A Model Mechanism for the Enzymatic Synthesis of Lupin Alkaloids

Michael Wink, Thomas Hartmann,

Institut für Pharmazeutische Biologie der Technischen Universität Braunschweig, Pockelsstraße 4, D-3300 Braunschweig

and

Hans-Martin Schiebel

Institut für Organische Chemie der Technischen Universität, D-3300 Braunschweig

Z. Naturforsch. **34 c,** 704 – 708 (1979); received June 8, 1979

Lupinus polyphyllus, Cell Suspension Cultures, Quinolizidine Alkaloids, Enzymatic Synthesis, Biosynthetic Model Mechanism

A crude enzyme preparation obtained from cell suspension cultures of *Lupinus polyphyllus* catalyzes the pyruvate dependent conversion of cadaverine into the tetracyclic lupin alkaloids. As the first reaction product 17-oxosparteine could be identified by gas-liquid chromatography and mass spectroscopy. In some experiments sparteine was found additionally. A participation of diamine oxidase could be ruled out. The cadaverine-pyruvate transaminating enzyme system (17-oxosparteine synthase) catalyzes the formation of 17-oxosparteine from three cadaverine units without releasing free intermediates. These results are inconsistent with the hypothetical mechanism thus far formulated for the lupin alkaloid biosynthesis. A new enzymatic model mechanism is proposed regarding both the results of the enzymatic experiments and those of the *in vivo* tracer studies.

Introduction

The C_{15} lupin alkaloids, such as sparteine and lupanine, consist of three C_5 units derived from lysine via its decarboxylation product cadaverine [1, 2].

In a preceding publication the conversion of cadaverine into the tetracyclic alkaloids had been demonstrated by a crude cadaverine-pyruvate transaminating enzyme system obtained from cell suspension cultures of *Lupinus polyphyllus* [3].

In this communication we report on the identification of the enzymatic reaction products and on a plausible model mechanism which accounts for the results of the enzymatic experiments and the findings of the *in vivo* tracer studies.

Materials and Methods

Enzyme preparations were obtained from acetone powders of *L. polyphyllus* cell suspension cultures as described previously [3]. One *g* acetone powder was suspended in 40 ml 0.1 M sodium-phosphate buffer, pH 7.8, containing 1 mM dithioerythritol, 10 mM diethyldithiocarbamate (DIECA), 10 mM pyruvate, 5 mM cadaverine. The reaction flasks were gassed with nitrogen for 2 min, sealed and shaken for 2 h at 30 °C.

Alkaloids were extracted from the alcalized reaction mixture with methylene chloride, dried over Na_2SO_4 and concentrated in vacuo. Alkaloid mixtures were separated by gas-liquid chromatography, using a Perkin Elmer F 22 gas chromatograph equipped with flame ionization and nitrogen detectors and a 25 m SE 30 glass capillary column (0.2 mm i. d.). For gas-liquid chromatography/mass spectroscopy a Perkin Elmer gas chromatograph, equipped with a 2 m glass column (2 mm i. d.), filled with 4% OV-17 on Chromosorb WAW 100/120 mesh, was combined with an AEI MS 30 and the Data System DS 50. Direct analysis of the alkaloid mixtures was performed alternatively with an AEI MS 9. A separation of the compounds was achieved by continuous temperature elevation from 30 ° to 180 °C. This method permits the detection of alkaloids with known fragmentation patterns in the p- to nmol range.

Results and Discussion

Gas-liquid chromatographic separation of the enzymatic reaction mixture is shown in Fig. 1. Lupin

Reprint requests to Prof. Dr. T. Hartmann.

