Tetrahedron Letters,Vol.24,No.47,pp 5197-5200,1983 0040-4039/83 $3.00 + .00
Printed in Great Britain ©1983 Pergamon Press Ltd.

BIOGENETIC LINK BETWEEN SARPAGINE AND AJMALINE TYPE ALKALOIDS

Artur Pfitzner and Joachim Stöckigt*

Institut für Pharmazeutische Biologie, Universität München, Karlstr. 29, D-8000 München 2 (FRG).

Abstract: A new enzyme -vinorine synthase- isolated from cell suspension cultures of Rauwolfia serpentina, links sarpagine and ajmaline alkaloids by catalyzing the acetyl-coenzyme-A dependent biosynthesis of the ajmalan skeleton from sarpagan type compounds.

Previous investigations on the biosynthesis of sarpagine/ajmaline type alkaloids in cell suspension cultures of Rauwolfia serpentina Benth. led to a detailed understanding of several of the involved biochemical mechanisms [1,2].

Whereas most of the enzymes catalyzing reaction steps of the sarpagan or ajmalan route are now identified, the biosynthetic connection between the sarpagine and ajmaline types was based entirely on theoretical considerations. As early as 1956 Woodward suggested[3] that sarpagan alkaloids 1 bearing an endo aldehyde function at C-16 might act as biogenetic precursor of the ajmalan skeleton (compounds 2, 3). Indeed, the formation of the ajmalan characteristic C-7-C-17 bond can be chemically achieved under strongly acidic conditions (5 N HCl) by generation of the labile alkaloids 2, which subsequently can be stabilized by acetylation forming 3[4] (Scheme 1). This process exactly simulates the biochemical reaction providing the biosynthetic link between sarpagine and ajmaline alkaloids, which is catalyzed by the acetyl-CoA dependent vinorine synthase, described in this publication.

Sarpagan Type → (HCl, Ac_2O) → Ajmalan Type (Indolenine group)

1 (R^1 = H or CO_2CH_3)

2 (R^1 = H or CO_2CH_3) (R^2 = H)

3 (R^1 = H or CO_2CH_3) (R^2 = Ac)

Tetraphyllicine 4 (R = H)

Acetyltetraphyllicine 5 (R = Ac)

Scheme 1

Our previous characterization of polyneuridine aldehyde esterase (PNA-esterase)[1,5] resulted in the isolation of the novel indole alkaloid 16-epi-vellosimine 7. The alkaloid 7 meets the indispensable steric requirement [16(S) configuration] for the elaboration of the C-7-C-17 bond of ajmaline compounds. Therefore 7 (1.4 μmol) was incubated in 6 ml KPi (0.1 M, pH 6.2) for 90 min in presence of acetyl-CoA (30 μmol) and 12 mg of a "crude enzyme extract" obtained from R. serpentina cell suspensions as recently described[6]. Ethyl acetate extraction of this mixture and TLC (solvent: acetone/diethylamine/petroleum ether = 1/1/3) showed two compounds; vellosimine [16(R)]-spontaneously formed by epimerisation of 7- and an alkaloid exhibiting identical TLC behaviour and CAS (ceric ammonium sulfate)-reaction as vinorine 9. The fact that 9 isolated from Vinca minor plants[7] was optical inactive in contrast to 9 found in cultured R. serpentina cells[8], prompted us to carry out a detailed spectroscopic analysis of the in vitro synthesized 9. To obtain sufficient amounts of 9 the above incubation was scaled up 12-fold, and 7 was generated in situ during the incubation, from 15 μmol polyneuridine aldehyde 6 and 65-fold purified PNA-esterase (10 nkat). Vinorine 9 (1.5 mg, enzyme activity 150 pkat/mg protein) was isolated, purified by TLC and spectroscopically investigated: UV λ_{max} 220,266 nm, λ_{min} 241 nm; MS (70 eV) 334 (M^+, 32), 291 (M^+-43, 51), 275 (30), 182 (32), 169 (72), 168 (100), 154 (20) m/z; ^{1}H-NMR[9], $[\alpha]_D^{20} = -30^o \pm 3^o$ (c=0.108[10], $CHCl_3$). All spectra are superimposible with those obtained for vinorine 9 isolated from R. serpentina cells or synthesized from acetyl tetraphyllicine 5[11,12]. They are also in agreement with published values[7,11] except for the reported [α]-value.

To obtain further information on the enzymatic conversion of 7 ⟶ 9 vinorine synthase was partially purified by two chromatographic steps employing DEAE-cellulose and AcA 54 gel chromatography. Protein fractions were routinely monitored for synthase activity in the presence of 10 nmol 6, 40 pkat PNA-esterase, [^{3}H]-acetyl-CoA (10 nmol, 2.5 x 10^{-2} μCi) in a total volume of 0.2 ml. After 30 min of incubation at pH 7.5 the mixture was extracted with ethyl acetate and 9 determined by scintillation counting of the organic phase. At this purification stage the 46-fold enriched vinorine synthase exhibited the following enzyme characteristics; an apparent molecular weight of 32000± 10% (determined by gel chromatography), an isoelectric point at pH 4.4 and a pH optimum at pH 8.5. It is interesting to note, that in addition to published experiments[4] the non-enzymatic ring closure of 7 and of related compounds can also be achieved under rather physiological conditions but obviously not at pH 8.5[13].

In order to obtain evidence for the complete sequence 7 ⟶ 9, we attempted to identify the expected intermediate 8. However 8 thus far has not been isolated from the enzyme reaction mixture containing only 7 and the synthase. This suggests a high lability of the 17-OH-indolenine 8. This observation is in accord with the fact that alkaloids of type 8 have never been synthesized nor they have been isolated from plants[14].

