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•Commentary•

# Carbonic anhydrase-like enzymes in the formation of *Lycopodium* alkaloid

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Plant alkaloids, renowned for their structural diversity and bioactivity, are prominent in both modern and traditional medicine [1]. Unraveling the intricacies of plant alkaloid biosynthesis could pave the way for the discovery of new natural products and pathways. It may also shed light on the roles of existing pathways in host biology and provide valuable tools for metabolic engineering in plants and microbes [2]. Unlike other major classes of plant natural products, such as terpenoids and polyketides, the formation of alkaloid scaffolds does not conform to a uniform chemical theme or depend on a singular enzyme class [3]. A case in point is the *Lycopodium* alkaloids in the Lycopodiaceae family, where the enzymes responsible for their core scaffold construction remain unidentified [4].

*Lycopodium* alkaloids, with their distinctive structures, biogenesis, and wide-ranging biological activities, have attracted immense interest worldwide. Despite extensive research into their total synthesis, the underlying biosynthetic mechanisms that plants use to create these alkaloid scaffolds are largely uncharted, hinting at the involvement of as-yet-unknown enzymes [4-6]. Within this family, huperzine alkaloids stand out. Huperzine A, in particular, is notable for its unique structure and potent inhibitory effect on acetylcholine esterase (AChE), leading to its approval as a novel Alzheimer's disease (AD) treatment in China and its use as a dietary supplement in the USA [7,8].

The article *Plant carbonic anhydrase-like enzymes catalyze neuroactive alkaloid biosynthesis* [3] recently illuminated the biosynthetic pathway of huperzine A. The study involved RNA-seq and differential expression analysis across various tissues of *Phlegmariurus tetrastichus* (the huperzine A producer). To generate co-expressed transcript clusters, the authors conducted Pearson correlation analysis of 2227 transcripts enriched in bioactive tissues using lysine decarboxylase (*LDC-1*) as the query gene. This analysis identified a specific co-expressed cluster of 131-transcript (cluster131), which includes all previously identified biosynthetic genes, pointing to the likelihood that this cluster contains crucial enzymes for assembling *Lycopodium* alkaloid scaffolds [9].

Compound **3** has been confirmed as a co-substrate in scaffold formation, with a diene (**8**) proposed as another key component (Fig. 1). The authors hypothesized that the formation of **8** requires two key steps: first, the oxidation of **4** (which is the spontaneous decarboxylation product of **3**) to form an imine; second, the reduction and elimination of the ketone oxygen. To facilitate these reactions, the team employed Agrobacterium-mediated DNA delivery in *Nicotiana benthamiana* as a transient expression platform, leading to the identification of SDR-1/2, ACT-1, and CYP782C1 as enzymes capable of catalyzing the necessary transformations. In the quest to identify enzymes responsible for "dimerization", the authors screened candidates from cluster131. This screening was conducted in grouped batches by enzyme family, leading to the discovery of three neofunctionalized CAL enzymes (CAL-1, CAL-2, and CAL-3). CAL-1/2 cooperatively catalyzed the formation of phlegmarane scaffold (**9**), while CAL-3 stereospecifically facilitated the combination of substrates to form (*S*)-**3**. However, the absence of enzymes capable of acting on **9** left the biosynthetic pathway from this dimerized compound to the tetracyclic compound **10** undetermined. To further elucidate the pathway from compound **10** to HupB, which involves the oxidation at C-8 and C-15 to create a double bond and the desaturation of the A ring, the authors expanded their search to include oxidases that could