0341-0382/79/0900-0704 $ 01.00/0

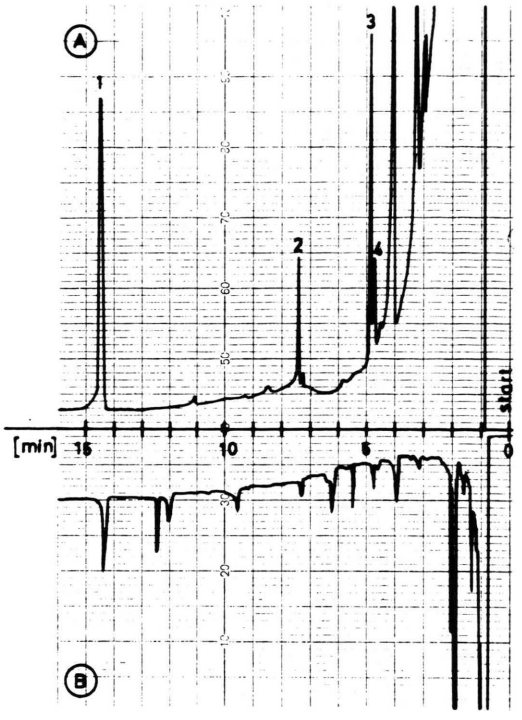


Fig. 1. Gas-liquid chromatographic separation of the reaction products of 17-oxosparteine synthase. SE-30 capillary column (WCOT); temperature: 190 ° – 270 °C/min. A: nitrogen detection; B: flame ionization detection. Peak 1: degradation product of DIECA; 2: 17-oxosparteine; 3: sparteine; 4: unidentified.

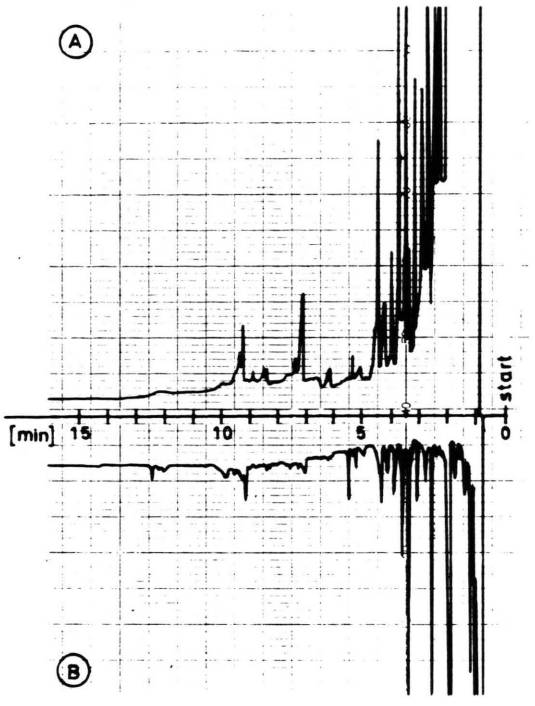


Fig. 2. Gas-liquid chromatographic separation of the reaction products of diamine oxidase. Acetone powder preparations of *Pisum sativum* were incubated with 10 mM cadaverine under aerobic conditions for 2 h. Chromatographic conditions as in Fig. 1. Peaks in the range between 4.5 and 10 min are not identical with the products of 17-oxosparteine synthase (compare Fig. 1).

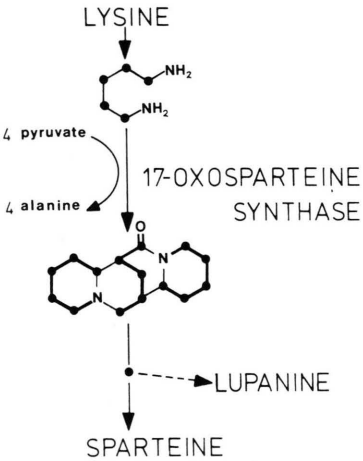
alkaloids such as sparteine, angustifoline, lupanine, 17-oxolupanine, and 13-OH-lupanine display retention times of 5 to 15 min under these conditions. In this range 4 nitrogen containing compounds could be detected. Gas-liquid chromatography/mass spectroscopy and direct mixture analysis (MS 9) indicated that peak 1 contained sulfur; it was considered to be a derivative of DIECA. Peak 2 showed the following characteristic ions (MS 30) m/z 248 (M^+) (5.5% relative intensity), 220 (8%), 191 (16%), 150 (8%), 136 (33%), 110 (63%), 97 (100%), 84 (25%). This fragmentation pattern is identical with that of 17-oxosparteine [4]. Peak 3: m/z 234 (M^+) (14%), 193 (20%), 137 (100%), 110 (20%), 98 (94%), 84 (20%). This compound could be identified as sparteine. No characteristic fragment ions of lupin alkaloids could be found between start and 4.5 min. Peak 4 could not be identified so far.

