^{2}H NMR Spectroscopy as a Probe of the Stereochemistry of Biosynthetic Reactions: The Biosynthesis of Lupinine

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Abstract: The mode of incorporation of ^{2}H from (*R*)- and (*S*)-(1-^{2}H)cadaverine into lupinine in *Lupinus luteus* was determined by ^{2}H NMR spectroscopy. The results establish the stereochemistry of five of the steps in the biosynthetic conversion of cadaverine into lupinine (Scheme I).

The carbon skeleton of lupinine (**8**), the major alkaloid of *Lupinus luteus* (yellow lupin),[1,2] is derived from two C_5 units related to lysine.[3-5] Label from lysine[3] and from cadaverine[4,5] (**1**), its decarboxylation product, is incorporated nonrandomly into lupinine (**8**). Two fundamentally different routes from cadaverine into lupinine have been postulated. One of these routes passes through a "dimeric" compound with C_{2v} symmetry (e.g., OHC-$(CH_2)_4$NH$(CH_2)_4$CHO (**9**)).[6] The key intermediate of the other route is tetrahydroanabasine (**6**),[7] the dimer of Δ^1-piperideine (**5**).

We have recently shown by means of a tracer experiment with intramolecularly ^{15}N,^{13}C-labeled cadaverine ($NH_2CH_2CH_2CH_2CH_2{}^{13}CH_2{}^{15}NH_2$) that a dimeric molecule with C_{2v} symmetry cannot be an intermediate between cadaverine and lupinine.[8] We have now examined the stereochemistry of the conversion of cadaverine into lupinine by means of ^{2}H NMR. The results here presented clarify the stereochemistry of the steps of lupinine biosynthesis which involve transformations at the carbon atoms derived from C-1 of cadaverine. They provide further evidence against the intermediacy of a compound with C_{2v} symmetry and lend support to the intermediacy of tetrahydroanabasine (**6**).

In two experiments, each with 40 plants of *L. luteus*, (*R*)-(1-^{2}H)cadaverine dihydrochloride[9] (**2**) (99 atom % ^{2}H, 150 mg) (experiment 1, March 1983) and (*S*)-(1-^{2}H)cadavarine dihydrochloride[9] (**3**) (93 atom % ^{2}H, 93 mg) (experiment 2, December 1982) were administered by the wick method over a period of 6 days. From each experiment the alkaloid fraction was isolated and lupinine (**8**) was separated and purified by standard methods.

The ^{2}H NMR spectra of the lupinine samples (in C_6H_6) from the two experiments are shown in Figure 1. Chemical shifts were assigned by comparison with the corresponding ^{1}H NMR chemical shifts (Table I) and on the basis of two assumptions which follow from the observation that the samples of lupinine from (*R*)- and from (*S*)-(1-^{2}H)cadaverine give different ^{2}H NMR spectra and that incorporation is thus stereospecific. The assumptions are as follows: (i) The α and β protons at any one carbon atom cannot both be deuterated within the same sample of lupinine (e.g., lupinine from (*R*)-(1-^{2}H)cadaverine cannot bear ^{2}H at both the 4α and the 4β position, but only at one or the other). (ii) The two samples of lupinine derived from the two enantiomers of (1-^{2}H)cadaverine cannot bear deuterium at the same site (e.g., only one or the other, but not both samples of lupinine, derived from (*R*)- and (*S*)-(1-^{2}H)cadaverine, respectively, can carry deuterium at C-10). It is further assumed, on the basis of earlier results on the incorporation of ^{14}C-labeled[4,5] and ^{13}C-labeled[8] cadaverine, that, in every case, each of the two cadaverine-derived C_5 units of lupinine (C-6 to C-10 and C-11, C-1 to C-4, respectively) will carry at least one deuterium atom, so that structures which bear two ^{2}H atoms in one of the C_5 units and none in the other (e.g., (6β-^{2}H,10-^{2}H)lupinine) need not be considered as biosynthetic products. Then, in the ^{2}H spectrum of the product from (*R*)-(1-^{2}H)cadaverine (experiment 1), the signal at 4.14 ppm is due to ^{2}H at the 11-*si* position, the signal at 2.50 ppm is due to ^{2}H at one of the equatorial positions, 4α or 6α, and the signal at 1.77 ppm is due to ^{2}H at C-10. In the spectrum of the product from (*S*)-(1-^{2}H)cadaverine (experiment 2), the two signals at 1.73 and 1.54 ppm are due to deuterium at the axial positions 4β and 6β, respectively.

