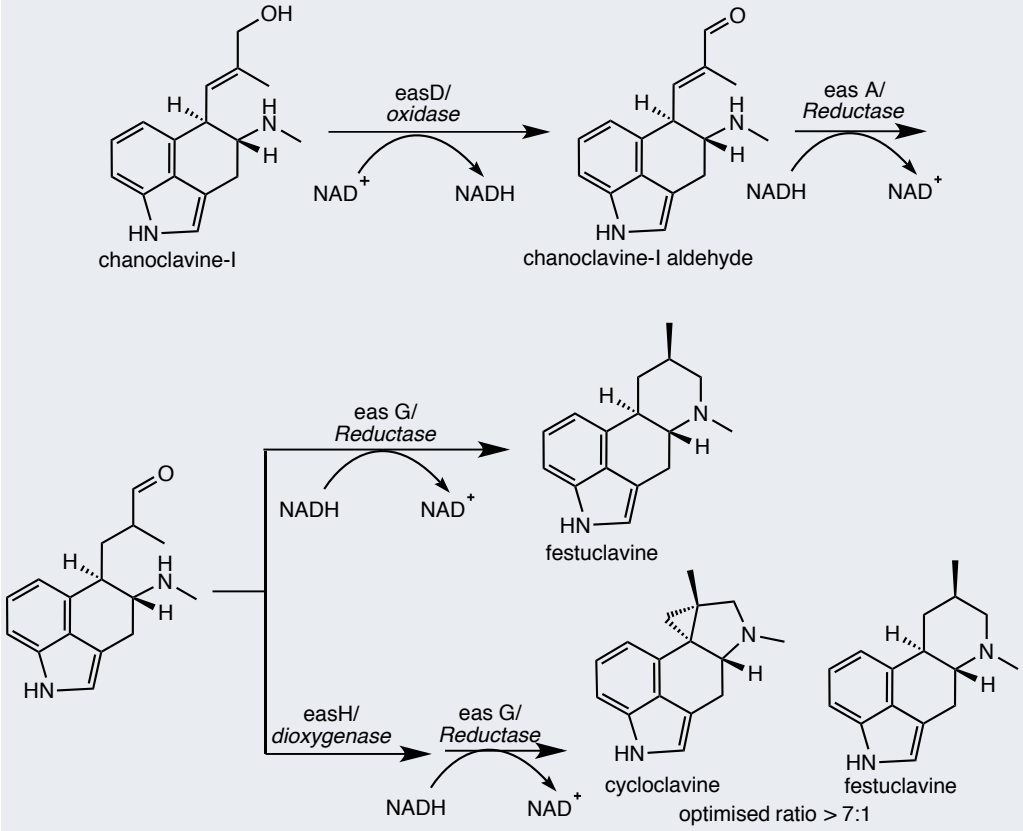
- This paper aimed to validate whether this cluster was responsible for cycloclavine biosynthesis by reconstitution of the eight genes in *S. cerevisiae*
- Chanoclavine-I was synthesized by heterologous expression of *dmaW*, *easF*, *easE* and *easC* in a *S. cerevisiae* strain

