Cathenamine, a Central Intermediate in the Cell Free Biosynthesis of Ajmalicine and Related Indole Alkaloids

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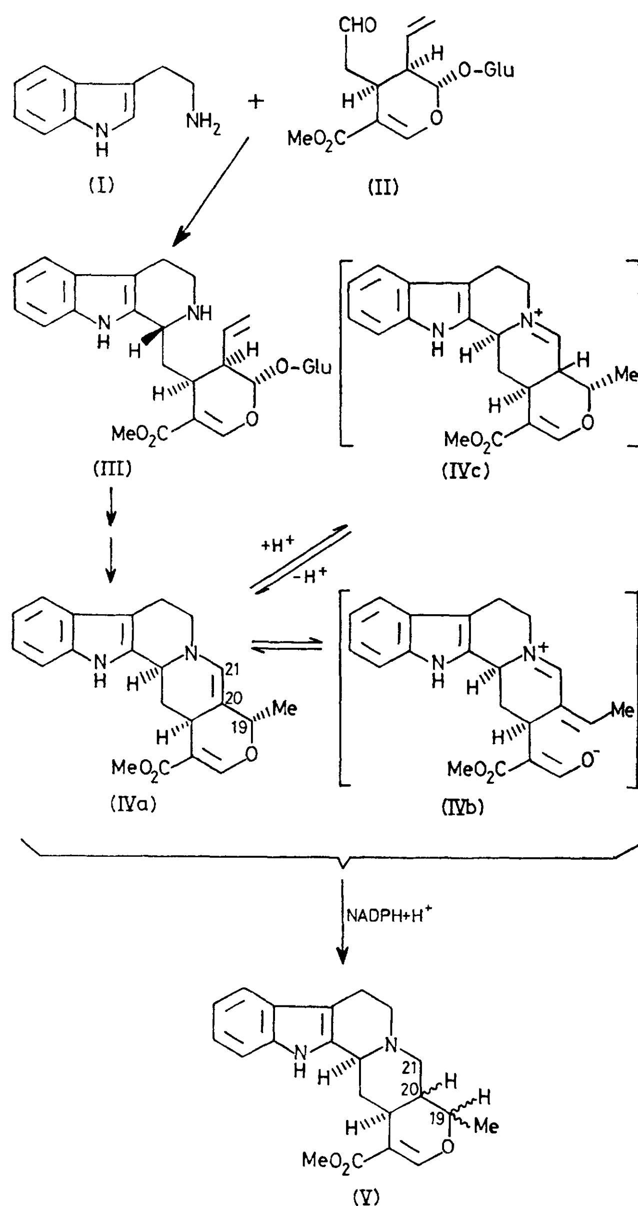
Summary A compound, which accumulates when tryptamine (I) and secologanin (II) are incubated with an enzyme preparation from *Catharanthus roseus* cell suspension cultures, was identified as 20,21-didehydroajmalicine (cathenamine) (IVa), and was shown to be a central intermediate in the enzymatic production of ajmalicine (Va), 19-*epi*-ajmalicine (Vb) and tetrahydroalstonine (Vc).

In *vivo* feeding experiments with labelled potential precursors have led to the formulation of generally accepted schemes for the biosynthesis of indole alkaloids.[1,2] The limitations of *in vivo* experiments were recently overcome when it was shown for the first time that complex alkaloids, such as geissoschizine and ajmalicine (Va), were synthesized by crude cell-free preparations of *Catharanthus roseus*

callus tissue from tryptamine (I) and secologanin (II).[3] These experiments were extended and it was shown that a cell-free preparation from fermenter-grown *C. roseus* cells



gave (Va), 19-*epi*-ajmalicine (Vb), and tetrahydroalstonine (Vc) from (I) and (II) in the presence of either NADPH or NADH.[4] It was observed, however, that in the absence of reduced pyridine nucleotides, a precursor of the ajmalicine isomers (Va—c) accumulated.[4] We consider that this precursor is a central intermediate in the biosynthesis of these alkaloids and report its isolation and identification.