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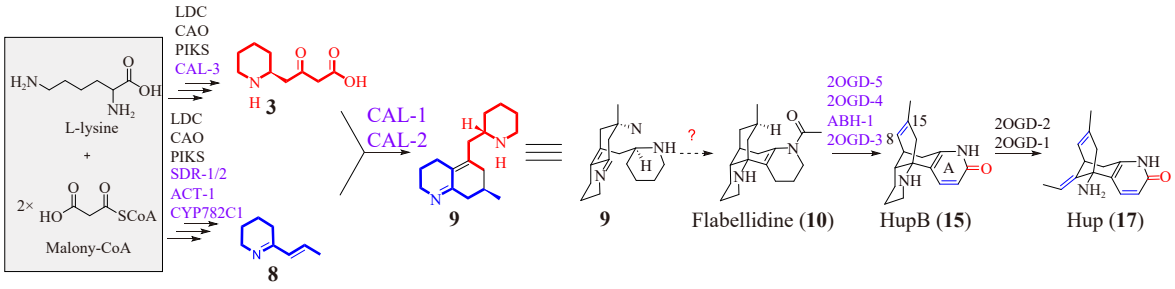


**Fig. 1 The enzymes in *Lycopodium* alkaloid biosynthesis. New enzymes or new reactions for previously described enzymes are colored purple.**

act on **10**. This exploration revealed that a Fe(II)/2-oxoglutarate-dependent dioxygenase (2OGD-4) could oxidize the carbon on the A ring to form a carbonyl group. Additionally, 2OGD-5 was responsible for the formation of the 8,15-double bond, an *α*/*β* hydrolase family enzyme (ABH-1) facilitated the removal of acetyl groups, and 2OGD-3 catalyzed the desaturation of the A ring (Fig.1). Therefore, the biosynthetic pathway of huperzine A has been largely elucidated.

In conclusion, three novel functional carbonate dehydratases (CAL-1, CAL-2, and CAL-3) were identified in this study, alongside the discovery of three additional enzyme classes (SDR-1/2, ACT-1, and CYP782C1), which play a crucial role in the synthesis of the *Lycopodium* alkaloid backbone. Additionally, three Fe(II)/2-oxoglutarate-dependent dioxygenases (2OGD-3, 2OGD-4, and 2OGD-5) and an *α*/*β* hydrolase (ABH-1) were found to participate in the downstream modification reactions. These findings greatly expand our understanding of the diverse enzymatic functions within the plant kingdom.

## References

[1] Lichman BR. The scaffold-forming steps of plant alkaloid biosynthesis [J]. *Nat Prod Rep*, 2021, **38**(1): 103-129.

[2] Anarat-Cappillino G, Sattely ES. The chemical logic of plant natural product biosynthesis [J]. *Curr Opin Plant Biol*, 2014, **19**, 51-58.

[3] Nett RS, Dho Y, Tsai C, *et al*. Plant carbonic anhydrase-like enzymes in neuroactive alkaloid biosynthesis [J]. *Nature*, 2023, **624**(7990): 182-191.

[4] Ma X, Gang DR. The *Lycopodium* alkaloids [J]. *Nat Prod Rep*, 2004, **21**(6): 752-772.

[5] Siengalewicz P, Mulzer J, Rinner U. *Lycopodium* alkaloids: synthetic highlights and recent developments . *In*: Knölker HJ. *The Alkaloids: Chemistry and Biology* [M]. Elsevier, 2013, **72**: 1-151.

[6] Kitajima M, Takayama H. *Lycopodium* alkaloids: isolation and asymmetric synthesis [J]. *Top Curr Chem*, 2012, **309**: 1-31.

[7] Liu JS, Zhu YL, Yu CM, *et al*. The structures of huperzines A and B, two new alkaloids exhibiting marked anticholinesterase activity [J]. *Can J Chem*, 1986, **64**(4): 837-839.

[8] Cheng BC, Song LL, Chen FE, *et al*. Huperzine alkaloids: forty years of total syntheses [J]. *Nat Prod Rep*, 2024: D3NP00029J.

[9] Nett RS, Dho Y, Low YY, *et al*. A metabolic regulon reveals early and late acting enzymes in neuroactive *Lycopodium* alkaloid biosynthesis [J]. *Proc Natl Acad Sci*, 2021, **118**(24): e2102949118.

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