Steric Course of Hydrogen Transfer during Enzymatic Formation of 3α-Heteroyohimbine Alkaloids†

Joachim Stöckigt,* Thomas Hemscheidt, Gerd Höfle,‡ Peter Heinstein,§ and Victor Formacek‖

ABSTRACT: With a crude enzyme extract obtained from *Catharanthus roseus* cell-suspension cultures, deuterium has been used to follow the cell-free biosynthesis of 3α-heteroyohimbine alkaloids. In the presence of D_2O one D^+ is incorporated into the C-19 methyl group and the other D^+ into the C-20 position of the heteroyohimbines ajmalicine, 19-epiajmalicine, and tetrahydroalstonine, respectively. During the final reduction step, cathenamine reductase(s) stereospecifically transfer(s) D^- from NADPD into the C-21α position of the three ajmalicine isomers. Formation of these 3α-heteroyohimbines, starting from geissoschizine as a precursor, proceeds by channeling the precursor into the main pathway by a stereospecific dehydrogenation. Deuterium incorporation was established by spectroscopic methods.

The overall biosynthetic sequence leading to 3α-heteroyohimbine alkaloids has been extensively studied over the past 6 years with enzymes isolated from cell-suspension cultures of *Catharanthus roseus* (Zenk, 1980, and references cited therein; Stöckigt, 1980, and references cited therein). So far, most of the biogenetic intermediates of the major pathway (tryptamine + secologanin → 3α-heteroyohimbines) or of the side reaction leading to the major pathway (geissoschizine → 4,21-dehydrogeissoschizine → 3α-heteroyohimbines) have already been identified (Figure 1). Moreover, the enzymes involved are known and most have been characterized in detail (Treimer & Zenk, 1979; Hemscheidt & Zenk, 1980; Pfitzner & Stöckigt, 1982). However, several questions concerning some outstanding mechanistic problems still need to be answered; for instance, the extent of incorporation of H^+ during the intermediary formation of the key compound cathenamine and the stereochemistry of the NADPH-dependent reduction of this intermediate are entirely unknown.

A study of these aspects, therefore, appeared to be necessary. After the complete ^1H NMR[1] analyses had been performed for ajmalicine, its C-19 epimer, tetrahydroalstonine, and geissoschizine (Höfle et al., 1980; Lounasmaa & Kan, 1980), an analytical method was thus available to investigate the steric mode of deuterium incorporation during the cell-free biosynthesis of the three ajmalicine isomers. Here, we report that, during the enzymatic formation of cathenamine, one proton is incorporated into the C-19 methyl group. In the subsequent reduction step, a further proton ends up at C-20 while the hydride ion of NADPH is stereospecifically transferred into the C-21 position, furnishing the three heteroyohimbine alkaloids, ajmalicine, 19-epiajmalicine, and tetrahydroalstonine. For the sequence geissoschizine → 3α-heteroyohimbine alkaloids, the stereochemistry of the involved reduction step was found to be the same. This fact also supports the finding (Pfitzner & Stöckigt, 1982) that geissoschizine is biogenetically converted into 4,21-dehydrogeissoschizine by a dehydrogenation step that leads to the main pathway of heteroyohimbine synthesis.

Materials and Methods

Chemicals and Biochemicals. Dowex AG 1-X4 was obtained from Bio-Rad; poly(vinylpyrrolidone) was from Fa. Heidinger. Deuterated water (99.7% atom % D) was from Roth and EGA-Chemie; [$^2H_6$]ethanol (99%) was purchased from Merck; deuterated chloroform (99.96%) for NMR analyses was obtained from Merck and from Merck Sharp & Dohme, respectively. Pyridine nucleotides, alcohol dehydrogenase from *Leuconostoc mesenteroides* (EC 1.1.1.2), and isocitrate dehydrogenase from pig heart (EC 1.1.1.42) were from Boehringer. Strictosidine was a gift of U. Pfitzner from our laboratory.