Table I. Incorporation of (*R*)- and (*S*)-(1-^{2}H)Cadaverine Dihydrochloride into Lupinine; ^{2}H NMR Analysis

H atom	^{1}H NMR chemical shifts,[a] ppm	^{2}H NMR chemical shifts,[b] ppm	
		expt 1[c]	expt 2[d]
4α (*si*)	2.57		
4β (*re*)	1.75		1.73
6α (*re*)	2.51	2.50	
6β (*si*)	1.57		1.54
10	1.78	1.77	
11-*re*	3.75		
11-*si*	4.16	4.14	

[a] Recorded in C_6D_6 at 250 MHz in the Fourier mode on a Bruker WM 250 spectrometer. [b] See caption to Figure 1. [c] Lupinine from (*R*)-(1-^{2}H)cadaverine (**2**). [d] Lupinine from (*S*)-(1-^{2}H)cadaverine (**3**).

The isotope enrichment at each labeled site can be calculated, using the area of the natural abundance ^{2}H signal of the solvent (C_6H_6) as a reference standard. The lupinine sample from (*R*)-(1-^{2}H)cadaverine showed an average enrichment of 2.3 atom % ^{2}H at each of the three labeled sites, whereas enrichment of the lupinine sample derived from (*S*)-(1-^{2}H)cadaverine was 0.6 atom % ^{2}H at each of the two labeled sites. As expected for a product resulting from a biosynthetic sequence which includes a dimerization process (**4** $\rightarrow\!\!\rightarrow$ **6**), the level of enrichment is the same at corresponding labeled sites in the two "halves" of the product. Thus, the signal area due to ^{2}H-10 is the same as that due to ^{2}H-11-*si* in the lupinine sample derived from (*R*)-(1-^{2}H)cadaverine, and the area due to ^{2}H-4-*re* is the same as that due to ^{2}H-6-*si* in the sample derived from (*S*)-(1-^{2}H)cadaverine.

The two deuteriated centers of the ^{2}H labeled product from (*S*)-(1-^{2}H)cadaverine, C-4 and C-6, are of opposite configuration: ^{2}H at C-4 is in the re position and ^{2}H at C-6 is in the *si* position. The labeled product from (*R*)-(1-^{2}H)cadaverine bears ^{2}H at only one of these two C atoms, either C-4-*si* or C-6-*re*. These results

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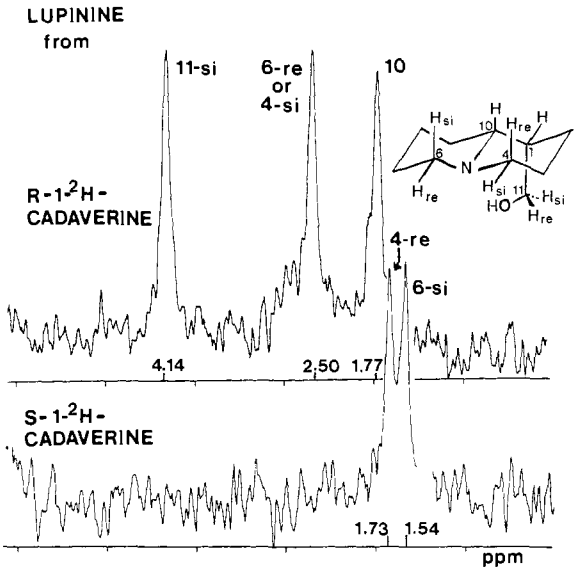