Since 17-oxosparteine and sparteine are not detectable in the cell suspension cultures of *L. polyphyllus* (Wink and Hartmann, in preparation) used for these enzyme preparations, any interference with background alkaloids can be ruled out. In enzymatic time course experiments 17-oxosparteine was always found to be the first product. Its concentration increased with progressing incubation time. Only in 2 out of 14 preparations incubated for 2 h, sparteine could be detected additionally (Fig. 1). Therefore, we consider 17-oxosparteine as the primary enzymatic product and precursor of sparteine.

Several lines of evidence have been presented that the reaction products of cadaverine-pyruvate transamination are converted into 17-oxosparteine without release of free intermediates [3]. If 5-aminopentanal, the deamination product of cadaverine, would be released as a free intermediate, Δ1-piperideine and other spontaneously formed cyclization products such as tetrahydroanabasine or tripiperidine should be expected. Gas-liquid chromatographic analysis of a reaction mixture of diamine oxidase and cadaveri-

ne indicates the occurence of various nitrogen containing compounds (Fig. 2). None of these products was found to be identical with the reaction products of the pyruvate-cadaverine transamination (Fig. 1).

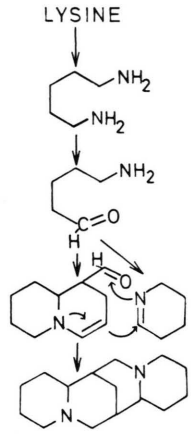
These results indicate that a cadaverine-pyruvate transaminating enzyme system, now called *17-oxosparteine synthase* catalyzes the formation of 17-oxosparteine from three cadaverine units without releasing free intermediates. The overall reaction can be formulated as follows (Scheme 1):



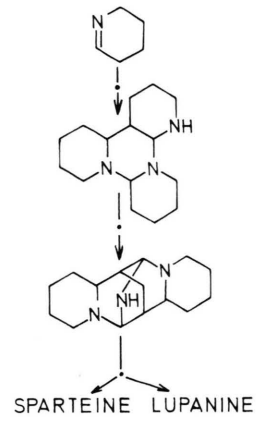
Scheme 1. Enzymatic synthesis of lupin alkaloids by enzyme preparations of *L. polyphyllus* cell suspension cultures.

The results of the enzymatic studies are inconsistent with the hypothetical mechanism formulated for quinolizidine alkaloid biosynthesis on the basis of *in vivo* tracer studies. Both mechanisms, summarized in Scheme 2 and 3, postulate piperideine as an intermediate.

