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

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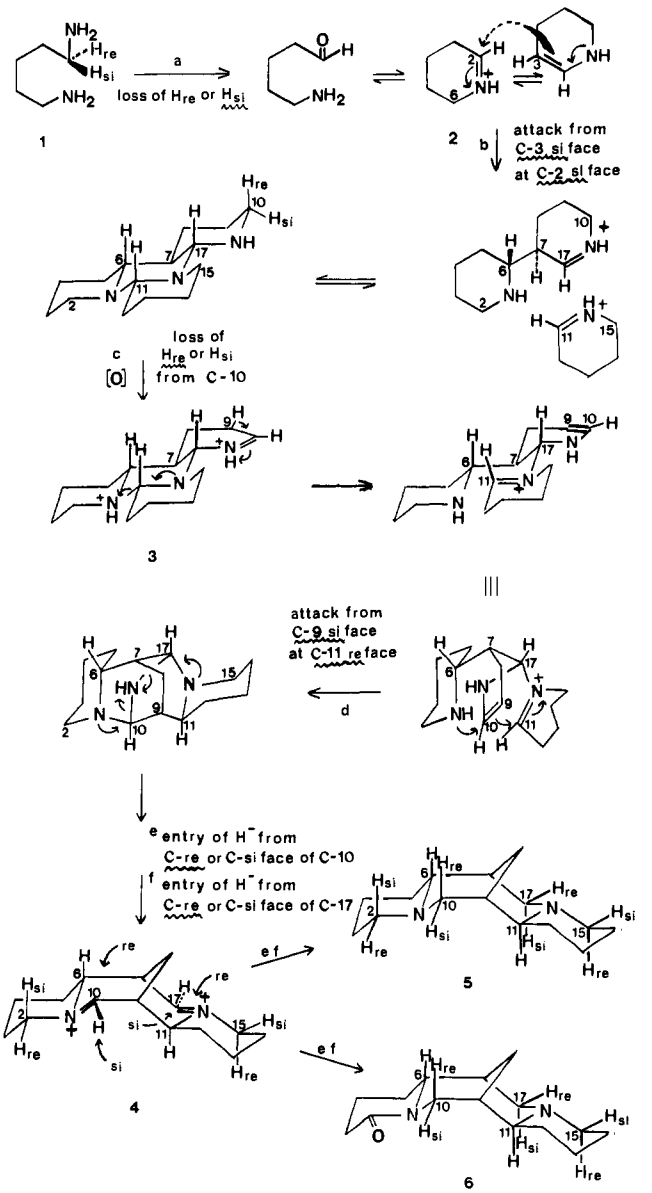
Abstract: The mode of incorporation of label from (1-^{13}C,1-^{15}N)cadaverine, (*R*)- and (*S*)-(1-^{2}H)cadaverine and L-(2-^{2}H)lysine into lupanine in *Lupinus angustifolius* and from (*R*)- and (*S*)-(1-^{2}H)cadaverine, L-(2-^{2}H)lysine, and (2-^{2}H)-Δ^1-piperideine into sparteine in *L. luteus* establishes the regiochemistry, the chirality, and the prochirality of the steps in the biosynthesis of lupanine and sparteine. The results constitute new evidence in support of the piperideine trimer model of the biosynthesis of the $C_{15}N_2$ lupine alkaloids. They invalidate other hypotheses that have recently been advanced.

