Polyneuridine Aldehyde Esterase: an Unusually Specific Enzyme involved in the Biosynthesis of Sarpagine Type Alkaloids

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Polyneuridine aldehyde esterase is a highly substrate specific enzyme which catalyses the conversion of the monoterpenoid C_{10}-unit into the C_9-unit at the stage of polyneuridine aldehyde in the biosynthesis of sarpagine type alkaloids.

Our present knowledge on the elaboration of the sarpagine skeleton is entirely based on hypotheses[1] derived from structure comparison of sarpagine alkaloids isolated from differentiated plants. During the course of a phytochemical screening of Apocynaceae cell suspension cultures we found that cultured cells of *Rauwolfia serpentina* Benth. produce only sarpagine/ajmaline alkaloids as major constituents.[2] We therefore used Rauwolfia cells for the first detailed enzymatic investigation on the biosynthesis of sarpagine/ajmaline type alkaloids.

The biogenetic formation of sarpagine (**6**) can be formally described by three general reaction steps: (a) generation of the characteristic C(5)–C(16) bond; (b) conversion of the monoterpenoid part (C_{10}) into a C_9-unit; (c) 'late stage' reactions, *e.g.* reduction, hydroxylation.

Of these main features we investigated (b) by incubation of polyneuridine aldehyde (**1**) with enzyme extracts of *R. serpentina* cells: 3.5 nmol (3.37×10^4 d.p.m.) of (**1**) were incubated in 50 μl of 0.1 M KPi buffer (pH 7; 2 h; 30 °C) with 10 μg of enzyme from Rauwolfia cell suspensions. T.l.c. analysis of this mixture showed the enzymatic conversion of (**1**) into a single compound, specific enzyme activity 0.26 nkat/mg protein. From an incubation mixture which was scaled up 2×10^4-fold (but of total volume 40 ml), 10 mg of this unknown product were isolated and purified by t.l.c. ($[\alpha]_D^{20}$ +52°, MeOH). Its mass spectrum was identical with that described for vellosimine (**4**),[3,4] but did not allow a distinction to be made between (**4**) and (**3**). ^{1}H N.m.r. analysis, however, clearly identified the enzymatic product as (**4**) (aldehyde signal at δ 9.65[5]). Moreover, further n.m.r. data fully agreed with the assignment of structure (**4**), already isolated from *Geissospermum vellosii*[6] and *R. vomitoria* plants.[4] This cell-free conversion of (**1**) into (**4**) is catalysed by a new plant enzyme, which we have named polyneuridine aldehyde esterase (PNA-esterase). It has a relative molecular mass of 35 000 ±3 000; K_m for (**1**) 1.25 mM at a specific activity of 16.9 nkat/mg; a pH optimum at 7.5; and a surprisingly high stability; no loss of activity at 4 °C within 30 days. PNA-esterase hydrolyses the ester (**1**) with the formation of (**2**) in a cofactor-independent reaction. The acid (**2**) cannot be isolated and

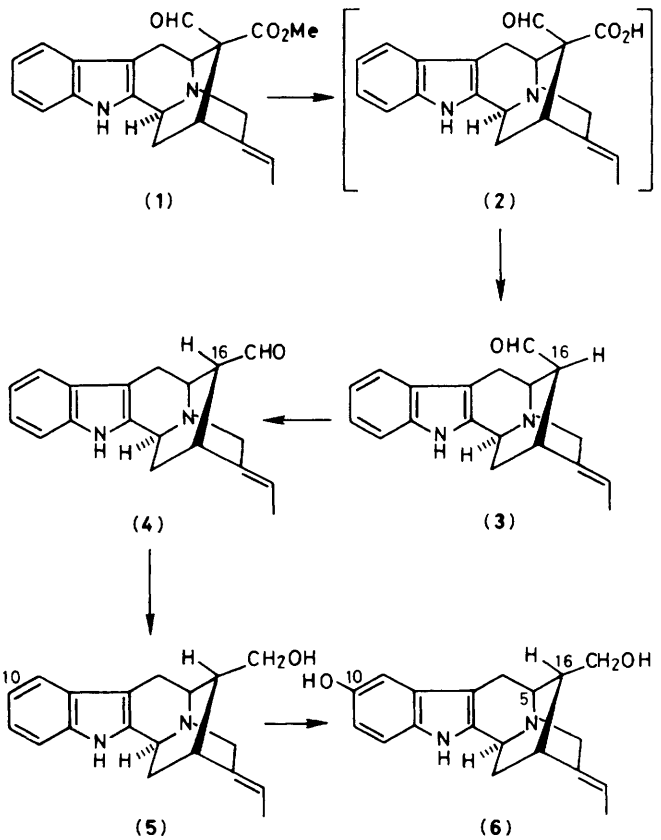

Table 1. Survey of polyneuridine aldehyde esterase (PNA-esterase) activities in plant cell cultures and, in parentheses, leaves (n.d. = not detected, detection limit 0.045 pkat).

