Indole Alkaloids

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Structural Basis and Enzymatic Mechanism of the Biosynthesis of C_9- from C_{10}-Monoterpenoid Indole Alkaloids**

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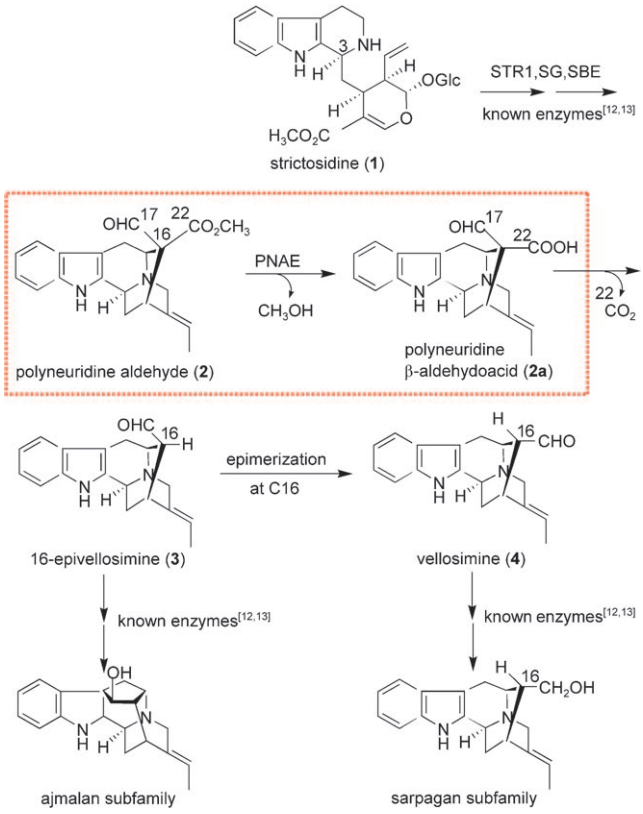
Dedicated to Professor Heinz G. Floss on the occasion of his 75th birthday.

Plants are an extremely important source of alkaloids, of which over 20000 have been identified to date and classified into a number of distinct families.[1] Thanks to their established therapeutic importance,[2,3] structural diversity,[4] and complex biosynthesis,[4,5] the monoterpenoid indole alkaloid family has attracted significant interest. Many alkaloids of the ajmalan and sarpagan subfamilies exhibit a C_9- instead of a C_{10}-monoterpenoid unit. Ajmalan-type alkaloids, such as ajmaline, are important therapeutics for the treatment of antiarrhythmic heart disorders. Since, like all monoterpenoid indole alkaloids, the members of these subfamilies are derived from the 3α(*S*)-glucoalkaloid strictosidine (**1**),[6,7] which displays the C_{10} secologanin skeleton, loss of a C_1 unit during their biosynthesis must take place somewhere downstream of **1**. We report herein the determination of the X-ray crystal structure of polyneuridine aldehyde esterase (PNAE),[8–10] which gives important insight into the biosynthesis of both subfamilies.

For the biochemical characterization of PNAE, the enzyme was isolated from plant cell cultures of the Indian medicinal plant *Rauvolfia serpentina*[10] and partially sequenced; the PNAE cDNA was then cloned and overexpressed in *Escherichia coli*, as previously described.[8–10] PNAE showed extremely high specificity for its natural substrate, polyneuridine aldehyde (**2**); out of 14 alkaloidal and aromatic esters only **2** and its ethylester were processed.[8] Sequence analysis led to the preliminary classification of PNAE as a new candidate of the large α/β hydrolase superfamily.[9,11] This classification is based in particular on the catalytic triad Ser87, Asp216, and His244, which was previously verified by single mutations and homology modeling.[9] Together with several well-known recently characterized enzymes,[12,13] the role of PNAE in alkaloid biosynthesis is shown in Scheme 1.