The steps from primary precursors into the tetracyclic lupine alkaloids (e.g., (−)-sparteine (**5**) and (+)-lupanine (**6**)) are still in dispute. Early hypotheses[2] did not address stereochemical aspects of the biosynthetic origin of these alkaloids. Almost 10 years ago[3] we advanced a biogenetic hypothesis of the origin of these compounds which could account for their stereochemistry, and we offered some evidence in its support. This hypothesis, which was entirely consistent with earlier evidence on the mode of incorporation of lysine[4,5] (or, more specifically, L-lysine[6]) and of cadaverine (**1**)[4,5,7,8] into the alkaloids, views the $C_{15}N_2$ lupine alkaloids as modified trimers of Δ^1-piperideine (**2**): The observed stereochemistry of these alkaloids is accounted for[3] by their derivation from the favored all-trans[9] stereoisomer of isotripiperideine,[10] one of the trimers of Δ^1-piperideine, in a four-step sequence (Scheme I). The observed[3] mode of incorporation of [2-^{14}C]-and [6-^{14}C]-Δ^1-piperideine into lupanine was consistent with this biogenetic model.[11] Our recent demonstration[14] that, in *Lupinus luteus*, intramolecularly doubly ^{13}C, ^{15}N-labeled cadaverine (NH_2–CH_2–CH_2–CH_2–CH_2–$^{13}CH_2$–$^{15}NH_2$) yields

Scheme I. The Biosynthetic Route from Cadaverine into (+)-Lupanine and (−)-Sparteine, and Its Stereochemical Ambiguities[a]



[a] The answers to the stereochemical questions posed in this investigation are underlined with a wavy line (e.g., H_{si}).

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(2) For a review of these early hypotheses see: Leete, E. In "Biogenesis of Natural Compounds", 2nd ed; Bernfeld, P., Ed.; Pergamon Press: Oxford, 1967; pp 972–975. Spenser I. D. In "Comprehensive Biochemistry"; Florkin, M., Stotz, E. H., Eds.; Elsevier: Amsterdam, 1968; Vol. 20, pp 262–267. Schütte, H. R. In "Biosynthesis of Alkaloids"; Mothes, K., Schütte, H. R., Eds.; VEB Deutscher Verlag der Wissenschaften: Berlin, 1969; pp 324–347.

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(11) A recent biogenetic proposal[12] is inconsistent with the observed mode of entry of label from [2-^{14}C]- and [6-^{14}C]-Δ^1-piperideine into lupanine, in that it predicts activity from [2-^{14}C]-Δ^1-piperideine to enter C-10 and from [6-^{14}C]-Δ^1-piperideine to enter C-17 of lupanine, whereas the converse was observed.[3] This observed mode of entry of Δ^1-piperideine into the C_5-unit, C-10, −9, −8, −7, −17, also invalidates the early notion,[2] which was recently revived,[13] that this C_5 unit is derived via glutardialdehyde.