Cell Cultures and Enzyme Preparation. Suspension cultures of *Catharanthus roseus* were grown for 8 days in a standard production medium (Zenk et al., 1977). Cells to be used as an enzyme source were harvested, immediately frozen with liquid nitrogen, and submitted to the following procedure: 160 g of frozen cells was crushed with an IKA Universalmühle M 20 in the presence of 110 g of PVP (prewashed with 50 mM phosphate buffer, pH 7.0). The mixture was stirred at 0 °C for 60 min in 500 mL of 50 mM phosphate buffer (pH 7.0, containing 10 μL of β-mercaptoethanol). After the resultant slurry was passed through cheesecloth and centrifuged at 27300*g* for 10 min, the protein in the supernatant was precipitated with 30–70% $(NH_4)_2SO_4$ (w/v). The residue was then redissolved in 17 mL of the above buffer (without β-mercaptoethanol). This solution was stirred for 15 min at 0 °C with 3 g of the anion-exchange resin Dowex AG 1-X4, 100–200 mesh, borate form. The centrifuge filtrate obtained was freed of endogenous alkaloids and cofactors by applying the solution to a Sephadex G-25 column (2.5 × 30 cm, flow rate 0.5 mL/min). The resulting enzyme solution was used in the experiments described below. For study of the incorporation of solvent protons (deuterium), the desalted protein solution was concentrated by further precipitation with $(NH_4)_2SO_4$ (30–70%) and the enzyme exhaustively dialyzed

† From the Department of Pharmazeutische Biologie, Universität München, 8000 München 2, FRG. *Received December 10, 1982.* This research was financially supported by the Deutsche Forschungsgemeinschaft (SFB 145). Part of this work was presented by J.S. in lectures held at the Phytochemical Society Symposium on Indole and Biogenetically Related Alkaloids, London, 1979, and at the 28th IUPAC Congress, Vancouver, 1981.

‡ Present address: Gesellschaft für Biotechnologische Forschung, 3300 Braunschweig, FRG.

§ Present address: Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907.

‖ Present address: Bruker Analytische Messtechnik GmbH, 7512 Rheinstetten-Fo., FRG.

[1] Abbreviations: PVP, poly(vinylpyrrolidone); NMR, nuclear magnetic resonance; TLC, thin-layer chromatography; MS, mass spectroscopy; Tris, tris(hydroxymethyl)aminomethane.