Mechanistically, the key chemical reaction of the enzyme is the hydrolysis of the methyl ester function of **2**, leading to the postulated polyneuridine β-aldehydoacid (**2a**). β-Ketoacids, such as oxalosuccinate in the Krebs cycle, are highly unstable owing to facile enzyme- or buffer-catalyzed decar-



Scheme 1. The central role of polyneuridine aldehyde esterase (PNAE) in the biosynthesis of the C_9-monoterpenoid ajmalan and sarpagan alkaloid subfamilies (biogenetic numbering; STR1 = strictosidine synthase, SG = strictosidine glucosidase, SBE = sarpagan bridge enzyme).

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boxylation, and occur rarely as intermediates in biosynthetic pathways. Decarboxylation of **2a** results in generation of the first C_9-monoterpenoid alkaloid 16-epivellosimine (**3**), the direct biosynthetic precursor of the ajmalan subfamily in *Rauvolfia*. Epimerization of **3** results in vellosimine (**4**), which functions as an initial precursor in the biosynthesis of the C_9 sarpagan alkaloids. It is the loss of CO_2 (C22) from the C_{10}-terpenoid skeleton which occurs as a result of PNAE action that leads to the biosynthesis of C_9 *Rauvolfia* alkaloids from the C_{10} progenitors **2** and **2a** (Scheme 1). Biochemical data provided evidence for the enzyme-catalyzed formation of both alkaloid groups, but we describe here direct insight into the three-dimensional structure and the mechanism of PNAE.[13]

The overall crystal structure (Figure 1) shows the core domain of PNAE, which consists of six β sheets flanked by five α helices. Together with the cap domain,[14] the three-dimensional structure unequivocally confirms for the first time that PNAE belongs to the well-known α/β hydrolase fold, but has a novel function. The opening of the reaction channel is located in the cap (see the Supporting Information), a region which in contrast to the canonical core of the α/β hydrolases, is structurally highly flexible. The deep channel leads **2** to the catalytic active site residues, which are in the order typical for the α/β fold: nucleophilic Ser87, acidic Asp216, and basic His244, suggesting a serine esterase mechanism (Scheme 2).

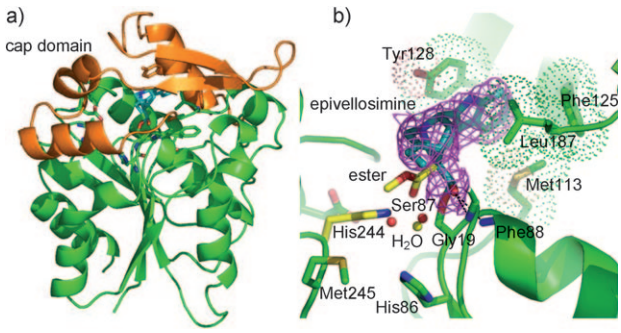
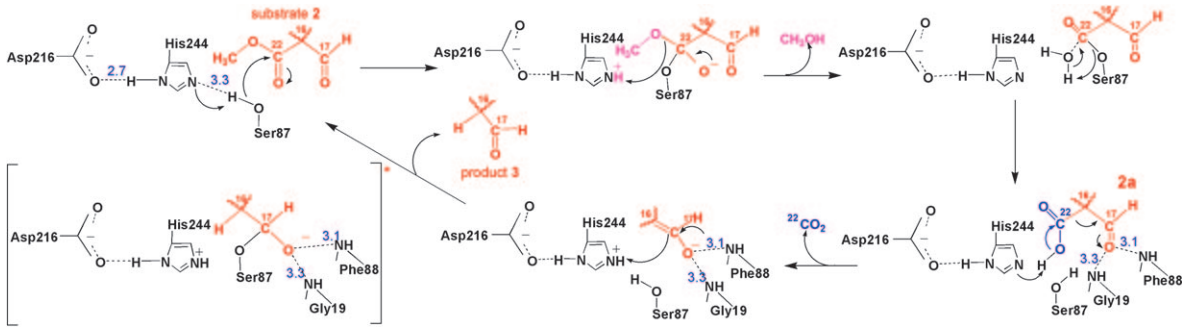


Figure 1. a) Three-dimensional structure of PNAE (PDB code: 2WFL). b) Enzyme product **3** (blue) covalently linked as a hemiacetal to Ser87 in the active site of the PNAE mutant His244Ala (PDB code: 3GZJ), overlaid with His244, the water molecules of native structure (yellow) and of complex structure (red). The ester group (yellow) is modeled. The cap domain is in orange.