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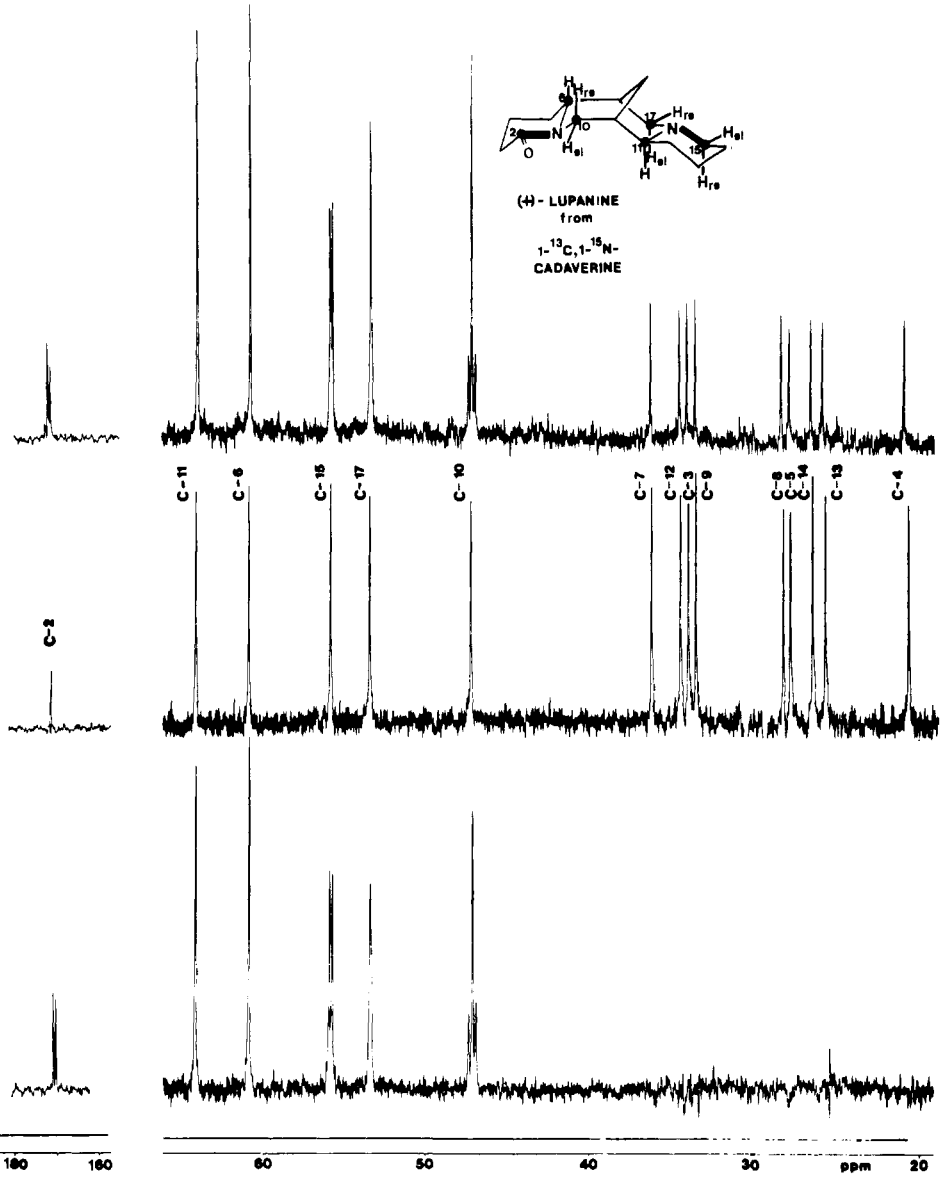

Figure 1. ^{13}C NMR spectra (20.15 MHz) of (+)-lupanine. Top: Proton noise decoupled (PND) spectrum of the $^{13}C,^{15}N$-enriched sample (30 mg in 1 mL of C_6D_6; 2-mm tube) (average specific incorporation of ^{13}C per C_5 unit of lupanine: 9.5%). Upfield region (10–70 ppm): narrow window spectrum, 5000 transients, 0.244 Hz/data point, acquisition time 4.096 s, 2-μs pulses. Downfield region (160–180 ppm): normal spectrum, 1600 transients, 1.471 Hz/data point, acquisition time 0.68 s, 2-μs pulses. Middle: PND spectrum of a natural abundance sample (30 mg in 1 mL of C_6D_6). Upfield region 17 000 transients; downfield region 90 000 transients. Bottom: Difference spectrum. The spectra were recorded in the Fourier mode on a Bruker WP 80 spectrometer. The natural abundance ^{13}C signal of C_6D_6 (128.4 ppm) was employed as internal reference. For assignment of signals see ref 16.

sparteine, bond labeled solely in two of its six C–N bonds (C-2,N-1 and C-15,N-16), is as predicted by the piperideine trimer model.

In further support of this model we now report the results of experiments on the incorporation into lupanine in *Lupinus angustifolius* of (1-^{13}C,1-^{15}N)cadaverine, (*R*)-(1-^2H)- and (*S*)-(1-^2H)cadaverine, and L-(2-^2H)lysine and into sparteine in *L. luteus* of (*R*)-(1-^2H)- and (*S*)-(1-^2H)cadaverine, L-(2-^2H)lysine, and (2-^2H)-Δ^1-piperideine. The labeled precursors were prepared as previously described[14,15] and were administered to 6-week-old plants of *L. luteus*[14] and *L. angustifolius*[3,6] by the wick method. The alkaloids were isolated by standard procedures.