0006-2960/83/0422-3448$01.50/0

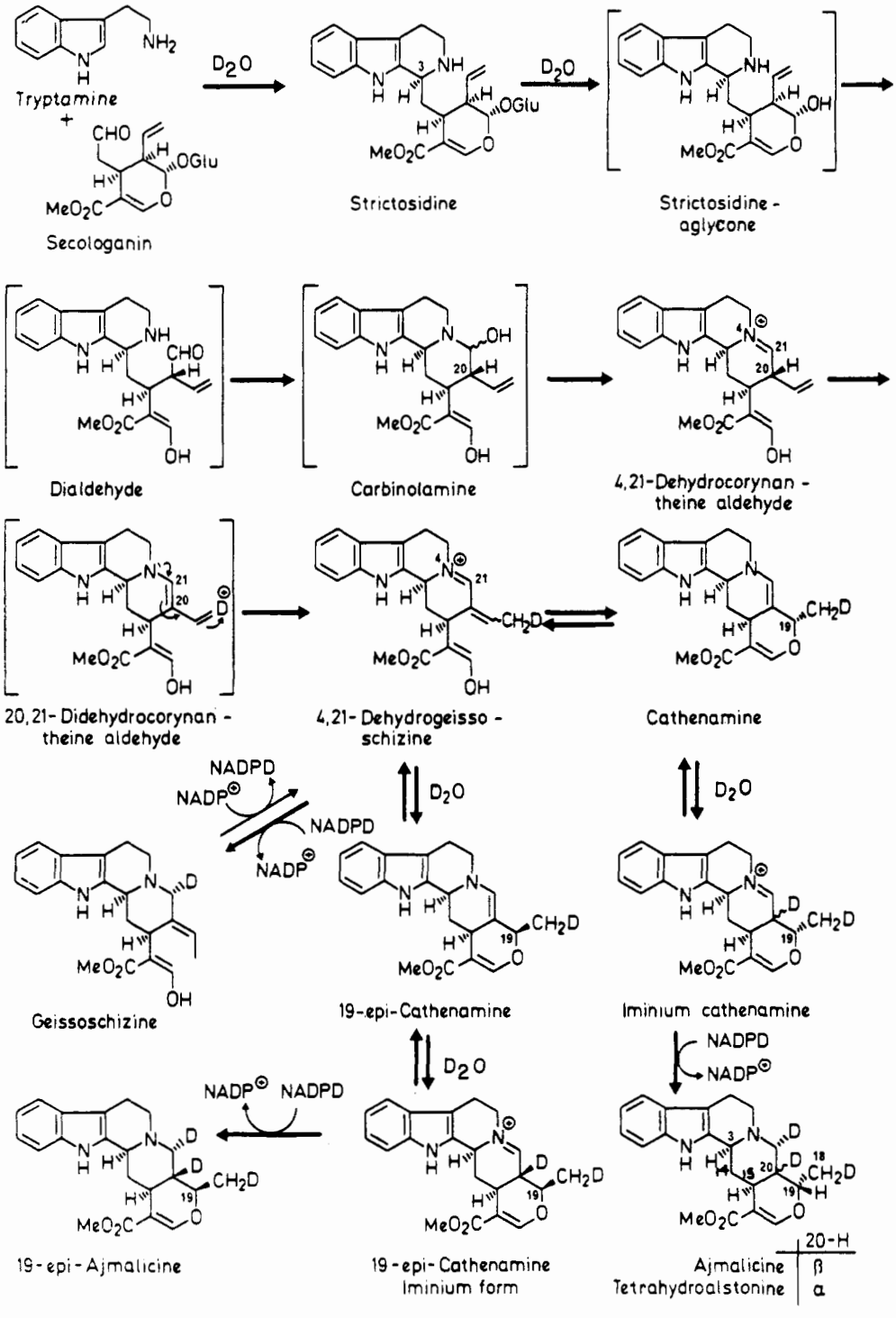

FIGURE 1: Hydrogen transfer during cell-free biosynthesis of 3α-heteroyohimbine alkaloids as demonstrated by deuterium incorporation.

against deuterated buffer. Protein concentrations were determined as previously described (Bradford, 1976).

Purification and Spectroscopic Analyses of Enzymatically Formed Products. Purification of cell free formed alkaloids was carried out by TLC chromatography on prewashed (methanol) silica gel plates (Polygram Sil G/UV_{254}; Machery-Nagel) in the following solvent systems: (A) acetone–petroleum ether (40–60 °C)–diethylamine, 2:7:1; (B) ethyl acetate–ether–*n*-hexane, 2:2:1; (C) chloroform–methanol–ammonia, 90:10:0.1. All solvents were of the highest purity commercially available. Yields of the synthesized alkaloids were determined by UV spectrophotometry.

The structural determination of the products was based on UV, MS, and ^{1}H NMR data. UV spectra were obtained on a Perkin-Elmer 551 S spectrophotometer in methanol (Uvasol) as solvent; mass spectra (MS) and deuterium incorporation were measured on a Finnigan MAT 44 S at 70 eV (EI mode). The ^{1}H NMR spectra were obtained with a Bruker WH 400 or WM 500 FT spectrometer in $CDCl_3$ or [$^2H_6$]acetone, using 30–1000 μg of sample in 0.4 mL of solvent. Depending on the isolated amounts of alkaloids, 500–19 000 transients were accumulated in a 32K memory.

Results

Cell-Free Formation of 3α-Heteroyohimbine Alkaloids. For the synthesis of [18-^{2}H]cathenamine (analyzed as its BH_4 reduction product tetrahydroalstonine), 8.4 mg (42.8 μmol) of tryptamine hydrochloride was incubated with 78.6 mg (202

μmol) of secologanin in the presence of 0.1 M phosphate buffer (pH 7.5) and 35.3 mg of protein in a total volume of 45 mL of D_2O for 120 min at 30 °C. For the reduction of the enzymatically formed cathenamine to tetrahydroalstonine (Husson et al., 1977), an excess of $NaBH_4$ and 2 mL of [^{2}H]methanol were added. After 40 min, the mixture was extracted with ether. The organic layer was washed with water and concentrated, and the residue was purified by TLC (systems A, B, and C), yielding tetrahydroalstonine (5 μmol).