The PNAE His244Ala mutant shows extremely reduced hydrolase activity (ca. 1.3 % relative activity compared to that of the wild-type enzyme). As now demonstrated by the crystal structure of His244Ala in complex with the enzyme product **3**, the indolic part in the molecule interacts with Met113, Phe125, Tyr128, and Lys187. This arrangement enables the alkaloid to be fixed by hydrophobic, sandwichlike interactions providing optimal structural accommodation for catalysis. The optimized geometry of the active center, the substrate and C17 aldehyde group are crucial for PNAE activity, since slight changes in structure or functionality result in loss of enzymatic hydrolysis, for example if **2** is compared with the indole alkaloid picralinal or with polyneuridine (reduced CHO group at C17) (see the Supporting Information).

In addition to the necessity of the catalytic triad as proven by site-directed mutagenesis,[9] residue Met245 located in the binding pocket of PNAE is also indispensable for hydrolysis, since the Met245Ala mutant has a relative activity of only approximately 0.4 %. Met245 is far (ca. 5 Å) from the water molecules in the active center and as the neighbor residue of the catalytic His244, it might therefore be of structural rather than of catalytic significance. This situation seems to be similar for His86, the neighbor of Ser87, since mutant His86Ala also exhibits only approximately 0.1 % relative activity. After Ser87-assisted hydrolysis, **2a** (Scheme 2) decarboxylates to the enolized enzyme product **3**, in which the enolate anion is stabilized by hydrogen bonds of the backbone amides of Gly19 and Phe88 forming an oxyanion hole. Of the three reasonable mechanisms for β-ketoacid decarboxylation[15]—a) metal-ion catalyzed, b) Schiff base assisted and, c) hydrogen bond and/or electrostatic-based polarization of the keto group—a) and b) can be excluded. Neither cation-complexing ethylenediaminetetraacetate[10a] nor borohydride reduction in presence of **2** which would reduce an intermediate Schiff base and block the catalysis, affected PNAE activity. Moreover, the three-dimensional structures of the enzyme and complex do not display Schiff base forming residues in the active site, and the complex contains no metal



Scheme 2. The proposed serine esterase type reaction mechanism of PNAE is the basis for the biosynthesis of C_9- from C_{10}-monoterpenoid indole alkaloids of ajmalan and sarpagan subfamilies in the Indian medicinal plant *Rauvolfia serpentina* (partial structures of substrate **2** and product **3** are shown; the covalently linked enzyme product **3** is marked with a star; average distances of residues are in Å).

ions (no significant peaks in anomalous Fourier map) in the active center; both of these results also favor mechanism (c).

The structural data now available for PNAE support the overall mechanism proposed in Scheme 2, which undoubtedly represents the key reaction for the biosynthesis of C_9-monoterpenoid *Rauvolfia* alkaloids. The data will also allow a rational, structure-based redesign of PNAE, similar what we have recently demonstrated for the Pictet-Spenglerase, strictosidine synthase,[16] which is now applied for chemoenzymatic synthesis of novel alkaloid libraries.

Because the cap domain (see the Supporting Information) and the architecture of the binding pocket of PNAE determines its exceptional high substrate specificity, future systematic structure-based, second-sphere, and random mutations should give PNAE mutants with altered, especially low, substrate specificity. Such enzymes then could be useful multipurpose catalysts for generation of novel alkaloid structures for biological screening.

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