The ^{13}C NMR spectrum (Figure 1) of the sample of (+)-lupanine derived from (1-^{13}C,1-^{15}N)cadaverine shows that, as in the case of sparteine,[13,14] only the signals due to C-2 and C-15 appear as doublets due to C–N coupling. This indicates that the two C–N bonds, C-2,N-1 and C-15,N-16, but none of the other four C–N bonds, are transferred intact from the precursor.

The ^2H NMR spectra of the (+)-lupanine samples (in C_6H_6) from the ^2H experiments are shown in Figure 2. Chemical shifts were assigned by comparison with the corresponding ^1H NMR chemical shifts[17–19] (Table I) and on the basis of three assumptions, previously discussed in a recent paper.[20] (i) The α and β protons at any one carbon atom of the sample of lupanine derived from one of the enantiomers of chiral (1-^2H)cadaverine cannot both be deuterated. (ii) The two samples of lupanine derived from the two enantiomers of (1-^2H)cadaverine cannot bear deuterium at the same site. (iii) Structures bearing two ^2H atoms in one of the three C_5 units of the alkaloid and no ^2H in the other two C_5 units need not be considered as biosynthetic products.

The ^2H NMR spectra of the (–)-sparteine samples (in C_6H_6)

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Table I. Incorporation of Deuterated Substrates into (+)-Lupanine and (−)-Sparteine: ^2H NMR Analysis

hydrogen atom	NMR chemical shifts (ppm) of reference samples		^2H NMR chemical shifts[b] (ppm) of alkaloid samples isolated from feeding experiments with			
	^1H[a]	^2H[b,c]	(*R*)-(1-^2H)-cadaverine	(*S*)-(1-^2H)-cadaverine	L-(2-^2H)-lysine	(2-^2H)-Δ^1-piperideine
			(+)-Lupanine (cf. ref 17–19)			
6β *R* (ax)	2.74		2.74			
10α *si* (*eq*)	4.80			4.75	4.75	
10β *re* (ax)	2.31					
11α *S* (ax)	1.70		1.67			
15α *re* (ax)	1.85		1.82			
15β *si* (eq)	2.66			2.61	2.60	
17α *si* (pseudo ax)	1.83	1.79	1.82			
17β *re* (pseudo eq)	2.55	2.52				
			(−)-Sparteine (cf. ref 19, 21, 22)			
2α *re* (eq)	2.62	2.58	2.61			
2β *si* (ax)	1.88	1.84		1.88	1.86	
6β *R* (ax)	1.62	1.63	1.63			1.64
10α *si* (eq)	2.47	2.43		2.48	2.48	
10β *re* (ax)	1.96	1.92				
11α *S* (ax)	2.11		2.08			2.08
15α *re* (ax)	2.04	1.97	2.00			
15β *si* (eq)	2.76	2.73		2.76	2.76	
17α *si* (pseudo ax)	2.47	2.44	2.48			2.47
17β *re* (pseudo eq)	2.67	2.63				

[a] ^1H NMR spectrum. Recorded in C_6D_6 at 250 MHz in the Fourier mode on a Bruker WM 250 spectrometer. [b] ^2H NMR spectra. Recorded in C_6H_6 at 38.40 MHz in the Fourier mode on a Bruker WM 250 spectrometer, operating at 5.872 T, in 10-mm tubes with natural abundance C_6DH_5 (7.19 ppm) as internal reference. Acquistion time 2.048 s. [c] To determine these chemical shifts, deuterated samples of sparteine (2,2-$^2H_2$-; 6-^2H-; 10,10-$^2H_2$-; 15,15-$^2H_2$-; 17,17-$^2H_2$-; 17α-^2H-; and 17β-^2H-) and lupanine (17α-^2H-; 17β-^2H-) were prepared by adaptation of known methods.[16,18,23,24]