Tetrahydro[18,20-$^2H_2$]alstonine: mass spectrum, *m/z* 354 (M^+, 96), 353 ($M^+ - H$, 100), 339 ($M^+ - CH_3$, 29), 225 (26); ^{1}H NMR 4.48 (t, J = 6.5 Hz, 19-H) ppm.

[18,20-$^2H_2$]-3α-Heteroyohimbine Alkaloids. For the enzymatic synthesis of 3α-heteroyohimbine alkaloids in deuterated medium, 22.5 mg (42.4 μmol) of strictosidine was allowed to react under NADPH-regenerating conditions in the presence of 124 mg (157 μmol) of $NADP^+Na_2$, 228 mg (880 μmol) of DL-isocitrate, 15 mg (750 nkat) of isocitrate dehydrogenase, 35.6 mg (373 μmol) of magnesium chloride, and 180 mg of *C. roseus* protein in 53 mL of deuterated phosphate buffer (50 mM, pH 7.5, 30 °C). After an incubation time of 120 min, the alkaloids produced were extracted with ethyl acetate at pH 7.5 and 8.5 (adjusted with $NaHCO_3$), respectively. The combined extracts were washed with water and concentrated, and the residue was twice chromatographed on TLC with systems A and B, yielding the deuterated alkaloids ajmalicine (1.6 μmol), 19-epiajmalicine (1.7 μmol), and tetrahydroalstonine (4.8 μmol).

[18,20-$^2H_2$]Ajmalicine: mass spectrum, *m/z* 354 (M^+, 87), 353 ($M^+ - H$, 87), 339 ($M^+ - CH_3$, 5); ^{1}H NMR ([$^2H_6$]-acetone) 2.27 (d, J = 11 Hz, 21α-H), 2.41 (dd, J = 12 Hz, J = 2 Hz, 15-H), 3.00 (d, J = 11 Hz, 21β-H), 4.49 (t, J = 7 Hz, 19-H) ppm. [18,20-$^2H_2$]-19-Epiajmalicine: mass spectrum, *m/z* 354 (M^+, 100), 353 ($M^+ - H$, 86), 339 ($M^+ - CH_3$, 3); ^{1}H NMR ($CDCl_3$) 2.23 (d, J = 11 Hz, 21α-H), 2.39 (br d, J = 11 Hz, 15-H), 3.89 (t, J = 6.5 Hz, 19-H) ppm. Tetrahydro[18,20-$^2H_2$]alstonine: mass spectrum, *m/z* 354 (M^+, 96), 353 ($M^+ - H$, 100), 339 ($M^+ - CH_3$, 29), 225 (26); ^{1}H NMR ($CDCl_3$) 2.73 (d, J = 12.5 Hz, 21α-H), 2.76 (dd, J = 4.5 Hz, J = 12 Hz, 15-H), 4.48 (t, J = 6.5 Hz, 19-H) ppm.

[21α-^{2}H]-3α-Heteroyohimbine Alkaloids. (a) Starting from strictosidine as precursor, in a total volume of 350 mL (1:1 mixture of 75 mM borate and 75 mM Tris buffer, pH 7.5), 40 mg (75.3 μmol) of strictosidine, 285 mg (361 μmol) of $NADP^+Na_2$, 5.9 mg (3000 nkat) of alcohol dehydrogenase (from *Leuconostoc mesenteroides*), 2.85 mL of [$^2H_6$]ethanol, and 171 mg of protein were incubated at 30 °C for 120 min. Under these NADPD-regenerating conditions, the three ajmalicine isomers were synthesized and isolated as described above. After repeated purification (3 times) on TLC (systems A and B), 3.0 μmol of ajmalicine, 3.3 μmol of 19-epiajmalicine, and 5.9 μmol of tetrahydroalstonine were finally obtained and analyzed for their deuterium content and for the location of the incorporated deuterium by MS and ^{1}H NMR.

