Tetrahedron Letters,Vol.24,No.24,pp 2485-2486,1983 0040-4039/83 $3.00 + .00
Printed in Great Britain

ENZYMATIC FORMATION OF AJMALINE

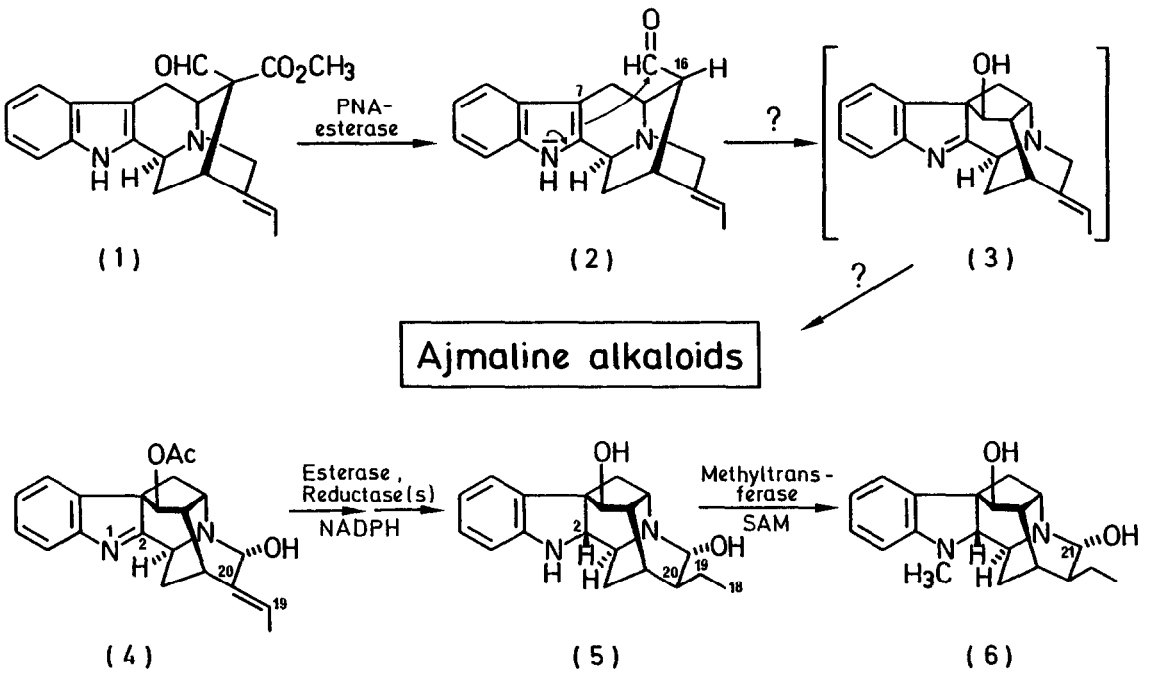
Joachim Stöckigt *, Artur Pfitzner and Paul J.Keller ‡

Institut für Pharmazeutische Biologie, Universität München and ‡ Lehrstuhl für Organische Chemie und Biochemie, Technische Universität München, D-8000 München 2 (FRG)

Abstract: An enzyme system from cell suspension cultures of Rauwolfia serpentina Benth. catalyzes the NADPH/S-adenosyl-methionine-dependent conversion of the indolenine alkaloid vomilenine into ajmaline.

Recently we characterized two specific enzymes, which are involved in the biosynthesis of sarpagine type alkaloids in cell suspension cultures and differentiated plants of Rauwolfia serpentina Benth.[1,2]. One of these new plant enzymes, the polyneuridine aldehyde esterase (PNA-esterase), converts polyneuridine aldehyde (1) into 16-epi-vellosimine (2)[1]. Compound (2) exhibits the aldehyde group in the endo-position, 16 (S) configuration. In a formal sense, it is this configuration at C-16 which would be required for the biogenetic formation of the ajmaline skeleton by addition of the carbonyl carbon to C-7 (scheme). If so, the immediate product synthesized can be expected to be an indolenine type compound (3), which presumably then serves as a biogenetic precursor for the elaboration of ajmaline alkaloids. In this paper we present evidence for the validity of this assumption. The conversion of the indolenine alkaloid vomilenine (4) into ajmaline (6) is shown to be catalyzed by an enzyme system, isolated from cultured Rauwolfia cells.

A crude enzyme mixture was isolated as previously reported[2]. 1,25 ml (10,7 mg protein) of the obtained enzymes were incubated for 2 hours (30°C) with 0,2 µmol vomilenine (4) and an excess of various cofactors in a tot. vol. of 3 ml. The incubation mixtures were then analyzed by an ajmaline specific radioimmunoassay (RIA) showing that (6) indeed was formed from (4) but only in the presence of active enzymes and both cofactors, NADPH and S-adenosyl-methionine (SAM). This incubation was scaled-up 10 fold but the SAM was omitted in an effort to intercept the sequence (4) → (6). After extraction of this mixture with ethyl acetate, t.l.c. in acetone/petroleum ether/diethylamine (1:2:1) showed a product (R_f=0,5) with orange CAS (ceric ammonium sulfate) reaction. UV- and MS-analysis of the cell-free formed compound indicated, that during the incubation (4) was deacetylated and reduced (1,2- and 19,20- double bond). UV and MS data clearly identified the enzymatic product to be norajmaline (5), which also is a naturally occurring alkaloid[3,4]. When NADPH was replaced by NADPD (regenerated from $NADP^+$, MeOH-d_4 and alcohol dehydrogenase from Leuconostoc mesenteroides EC 1.1.1.2) two deuterium atoms were incorporated into (5) indicating that reduction of both



double bonds takes place in a stereospecific manner. These described experiments demonstrate that the following enzymes are involved in the conversion of (4) → (5), an esterase and one or two NADPH-specific reductases.

The results also indicate, that (5) must be the immediate biosynthetic precursor for ajmaline (6). To check this point 3 ml enzyme (24 mg proteine) were incubated with 0,3 µmol (5) and 5 µmol SAM under the above conditions. When this mixture was chromatographed (t.l.c.) a red compound was observed after spraying with CAS (R_f identical to ajmaline). UV, MS fragmentation as well as chromatographic behaviours of this product fully coincided with that of authentic ajmaline, which verifies that a methyl transferase is the final enzyme in the biosynthesis of (6) in R. serpentina. The results do, however, not exclude the further possibility that 21-deoxyajmaline might be also an immediate precursor of (6). The in vivo incorporation of radioactive labelled 21-deoxyajmaline into ajmaline (6) however found with R. verticillata plants[5] would support the latter assumption.

We thank Miss S.Mümmler for RIA analysis and the Deutsche Forschungsgemeinschaft for a Finnigan MAT 44 S instrument and financial support (SFB 145). P.J.K. thanks the Alexander von Humboldt foundation for a fellowship.

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(Received in Germany 24 March 1983)