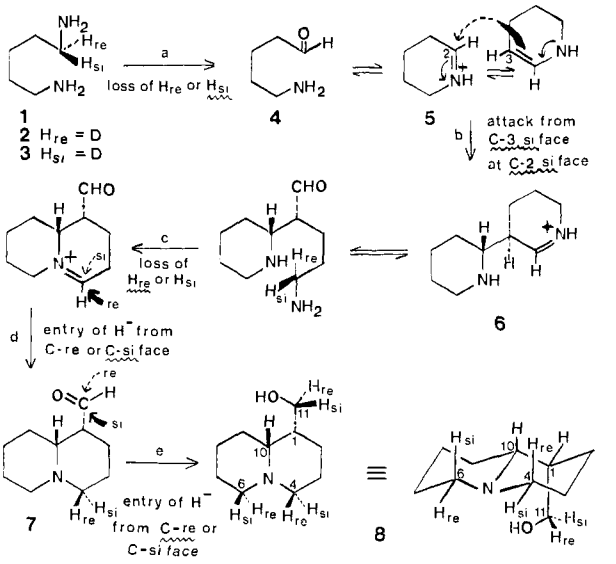
Figure 1. ^2H NMR spectra (38.40-MHz) of (top) lupinine (2.0 mg in 1 mL of C_6H_6, 29 632 transients) obtained from administration of (*R*)-(1-^{2}H)cadaverine dihydrochloride (**2**) and of (bottom) lupinine (2.6 mg in 1 mL of C_6H_6, 34 000 transients) obtained from administration of (*S*)-(1-^{2}H)cadaverine dihydrochloride (**3**). The spectra were recorded in the Fourier mode on a Bruker WM 250 spectrometer, in 10-mm tubes, with natural abundance C_6DH_5 (7.19 ppm) as internal reference. The acquisition time was 2.048 s.

provide independent evidence that a "dimeric" compound with C_{2v} symmetry, such as **9**, cannot be an intermediate between cadaverine and lupinine. The signal at 2.50 ppm in the ^2H NMR spectrum of lupinine derived from (*R*)-(1-^{2}H)cadaverine is due to the presence of ^2H either at the 4-*si* or the 6-*re* position. A biogenetic sequence which would account for the presence of ^2H at C-4 of the lupinine samples from both enantiomers of (1-^{2}H)cadaverine, but at C-6 of only one of them, is shown in Scheme II.[10] This hypothetical sequence is disproven not only by the observation[8] that the C-6,N bond but not the C-4,N bond of lupinine represents an intact C-N bond of cadaverine but also because it leads to stereochemically contradictory inferences. Assignment of the signal at 2.50 ppm to ^2H at C-6-*re* yields conclusions that are internally consistent and that agree with all other available results.

The deuteration pattern at C-4 shows that the *re* hydrogen is lost stereospecifically from the site destined to become C-4 of lupinine, whereas the *si* hydrogen is retained in the course of entry of C-1 of cadaverine into C-4 of lupinine (step c, Scheme I). This provides evidence that formation of the C-4,N bond occurs by an oxidation–condensation–reduction process (steps c and d), rather than by intramolecular displacement of the primary amino group. Since the 1-*si* hydrogen of cadaverine is located at the 4-*re* position of lupinine, reductive attack by a hydride donor must have taken place from the 4-*si* face of an intermediary immonium ion (step d).

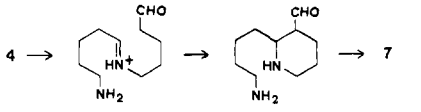
(10) See Scheme II.

Scheme I. The Biosynthetic Route from Cadaverine into Lupinine, and Its Stereochemical Ambiguities[a]



[a] The stereochemical questions answered in this investigation are printed in boldface (e.g., H_{si}).

Scheme II



Deuterium enters positions C-10 and C-11 of lupinine when (*R*)-(1-^{2}H)cadaverine serves as precursor but not when (*S*)-(1-^{2}H)cadaverine is the substrate. Stereospecific loss of the 1-*si* proton in the course of enzymic transformation of a primary amine into an aldehyde (step a) is consistent with the stereochemistry of a reaction catalyzed by a diamine oxidase.[9]

Lupinine, derived from (*R*)-(1-^{2}H)cadaverine, carries label at the *si* site of the primary alcohol group, C-11. This stereochemistry is consistent with attack by a hydride donor from the C-*re* face of the C=O bond of an aldehyde intermediate (step e).

The stereochemical outcome is thus determined for all the transformations in the course of lupinine biosynthesis (steps a, c, d, e), which occur at the four carbon atoms of lupinine which are derived from the α-carbon atoms of cadaverine. The results support the biosynthetic sequence shown in Scheme I. The stereochemistry of another transformation, the dimerization (step b), follows from the absolute configuration of (−)-(1-*R*,10-*R*)lupinine.

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