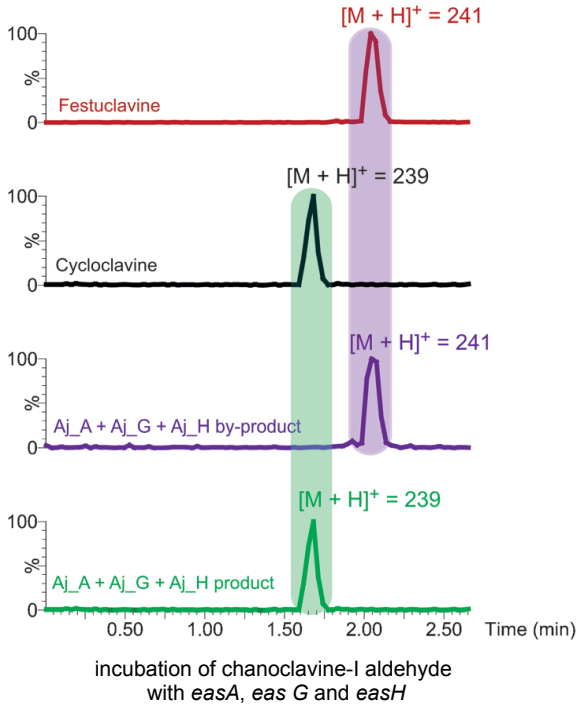


Microbial Cell Factories, **2014**, *13*, 95
Nat. Prod. Rep., **2011**, *28*, 496

Biosynthetic Pathway Elucidation: Reconstitution of the Ergot Alkaloid Synthesis (EAS) Genes

- *In Vivo* transformation of the chanoclavine-I producing strain with *easD, easA,* and *easG* produced festuclavine
- Addition of *easH* produced cycloclavine as the major product along with festuclavine
- *In vitro* incubation of chanoclavine-I aldehyde with *easA*, *eas G easH* produced cycloclavine
- Cycloclavine was reconstituted in *S. cerevisiae* with a final concentration of 529 mg. L^{-1}