[21α-^{2}H]Ajmalicine: mass spectrum, *m/z* 353 (M^+, 93), 352 ($M^+ - H$, 100), 338 ($M^+ - CH_3$, 6), 185 (35); ^{1}H NMR ($CDCl_3$) 2.17 (br d, J = 11 Hz, 20-H), 2.45 (t, J = 12 Hz, 15-H), 2.98 (br s, 21β-H) ppm. [21α-^{2}H]-19-Epiajmalicine: mass spectrum *m/z* 353 (M^+, 95), 352 ($M^+ - H$, 100), 338 ($M^+ - CH_3$, 5), 185 (33); ^{1}H NMR ($CDCl_3$) 2.40 (t, J = 11 Hz, 15-H) ppm (21α-H disappeared). Tetrahydro[21α-^{2}H]alstonine: mass spectrum, *m/z* 353 (M^+, 80), 352 ($M^+ - H$, 100), 338 ($M^+ - CH_3$, 25); ^{1}H NMR ([$^2H_6$]acetone) 3.18 (br s, 21β-H) ppm.

(b) Starting from geissoschizine as precursor, under NADPD-regenerating conditions, as described for (a), the sequence geissoschizine → heteroyohimbines was investigated. The reaction mixture contained, in 43 mL, 70 mM phosphate buffer (pH 7.5), 4.5 mg (12.8 μmol) of geissoschizine, 78 mg (99 μmol) of $NADP^+Na_2$, 2.6 mL of [$^2H_6$]ethanol, 0.3 mg (153 nkat) of alcohol dehydrogenase from *L. mesenteroides*, and 94 mg of protein from *C. roseus* cell cultures. The incubation was carried out at 30 °C for 120 min, and the alkaloids formed were isolated and purified as described above. MS and ^{1}H NMR data for the three isomers were identical with those obtained by the procedure as described for (a).

Discussion

A biogenetic pathway can be elucidated by the cell-free investigation of single-reaction steps involved in the whole sequence. With this methodology, the enzymatic synthesis of the corresponding biogenetic intermediates can usually be achieved, and structural determination will provide information on the mechanistic aspects of a specific biogenetic step. However, this approach requires the usually tedious purification of a number of enzymes. An alternative approach is to use stable isotopes and spectral analysis of the synthesized intermediates and final products to elucidate the mechanisms and the stereochemistry of the majority of steps in a biosynthetic sequence. This allows the use of crude enzyme mixtures or cell-free homogenates that are incubated with and without cofactors and with the appropriate substrates, i.e., intermediates in the pathway. We, therefore, used deuterium, in the form of D_2O, in the medium and deuterated cofactors for a more detailed understanding of the biogenetic route leading to monoterpenoid indole alkaloids of the 3α-heteroyohimbine type. By use of a crude enzyme extract from *C. roseus* cell-suspension cultures, the incorporation of D^+ and D^- was investigated by MS and ^{1}H NMR for the enzymatic conversion of strictosidine and geissoschizine into ajmalicine, 19-epiajmalicine and tetrahydroalstonine, the end products of the heteroyohimbine pathway.

During the course of the reactions in the main pathway, the vinyl function of strictosidine is converted into a methyl group. Pathway intermediates exhibiting this methyl group, e.g., 4,21-dehydrogeissoschizine and the cathenamine isomers, are enzymatically formed in the absence of any cofactors. This indicates a nonreductive sequence, in which a proton of the ambient water might be added to the vinyl function. To clarify this assumption, the cell-free synthesis of cathenamine was carried out in D_2O instead of H_2O, starting with tryptamine and secologanin and using the crude enzyme preparation. To stabilize the rather reactive intermediate, cathenamine, its enamine double bond was immediately reduced by BH_4^- after the incubation and the resulting stable tetrahydroalstonine analyzed by MS and ^{1}H NMR. The mass spectrum of the tetrahydroalstonine so produced showed the incorporation of two deuterium atoms by the increase of the mass ions *m/z* 352, 351, 337, and 223 to *m/z* 354, 353, 339, and 225, respectively. For the C-19 proton, a clear triplet was observed in the NMR spectrum at 4.48 ppm, demonstrating the quantitative incorporation of one D^+ into the C-19 methyl group and, during the chemical reduction step, into the C-20 position. Therefore, during the biogenetic reactions of the main pathway, D^+ is incorporated at the stage of 20,21-dehydrocorynantheine aldehyde with the concomitant formation of 4,21-dehydrogeissoschizine.