For the isolation of the precursor, a solution of [2-^{14}C]-(I) (1·54 mM, 20 μCi) and (II) (7·7 mM) in phosphate buffer (pH 7·5, 77 mM) was incubated in the presence of 2 mg/ml protein which was prepared as previously reported.[4] Total volume of the incubation mixtures was 200 ml. After 90 min of incubation at 29 °C, the solution was extracted with ethyl acetate which removed 80% of the radioactivity from the aqueous phase. After evaporation of the solvent, the residue was chromatographed on silica gel 60 PF_{254} plates using chloroform–ethanol (90 : 5). 52% of the radioactivity was located in a band (R_f 0·78) which was previously[4] designated compound D. This band was eluted with chloroform–methanol, the solvent was evaporated off and the residue, λ_{max} (EtOH) 228, 274, 280, and 290 nm (shoulder); *m/e* (80 eV) 350 (M^+, 65%), 335(9), 332(15), 331(18), 330(12), 329(21), 322(20), 321(24), 289(12), 249(100), 170(41), 169(47), and 156(46); δ ($CDCl_3$, Me_4Si, 240 MHz[5]) 1·42 (d, *J* 6Hz, *Me*-CH-O-), 3·73 (s, -CO_2*Me*), 4·63 (q, *J* 6 Hz, Me-*CH*-O-), 6·18 (s, >N-*CH*=C<), 7·55 (s, -O-*CH*=), and 8·02 (s, >N-*H*), was stored in the dark under N_2. The spectroscopic data are in agreement with the structure 20,21-didehydroajmalicine (IVa); the compound was given the trivial name cathenamine.

When this compound was reduced with $NaBH_4$ in methanol, it gave, in nearly quantitative yield, tetrahydroalstonine (Vc) which was characterized by chromatography and mass (80 eV) and ^{1}H n.m.r. spectrometry. This suggests that the configuration of the C-19-methyl group in (IVa) is α.

In order to establish that (IVa) is an intermediate in the enzymic reaction, it was prepared with high specific activity (48·5 μCi^{14}C/μmol) using the *C. roseus* enzyme preparation. The purified compound was incubated in phosphate buffer (pH 7·5) containing 4 mM NADPH for 60 min in the presence of 1·8 mg/ml protein. The mixture was then analysed for its alkaloid content and (Va—c) were identified in a total yield of 68%. In control experiments with heat-denatured enzyme or in the absence of NADPH these compounds were not formed.

The spectroscopic and chemical data as well as the enzymatic conversion shown by (IVa) isolated from cell-free extracts of *C. roseus* are exactly identical with those shown by a compound isolated previously from a plant source.[6]

During the course of these experiments it was observed that a compound more polar than (IVa) was produced in the cell-free extracts. Experiments are in progress to establish whether this compound is a precursor of (IVa).

On the basis of these results the following scheme can be formulated for the biosynthesis of *Corynanthe* type indole alkaloids. The condensation product of (I) and (II) is vincoside[2] which is converted into (IVa) in several steps. Compound (IVa) accumulates in the absence of reduced pyridine nucleotides, but in their presence it is reduced to (Va—c).

Since ajmalicine (Va) is not converted into isomers (Vb) and (Vc) by the enzyme mixture, a stereochemical control should occur in the cathenamine stage (IVa). Therefore an enzyme-catalysed interconversion can be rationalised as proceeding *via* an equilibrium of (IVa) to give (IVc) and/or retro-Michael ring opening to yield (IVb).[7]

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[1] G. A. Cordell, *Lloydia*, 1974, **37**, 219.
[2] R. J. Parry, 'The Biosynthesis of Catharanthus Alkaloids' in 'The Catharanthus Alkaloids,' eds. W. I. Taylor and N. R. Farnsworth, Marcel Dekker, New York, 1975, ch. V, pp. 141—191.
[3] A. I. Scott and S. L. Lee, *J. Amer. Chem. Soc.*, 1975, **97**, 6906.
[4] J. Stöckigt, J. Treimer, and M. H. Zenk, *FEBS Letters*, 1976, **70**, 267.
[5] P. Gonord, C. Duret, C. Vibet, J. Saiset, and S. K. Kan, *Rev. Sci. Instr.*, 1973, **44**, 1725.
[6] H. P. Husson, C. Kan-Fan, J. P. Vidal, and Th. Sevenet, unpublished results.
[7] R. T. Brown, C. L. Chapple, D. M. Duckworth, and R. Platt, *J.C.S. Perkin I*, 1976, 160.