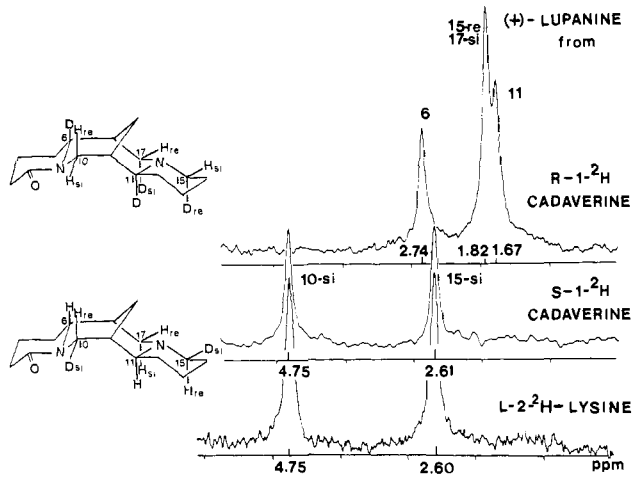

Figure 2. ^2H NMR spectra (38.40 MHz) of (+)-lupanine in C_6H_6. For details see Table I, footnote *b*. Top: (+)-Lupanine (43 mg in 1 mL of C_6H_6) (968 transients) obtained from administration of (*R*)-(1-^2H)cadaverine dihydrochloride to *L. angustifolius*. Middle: (+)-Lupanine (44 mg in 1 mL of C_6H_6) (712 transients) from (*S*)-(1-^2H)cadaverine dihydrochloride. Bottom: (+)-Lupanine (25 mg in 1 mL of C_6H_6) (32240 transients) from L-(2-^2H)lysine.

were assigned similarly[19,21,22] (Table I).

The incorporation of deuterium from the enantiomeric samples of (1-^2H)cadaverine into the samples (+)-lupanine and (−)-sparteine is in accord with the piperideine trimer hypothesis.[3] In addition the labeling pattern reveals the prochirality of each of the biosynthetic steps that involve sites adjacent to nitrogen (Scheme I). Furthermore, since L-(2-^2H)lysine and (*S*)-(1-^2H)cadaverine yield alkaloid samples with identical labeling patterns (Table I), it follows that decarboxylation of L-lysine within *L. angustifolius* and *L. luteus* takes place with net retention of configuration (cf. ref 25).

The fact that the samples of lupanine and sparteine retain deuterium from (*R*)-(1-^2H)cadaverine and from (2-^2H)-Δ^1-piperideine at the 17α position (Table I), at an enrichment equimolar with that at positions 11α and 6β, indicates that 17-oxosparteine cannot be a precursor of lupanine or of sparteine and invalidates a biogenetic hypothesis which was recently advanced.[26] Similarly, since the samples of lupanine and sparteine from (*S*)-(1-^2H)cadaverine and L-(2-^2H)lysine maintain ^2H at C-10α, the suggestion[3] that 10-oxosparteine may be an intermediate is invalidated. The fact that sparteine retains deuterium from (*R*)-(1-^2H)cadaverine at the 2α position and from (*S*)-(1-^2H)-cadaverine and L-(2-^2H)lysine at the 2β position controverts the suggestion that 1,2-dehydrosparteinium ion is a precursor of sparteine[27] or that sparteine originates by reduction of lupanine.[28]

The notion[3] that (+)-lupanine (**6**) and (−)-sparteine (**5**) are separately derived from 1, 10;16,17-didehydrosparteinium ion (**4**), a key intermediate of the piperideine trimer model,[3] is entirely consistent with the experimental findings presented here.

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Registry No. 1, 462-94-2; **2**, 505-18-0; **5**, 90-39-1; **6**, 550-90-3; L-lysine, 56-87-1.

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