The final steps in the pathway are the NADPH-dependent reductions of cathenamine and its C-19 epimer. Investigation of this part of the sequence, using the experimental conditions

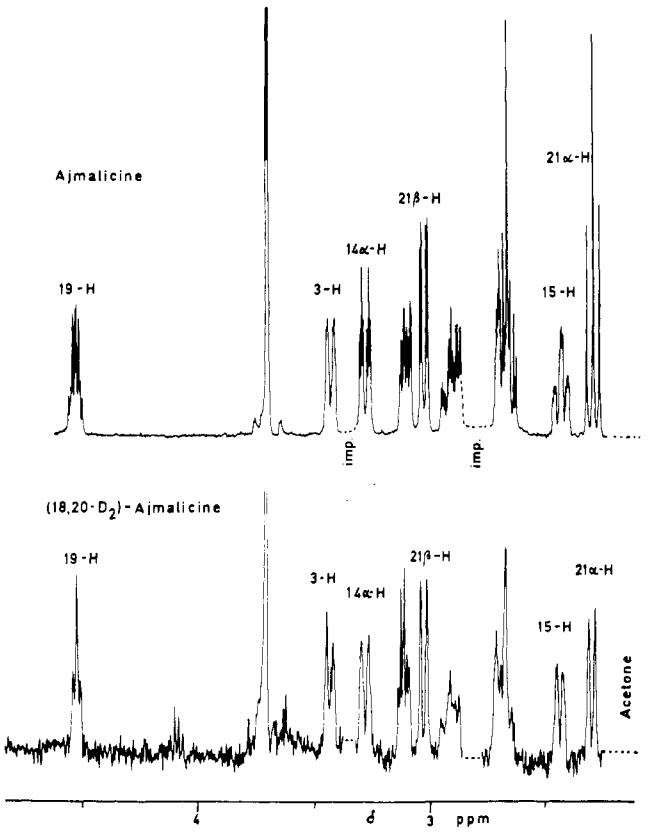

FIGURE 2: ^1H NMR spectra (in part) of ajmalicine and [18,20-$^2H_2$]ajmalicine in deuterioacetone at 400 MHz (imp. = impurities from solvent).

described above (except that BH_4^- was replaced by NADPH), allowed a comparison of the course of the chemical and enzymatic reduction of cathenamine. If both mechanisms, i.e., the chemical and enzymatic reduction, proceed in the same way, one would expect that one D^+ would appear in the C-20 position of each of the ajmalicine isomers. We, therefore, incubated strictosidine in the presence of D_2O, NADPH, and the crude *C. roseus* enzymes. Ajmalicine, 19-epiajmalicine, and tetrahydroalstonine extracted from these reactions were then analyzed by MS and ^1H NMR for their deuterium content. For all three isomers, the MS data were consistent with incorporation of two deuterium atoms, m/z 354, 353, and 339 instead of m/z 352, 351, and 337, respectively, for the unlabeled species. The fragment ion at m/z 184 clearly excluded a labeling at C-21 (Heinstein et al., 1980). The shift of the fragment ion from m/z 223 to m/z 225 indicated an uptake of D^+ into the C-19 methyl group and into the C-20 position, respectively (Hesse, 1974). Detailed ^1H NMR analyses unambiguously supported this suggestion. The three isomers ajmalicine, 19-epiajmalicine, and tetrahydroalstonine displayed a triplet for the well-separated signal of C-19 H by coupling of C-19 H with the C-19 CH_2D group, whereas the unlabeled alkaloids gave rise to a double quartet due to the coupling between C-19 H and C-19 CH_3 (Figure 2). Moreover, coupling between C-20 H, C-21α H, and C-15 H, which is found for the unlabeled alkaloids, disappeared completely. Therefore, the 18,20-dideuterio compounds would show a clean doublet for 21α-H due to the exclusive geminal coupling with the 21β proton, which indeed was observed. As fully expected, the signals for 15-H were also simplified, since no apparent coupling interactions occurred with 20-D (Figure 2). These results clearly demonstrate the chemical and enzymatic reduction of cathenamine to be identical, namely, addition of H^+ at C-20 followed by H^- transfer from BH_4^- or NADPH into the C-21 position.