Studies on the specificity of vinorine synthase clearly indicate, that 7 is the true biogenetic precursor of 9 and that only alkaloids bearing an endo aldehyde group act as substrate. The corresponding N_a-methylated alkaloids are however enzymatically inactive (table), supporting our earlier suggestion[15] that N_a-methylation takes place as a final step in the ajmalan biosynthesis.

Substrate	rel.Enzyme act. (%)	Substrate	rel.Enzyme act. (%)	Substrate	rel.Enzyme act. (%)
16-epi-Vellosimine	(100)	21-Deoxyajmalal A[16]	(n.d.)	Polyneuridine aldehyde	(n.d.)
Gardneral[17]	(85)	Methyl-gardneral[17]	(n.d.)	Voachalotine aldehyde	(n.d.)

Table: Substrate specificity of Vinorine synthase (n.d.=not detected, detection limit <1%)

Polyneuridine aldehyde 6 —PNA-esterase→ 16-epi-Vellosimine 7 ⇄ [Deacetyl-vinorine 8] —Acetyl-CoA, Vinorine synthase→ Vinorine 9

16-epi-Vellosimine 7 → 10-Deoxy-sarpagine

Scheme 2

As summarized in Scheme 2, 16-epi-vellosimine 7 is a branch point in the biosynthesis of sarpagine and ajmaline alkaloids leading on one hand to vinorine 9 and on the other to 10-deoxy-sarpagine[6].

Acknowledgements: We thank Prof.S.Sakai, Chiba, Dr.Zeugner and Dr.Kehrbach, Hannover, for most generous gifts of reference alkaloids, Dr.P.J.Keller, Munich, Dr.Makus and Dr.Kellner, Maisach for ^{1}H-NMR spectra and the Deutsche Forschungsgemeinschaft for a Finnigan MAT 44 S instrument and for financial support (SFB 145).

References

1 A.Pfitzner and J.Stöckigt, Planta med., in press and lit. cited therein.

2 J.Stöckigt, Proceedings of the Fourth International Meeting on Tryptophan Metabolism: Biochemistry, Pathology and Regulation, Martinsried 1983, in press.

3 R.B.Woodward, Angew. Chem. 68, 13 (1956).

4 M.E.Bartlett, B.F.Lambert, H.M.Werblood and W.I.Taylor, J.Amer.Chem.Soc. 85, 475 (1962).

5 A.Pfitzner and J.Stöckigt, J.Chem.Soc.Chem.Commun. 1983, 459.

6 A.Pfitzner and J.Stöckigt, Tetrahedron Letters 24, 1695 (1983).

7 H.Meisel, W.Döpke and E.Gründemann, Tetrahedron Letters 17, 1291 (1971). Unfortunately, wave length and concentration for the determination of [α] were not reported and the original alkaloid could not be obtained for a direct comparison with enzymatically formed 9.

8 J.Stöckigt, A.Pfitzner and J.Firl, Plant Cell Reports 1, 36 (1981), $[\alpha]_D^{20} = -29^o \pm 3^o$ (c=0.07, $CHCl_3$).

9 ^{1}H-NMR of 9 (200 MHz) in $CDCl_3$: 7.63, 7.45 (arom.2H, dd, J_1=8 Hz, J_2=1 Hz), 7.40, 7.23 (arom. 2H, td, J_1=8 Hz, J_2=1 Hz), 5.41 (C=CH-CH_3, q, J=7 Hz), 4.98 (H-17, d, J=1 Hz), 4.46 (H-3, brd, J=7 Hz), 3.72 (2 H-21, brs), 3.62 (1 H, m), 3.37 (1 H, brt, J=4 Hz), 2.78 (1 H, dd, J_1=12 Hz, J_2=5 Hz), 2.54 (1 H, t, J=6.5 Hz), 2.16 ($OCOCH_3$, s), 2.08 (1 H, brs), 2.04 (2 H, m), 1.67 (CH_3-CH=, dt, J_1=7 Hz, J_2=2 Hz) ppm.

10 The concentration was spectrophotometrically determined by log ε as published in[7].

11 A.K.Kiang, S.K.Loh, M.Demanczyk, C.W.Gemden, G.J.Papariello and W.I.Taylor, Tetrahedron 22, 3293 (1966).

12 The 17-β configuration is retained during the $Pb(OAc)_4$ oxidation of 5 yielding 9, as shown by oxidation of [^{3}H]-5, obtained from 4 by acetylation with [^{3}H]-Ac_2O.

13 A.Pfitzner and J.Stöckigt, unpublished. The synthesized 17-OH indolenine compounds were trapped by reduction with $NaBH_3CN$ and $NaBH_4$ respectively.

14 The proposed structure of compound A (12-methoxy analog of 7), which was isolated from R. vomitoria plants by N.N.Sabri and W.E.Court, Phytochemistry 17, 2023 (1978) should not be taken in consideration because of thus far only incompletely available spectroscopic data.

15 J.Stöckigt, A.Pfitzner and P.J.Keller, Tetrahedron Letters 24, 2485 (1983).

16 A mixture of 21-deoxyajmalal A and B (ratio 65:35) was used as a substrate.

17 ^{1}H-NMR analysis of both relatively instable alkaloids provided by Prof.Sakai clearly indicated the very high stereochemical purity; the 16-epimers were not observed. The data were found to be still exactly the same as determined in 1980: S.Sakai, Y.Yamamoto and S.Hasegawa, Chem.Pharm.Bull. 28, 3454 (1980).

(Received in Germany 25 July 1983)