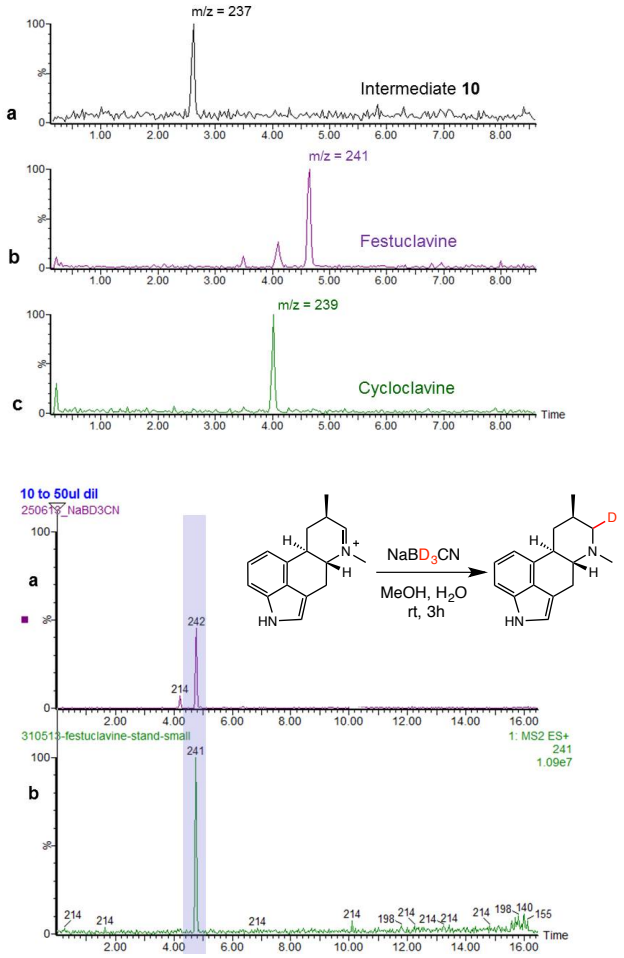


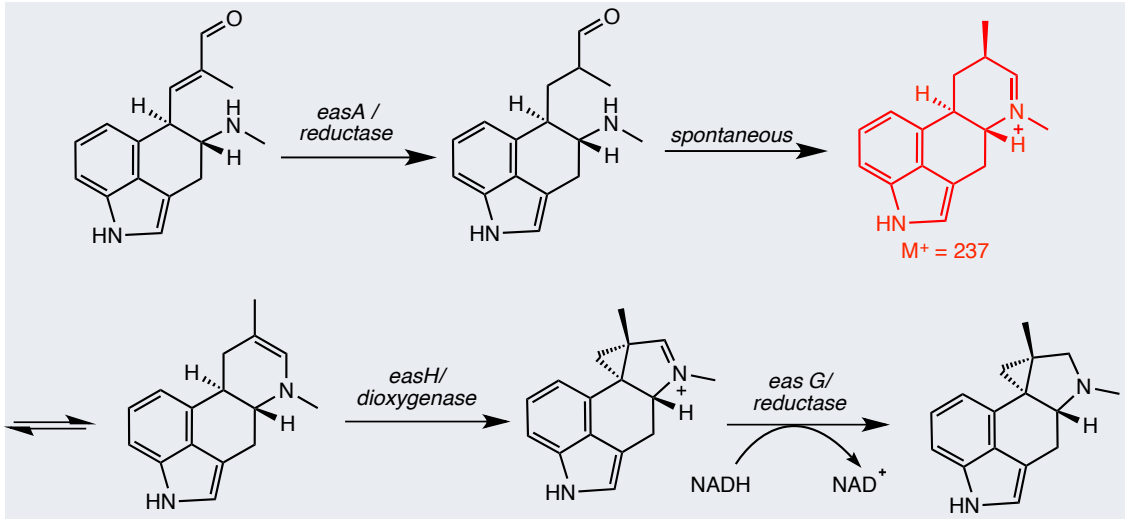
incubation of chanoclavine-I aldehyde with *easA*, *eas G* and *easH*

Role of *EasH* in the Biosynthesis of Cycloclavine

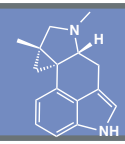
- Authors propose *EasH* generates the cyclopropyl moiety through an oxidative mechanism by intercepting one of the intermediates generated during the conversion of chanoclavine-I aldehyde into cycloclavine

- Hypothesis was supported by the detection of intermediate (M^+ = 237) upon incubation of chanoclavine-A aldehyde with Aj_EasA and Aj_EasH. Confirmed by trapping studies.

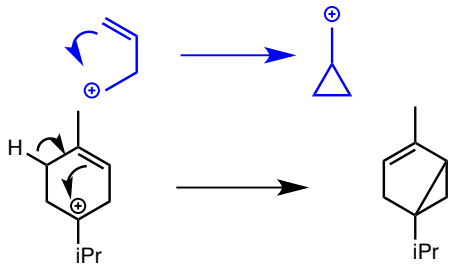




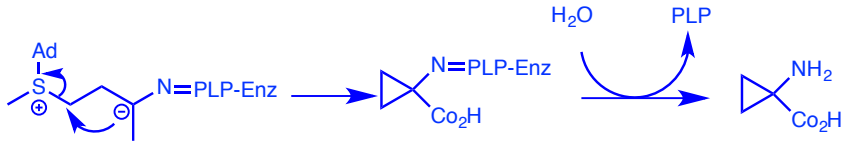
Common Biosynthetic Cyclopropanations



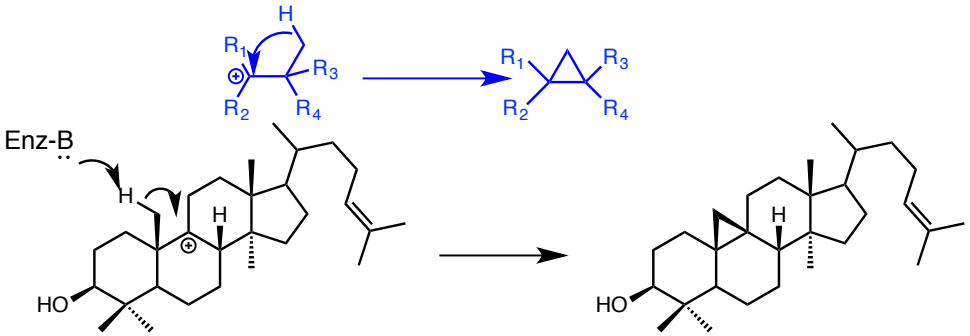
Electrophilic attack of a carbenium ion on a double bond
(most substrates are isoprenoid)