Therefore we suggest a new model mechanism for the biosynthesis of the lupin alkaloids covering both the results of the enzymatic studies and those of the *in vivo* tracer experiments. The model has to meet the following requirements: 1. a cadaverine-pyruvate transamination; 2. bound intermediates; and 3. release of 17-oxosparteine as the key product. 17-oxosparteine synthase is assumed to possess one coenzyme pyridoxal phosphate which accounts for the 4 transamination steps involved in the overall reaction. In addition a separate amino group is proposed as a carrier to which the intermediates remain bound until the final product is released *. The enzymatic synthesis is initiated by binding of the first cadaverine unit to pyridoxal phosphate (Scheme 4/2) and transfer of the resulting semi-aldehyde to the carrier amino group (4/3). The following steps include transamination of pyridoxamine phosphate, transamination of the second amino group of cadaverine I, binding of the second cadaverine unit and condensation of the cadaverine units by Schiff's base formation (4/3 – 7). The next steps (4/8 – 10) lead by cyclization to the quinolizidine ring system, involving tautomerization, Mannich reaction with formation of a reactive carbonium ion in C_7. After condensation of the third cadaverine unit the cyclization to the tetracyclic system occurs, involving a similar mechanism as above. After double bond shift, 17-oxosparteine is released by hydrolysis. The bicyclic lupinine might be obtained by terminating the reaction after step 4/10.



