Tetrahedron Letters Vol. 21, pp 1925 - 1926 0040-4039/80/0502-1925$02.00/0

MECHANISM OF THE BIOSYNTHETIC CONVERSION OF GEISSOSCHIZINE TO 19-EPI-AJMALICINE IN CATHARANTHUS ROSEUS

J. Stöckigt*, G. Höfle+ and A. Pfitzner

Lehrstuhl für Pflanzenphysiologie, Ruhr-Universität Bochum, D-4630 Bochum, and +Gesellschaft für Biotechnologische Forschung, D-3301 Stöckheim, Federal Republic of Germany

Abstract: Geissoschizine (8) is enzymatically converted to 19-epi-ajmalicine (7) first by oxidation to the 4,21-dehydro-intermediate (4) of the heteroyohimbine pathway followed by cyclisation and stereospecific reduction.

The biogenetic pathway leading from tryptamine (1) and secologanin (2) to the heteroyohimbine alkaloids, e.g. 19-epi-ajmalicine (7), has been well investigated[1]. However, the role of geissoschizine (8) in this sequence and the mechanism of its enzymatic conversion to (7) is still controversial. Some published results concerning the formation of (7) are completely contradictory [2,3]. Different mechanisms for the involvement of (8) were discussed in these reports[2,3] and, based on negative results, a stepwise mechanism was preferred, in contrast to other findings.[4]

To clarify this unsatisfactory situation, we have carried out four experiments using cell-free extracts of C. roseus cell suspension cultures. The enzyme system was capable of synthesizing besides ajmalicine and tetrahydroalstonine, mainly 19-epi-ajmalicine (7) starting from (1) and (2).

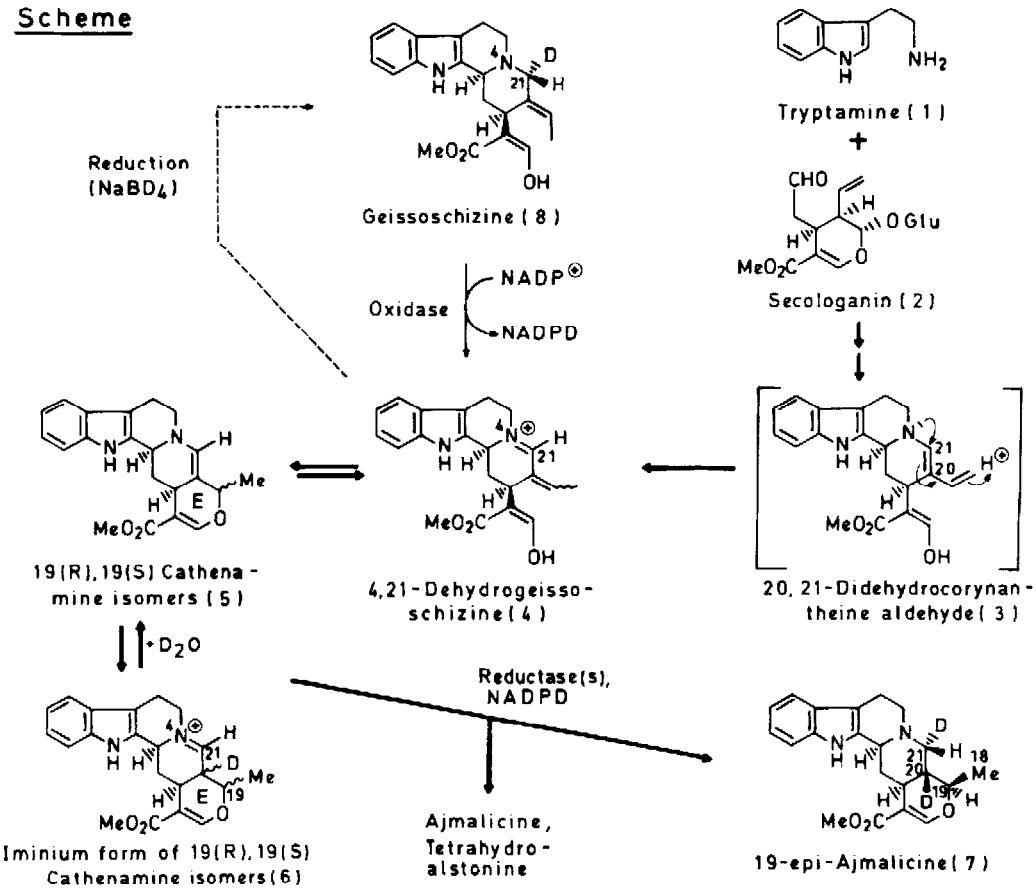
1. Exp.: (8) was incubated with the enzymes in the presence of NADP and NADPH. Of the ajmalicine isomers formed, the main product was clearly identified as (7) by TLC, UV and MS. Conclusion: (8) also acts as a biogenetic precursor for (7). This is in contrast with published results [3].

2. Exp.: This experiment was carried out as described in Exp. 1, but in D_2O instead of H_2O. MS analysis of the formed (7) showed the incorporation of one deuterium at position C-20. Conclusion: (8) is channelled into the pathway at a stage beyond the proposed 20,21-didehydrocorynantheine aldehyde (3) [1]. If channelling would take place at/or before (3), two deuterium atoms would be incorporated into (7) at C-18 and C-20 respectively [1].

3. Exp.: The experimental conditions were as in Exp. 1, but with incubation of (8) in the presence of NADPD instead of NADPH. MS analysis of the synthesized (7) again showed the incorporation of one deuterium atom. D was localized by MS at position C-21 [m/e 184 → m/e 185] . The NMR data of (7) clearly showed that $D^{\ominus}$ is transferred into the α-position of C-21. Conclusion: (8) is channelled into the pathway at the stage of its 4,21-dehydroderivative (4) as

proposed earlier[4]. After the formation of cathenamines (5), (6)[5] by E-ring closure, enzymatic reduction takes place in a stereospecific manner (scheme). 4. Exp.: The conditions were again as in Exp. 1, but (8) had been labelled with D at C-21- α . In the enzymatic conversion to (7), complete loss of D was observed (MS). Conclusion: This result again confirms the step (8)$\longrightarrow$(4) and disproves all other proposed mechanisms for the formation of (7).
The final mechanism of the enzymatic conversion of geissoschizine (8) is summarized in the scheme. A part of this investigation was possible only after Kan-Fan and Husson[6] had made available (4) for biosynthetic research.

Scheme



Acknowledgements: Generous gifts of (4) and (8) from Dr. H.-P. Husson, Gif-sur-Yvette, and Professor E. Winterfeldt, Hannover; helpful discussions by Prof. M.H. Zenk, Bochum, and financial support of the BMFT to Prof. Zenk are gratefully acknowledged.

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(Received in Germany 29 February 1980)