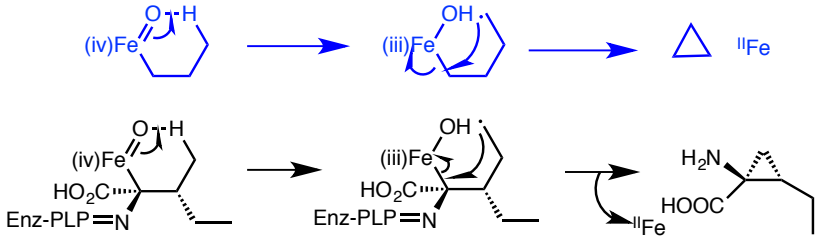
Internal nucleophilic substitution (S_Ni)



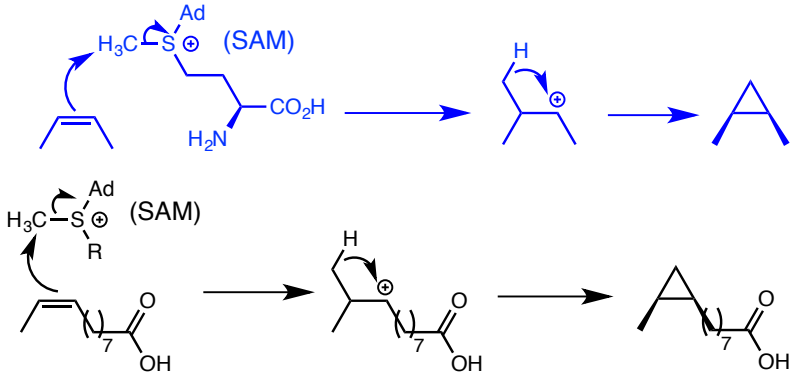
Reaction of an intermediate cation with an existing α-methyl group
(common in steroids)