Scheme 2



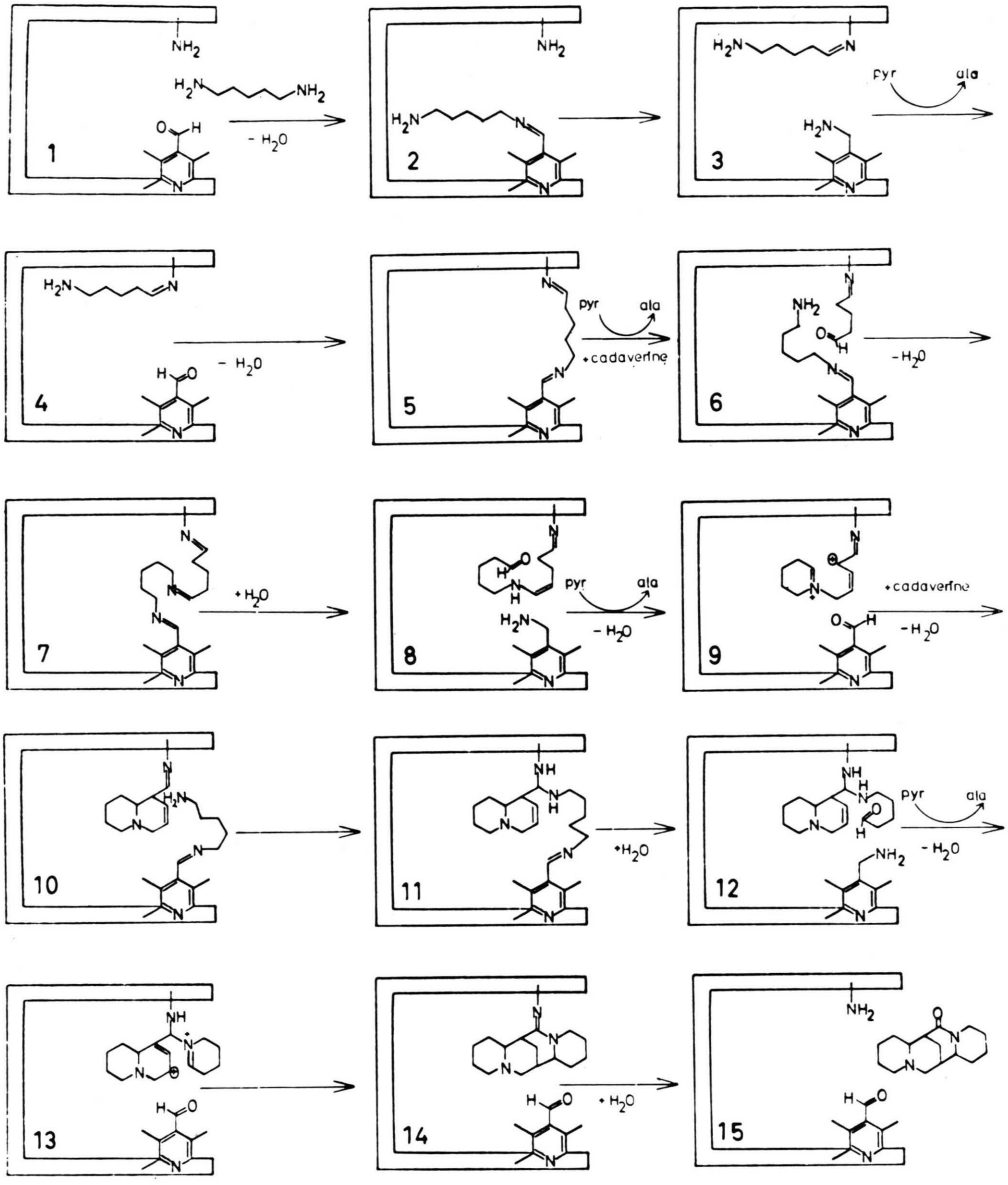
Scheme 3

Scheme 2. Proposed concept for the biosynthesis of lupin alkaloids on the basis of *in vivo* incorporation studies with lysine and cadaverine [1, 2].

Scheme 3. Golebiewski/Spenser hypothesis of lupin alkaloid biosynthesis [5]. Sparteine and lupanine as modified trimers of Δ1-piperideine.

The bound nature of the reaction intermediates explains the failure of the *in vivo* tracer studies to isolate intermediates between cadaverine and tetracyclic alkaloids [1, 2]. On the other hand Δ1-piperideine was found to be incorporated into sparteine and lupanine, thus leading to the Golebiewski Spenser hypothesis (Scheme 3). Since Δ1-piperideine can

* Alternatively a multienzyme complex involving more than 1 molecule pyridoxal phosphate might be assumed for this mechanism.



Scheme 4. Model mechanism for the synthesis of 17-oxosparteine catalyzed by 17-oxosparteine synthase.

be present in its open form, it might be argued that 5-aminopentanal binds to the enzyme leading to the incorporation observed.

Sparteine was found to be converted into lupanine and other lupin alkaloids in *in vivo* and in *in vitro* experiments [1, 2, 6]. Therefore sparteine was suggested to be the direct precursor of the lupin alkaloids. However, short time experiments with labelled CO_2 indicated, that sparteine and lupanine can be synthesized independently [7]. A precursor common for both sparteine and lupanine was therefore postulated [5, 7]. This compound might be dehydrosparteine [7], which could derive from 17-oxosparteine by reduction and water elimination. Recent results confirm this hypothesis (Wink and Hartmann, unpublished) as the enzymatic synthesis of lupanine and sparteine can be achieved from a precursor common to both alkaloids.

Acknowledgements

Collaboration of Dr. L. Witte, D. Döring, and H. Steinert (Gesellschaft für Biotechnologische Forschung, Braunschweig-Stöckheim) in mass spectroscopy are gratefully acknowledged. We would like to thank Dr. W. Kreiser (Institut für Organische Chemie, Braunschweig) for helpful discussion. The work was supported by a grant of the Studienstiftung des Deutschen Volkes (to M. W.) and by a research grant of the Land Niedersachsen.

[1] H. R. Schütte, Biosynthese der Alkaloide (K. Mothes and H. R. Schütte, eds.) pp. 324 – 343, VEB Berlin 1969.
[2] E. K. Nowacki and G. R. Waller, Rev. Latinoamer. Quim. **8,** 49 – 56, (1977).
[3] M. Wink and T. Hartmann, FEBS Lett. **101,** 343 – 346 (1979).
[4] D. Schumann, N. Neuner-Jehle, and G. Spiteller, Mh. Chem. **99,** 390 – 408 (1968).
[5] W. M. Golebiewski and I. D. Spenser, J. Amer. Chem. Soc. **98,** 6726 – 6728 (1976).
[6] E. K. Nowacki and G. R. Waller, Phytochemistry **14,** 165 – 171 (1975).
[7] V. D. Cho, R. O. Martin, and J. N. Anderson, J. Amer. Chem. Soc. **93,** 2087 – 2089 (1971).