This transfer of the hydride ion should also be easily monitored by MS and ^1H NMR and would be expected to clarify whether the enzymatic reduction steps catalyzed by cathenamine reductase(s) take place in a stereospecific manner. The cell-free conversion of strictosidine to 3α-heteroyohimbines, therefore, was carried out in the presence of NADPD, generated during the incubation by alcohol dehydrogenase from $NADP^+$ and [$^2H_6$]ethanol. The MS spectra of the alkaloids formed showed main fragments at m/z 353, 352, and 338, respectively, which is in agreement with one-deuterium incorporation. A major ion, usually observed at m/z 184 for the undeuterated isomers, was shifted to m/z 185, which suggested a transfer of D^- exclusively into the C-21 position of the three ajmalicine isomers. The MS data did not, however, allow a distinction to be made between D^- incorporation into the C-21α or C-21β position of the ajmalicine isomers. The stereochemistry of this reduction step, however, can be determined with ^1H NMR from the disappearance of the corresponding 21α or 21β signal, as well as by the collapse of the geminal coupling of the corresponding proton. No signal was observed for C-21α H in the spectrum of the 21-^2H-labeled alkaloids, ajmalicine and tetrahydroalstonine. 21β-H was displayed as a singlet, whereas in the undeuterated isomers, the coupling constant was 11–12 Hz for the 21α/21β hydrogens. Both observations are consistent with a complete and stereospecific transfer of D^- into the C-21 position. The spectrum of 19-epiajmalicine revealed no signal for C-21α H. Therefore, in the enzymatic reduction of cathenamine, the reductase(s) transfer(s) the H^- of NADPH in a highly stereospecific mode into the C-21α position of all three ajmalicine isomers.

During the last 4 years, the role of geissoschizine as an "intermediate" in the biogenetic formation of 3α-heteroyohimbines has been widely studied (Scott et al., 1978; Stöckigt, 1978; Lee et al., 1979; Stöckigt et al., 1980). The methods discussed above, utilized to elucidate the stereochemistry of the reactions of the main pathway, were also used for the final explanation of the precursor role of geissoschizine in the formation of the three ajmalicine isomers. The enzymatic formation of ajmalicine, 19-epiajmalicine, and tetrahydroalstonine from geissoschizine was investigated in the presence of $NADP^+$/NADPD. After isolation of the 3α-heteroyohimbine alkaloids, the spectroscopic analysis gave identical results with those obtained in the experiment when strictosidine was used as the precursor. Again, one deuterium was incorporated into the C-21 position, and the stereochemistry of this reduction step was in absolute agreement with the "strictosidine experiment". These results clearly show that geissoschizine is indeed converted into the ajmalicine isomers by the enzymes of the main pathway after channeling geissoschizine into the appropriate dehydro form (4,21-dehydrogeissoschizine) has occurred in a separate side reaction (Figure 1). Geissoschizine is, in contrast to previous assumptions, not a biogenetic intermediate in heteroyohimbine biosynthesis (Battersby & Hall, 1969; Lee et al., 1979) (see also Added in Proof).

The findings presented here, obtained with the isotope deuterium, are summarized in Figure 1 and confirm that a crude enzyme mixture can be successfully used for the investigation of mechanistic aspects, including the stereochemistry involved, of a complete biogenetic pathway. This methodology, therefore, should provide the possibiity to follow the overall enzymatic synthesis of 3α-heteroyohimbine alkaloids in an NMR tube, employing, e.g., ^{13}C-labeled precursors, to obtain evidence for so far suggested intermediates of the

pathway. This work is presently in progress.