Species	Tribe	Family	PNA-esterase activity pkat/mg protein	
Rauwolfia serpentina	Rauwolfieae	Apocynaceae	330	(2.9)
Rauwolfia vomitoria	Rauwolfieae	Apocynaceae	—	(2.3)
Catharanthus roseus	Alstonieae	Apocynaceae	2.1	(0.14)
Alstonia scholaris	Alstonieae	Apocynaceae	n.d.	(n.d.)
Tabernanthe iboga	Tabernaemontaneae	Apocynaceae	n.d.	(n.d.)
Nauclea latifolia	Naucleeae	Rubiaceae	n.d.	(n.d.)
Nicotiana sylvestris	—	Solanaceae	n.d.	(n.d.)
Daucus carota	—	Umbelliferae	n.d.	(—)

is assumed to decarboxylate spontaneously with generation of the monoterpenoid C_9-unit. This C_9-skeleton is typical of sarpagine alkaloids.

Formation of (**4**) implies that the decarboxylation step proceeds with inversion of configuration at C-16. In the case of retention of the C-16 configuration, (**3**) should be the immediate product of (**2**). Incubation of (**1**) for a short time in the presence of PNA-esterase supported the later assumption. Under these conditions the major product formed was found to be (**3**) (-C*H*O at δ 9.16), which thus far has not been described as a naturally occurring alkaloid. With purified PNA-esterase the ratio of (**3**) to (**4**) was found to be 4 : 1. This ratio was not only dependent on the incubation time but was strongly influenced by the amounts of enzyme used; formation of (**3**) was proportional to the esterase concentration. After the isolation of (**3**) its conversion into (**4**) with time was studied by incubation of (**3**) in the presence *and* absence of PNA-esterase, respectively. In both series of experiments the observed conversion rates of (**3**) into (**4**) clearly indicated, that under the conditions used, (**3**) spontaneously epimerizes to (**4**). From these experiments we conclude that the decarboxylation of (**1**) takes place with retention of configuration.

With only few exceptions,[7] esterases usually show a broad substrate specificity.[8] In order to determine the specificity of the PNA-esterase and thus clarify whether this enzyme plays a significant role in the biosynthesis of sarpagine type alkaloids, we examined the enzymatic hydrolysis of 15 different esters. Of these compounds, exclusively polyneuridine aldehyde (100% rel. act.) and akuammidine aldehyde (49% rel. act.), are accepted as substrates, which demonstrates that this esterase has an extraordinary specificity. We therefore conclude that PNA-esterase does indeed occupy a key function in the biosynthesis of the sarpagine skeleton.

This central role can also be demonstrated by the distribution of PNA-esterase within different plant families, tribes, and species (Table 1). In different species of plant families which do not synthesize monoterpenoid indole alkaloids (Solanaceae and Umbelliferae) the esterase could not be detected. Of the indole alkaloid-bearing species, high enzyme activities of PNA-esterase were only present in species which are known to contain (**4**) or derivatives of (**4**), *e.g.* (**5**) (10-deoxysarpagine). Obviously the occurrence of PNA-esterase is restricted particularly to plant species biosynthesizing sarpagine type alkaloids.

Thus, in the biosynthetic sequence to sarpagine (**6**) the next step would be the enzymatic reduction of (**4**), after the conversion of (**1**) into (**3**) and epimerisation of (**3**) to (**4**) has taken place. When (**1**) or (**4**) were incubated with a crude enzyme mixture in the presence of NADPH, 10-deoxysarpagine (**5**) was synthesized. We propose the name vellosimine reductase for the enzyme involved. The final purification and characterisation of this new enzyme catalysing one of the 'late-stage' reactions in the biosynthesis of sarpagine alkaloids is presently under investigation.

We thank Dr. N. Neuss, Eli Lilly Company Indianapolis, for generous gifts of reference alkaloids, Dr. P. Keller, Ohio State University, for n.m.r. spectra and for his help in preparing the English version of the manuscript, and the Deutsche Forschungsgemeinschaft for a Finnigan MAT 44 S instrument as well as for financial support.

Received, 29th December 1982; Com. 1485

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