Transition Metal-Mediated Radical Cyclisation



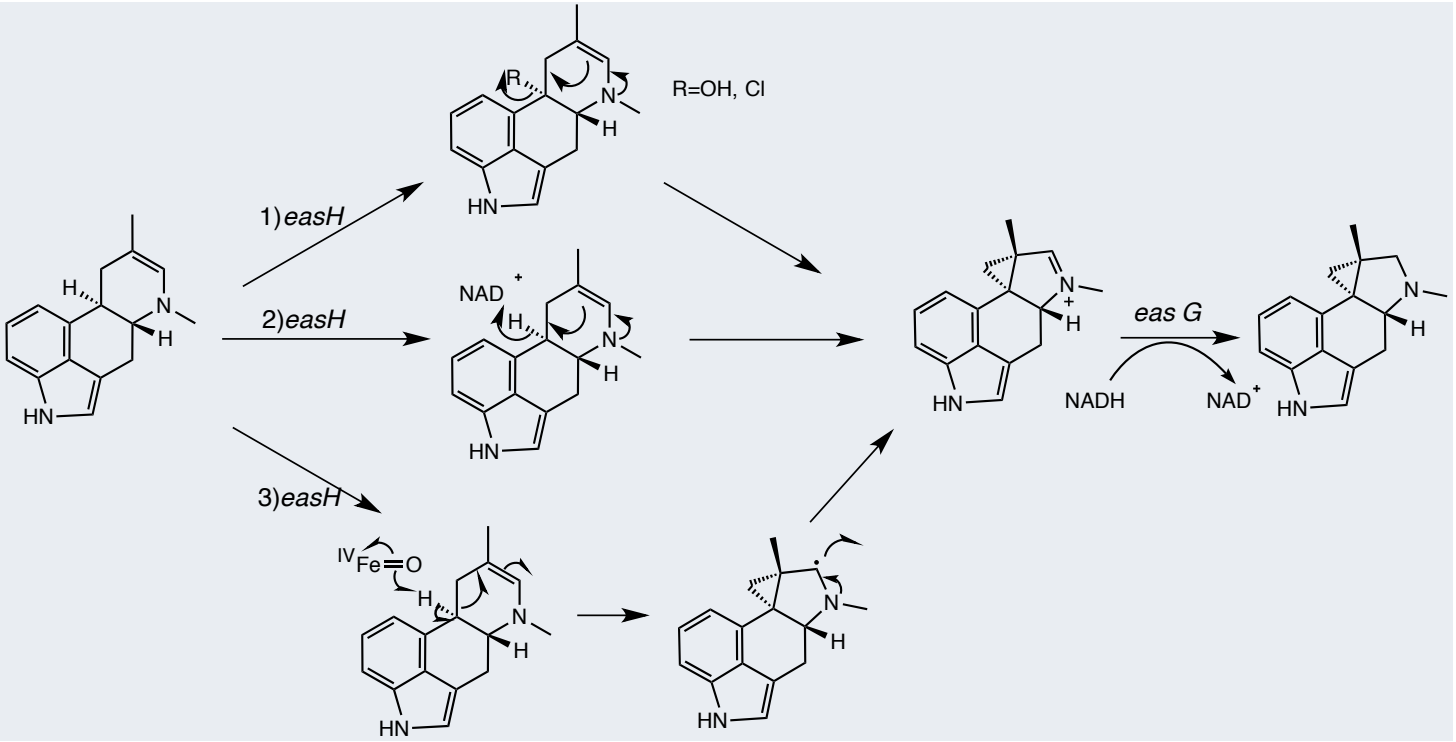
SAM mediated cyclopropanation



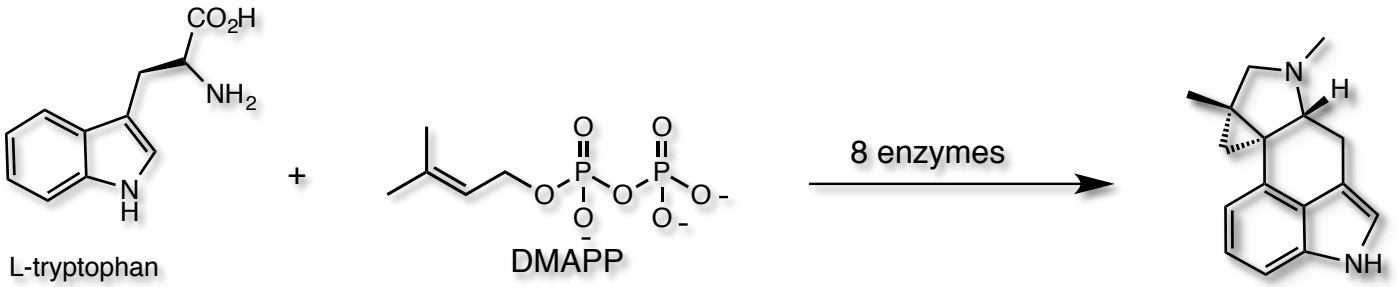
Wessjohann, L. A. and Brandt, W., *Chem. Rev.*, **2003**, *103*, 1625

Possible Mechanisms of Cyclopropanation

- 1) Hydroxylation/ Halogenation, loss of leaving group & cyclisation
- 2) Abstraction of a benzylic H by NAD^+, followed by cyclisation
- 3) Abstraction of a benzylic H by an iron cofactor followed by radical cyclisation
- Closest known *easH* homologues have demonstrated hydroxylase activity



Conclusions



- Cycloclavine was produced in high yields (529 mg. L^{-1}) through yeast fermentation
- First example of successful reconstitution of a complex, downstream ergot alkaloid
- Identification of *easH* as the gene responsible for generating the cyclopropyl moiety through an oxidative mechanism