Added in Proof

The most recent monograph on indole alkaloid biosynthesis (Atta-ur-Rahman & Basha, 1983) does not take this fact in consideration, because of incompletely reviewed literature.

Acknowledgments

We are grateful to B. Krausch for excellent technical assistance. Our thanks are due to Dr. B. Deus and the members of our cell-culture laboratory for providing us with cell-suspension cultures and to Dr. V. Wray (GBF) for NMR spectra. Geissoschizine was a kind gift of Prof. Winterfeldt (Hannover). Strictosidine was kindly supplied by U. Pfitzner of our laboratory. Helpful discussion and the continuous interest in this work by Prof. M. H. Zenk and Prof. H. G. Floss are acknowledged. J.S. thanks the Deutsche Forschungsgemeinschaft for a Finnigan MAT 44S instrument.

Registry No. Tryptamine, 61-54-1; secologanin, 19351-63-4; strictosidine, 20824-29-7; strictosidine aglycon, 85925-13-9; dialdehyde, 85955-83-5; carbinolamine, 85925-14-0; 4,21-dehydrocorynantheine aldehyde, 85925-15-1; 20,21-didehydrocorynantheine aldehyde, 85925-16-2; 4,21-dehydrogeissoschizine, 73385-56-5; cathenamine, 63661-74-5; cathenamine iminium form, 74924-24-6; 19-epicathenamine, 73326-87-1; geissoschizine, 439-66-7; 19-epiajmalicine, 25532-45-0; 19-epicathenamine iminium form, 85955-84-6; ajmalicine, 483-04-5; tetrahydroalstonine, 6474-90-4.

References

Atta-ur-Rahman & Basha, A. (1983) in *Biosynthesis of Indole Alkaloids* (Baldwin, J. E., Goodenough, J. B., Halpern, J., & Rowlinson, J. S., Eds.) International Series of Monographs on Chemistry, p 55, Clarendon Press, Oxford.

Battersby, A. R., & Hall, E. S. (1969) *J. Chem. Soc., Chem. Commun.*, 793.

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248.

Heinstein, P., Stöckigt, J., & Zenk, M. H. (1980) *Tetrahedron Lett. 21*, 141.

Hemscheidt, T., & Zenk, M. H. (1980) *FEBS Lett. 110*, 87.

Hesse, M. (1974) *Fortschr. Massenspektrometrie 1*, 130.

Höfle, G., Heinstein, P., Stöckigt, J., & Zenk, M. H. (1980) *Planta Med. 40*, 120.

Husson, H.-P., Kan-Fan, C., Sevenet, Th., & Vidal, J.-P. (1977) *Tetrahedron Lett.*, 1889.

Lee, S.-L., Hirata, T., & Scott, A. I. (1979) *Tetrahedron Lett. 20*, 691.

Lounasmaa, M., & Kan, S.-K. (1980) *Tetrahedron Lett. 36*, 1607.

Pfitzner, A., & Stöckigt, J. (1982) *Phytochemistry 21*, 1585.

Scott, A. I., Lee, S.-L., Hirata, I., & Culver, M. G. (1978) *Rev. Latinoam. Quim. 9*, 131.

Stöckigt, J. (1978) *J. Chem. Soc., Chem. Commun.*, 1097.

Stöckigt, J. (1980) in *Indole Biogenetically Related Alkaloids* (Phillipson, J. D., & Zenk, M. H., Eds.) Academic Press, London and New York.

Stöckigt, J., Höfle, G., & Pfitzner, A. (1980) *Tetrahedron Lett. 21*, 1925.

Treimer, J. F., & Zenk, M. H. (1979) *FEBS Lett. 97*, 159.

Zenk, M. H. (1980) *J. Nat. Prod. 43*, 438.

Zenk, M. H., El-Shagi, H., Arens, H., Stöckigt, J., Weiler, E. W., & Deus, B. (1977) in *Plant Tissue Culture and Its Biotechnological Application* (Barz, W., Reinhard, E., & Zenk, M. H., Eds.) Springer, Berlin, Heidelberg, and New York.