A ^{13}C N.M.R. Study of the Biosynthesis of Lupinine and Sparteine

W. Marek Golebiewski and Ian D. Spenser

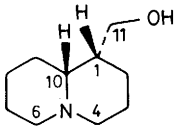
Department of Chemistry, McMaster University, Hamilton, Ontario, Canada L8S 4M1

The mode of incorporation, into lupinine and into sparteine, of cadaverine, intramolecularly doubly labelled with ^{15}N and with ^{13}C at the C-atom adjacent to ^{15}N, was determined by ^{13}C n.m.r. spectroscopy; lupinine is generated from two, and sparteine from three cadaverine-derived C_5-units, each by a route which excludes intermediates with C_{2v} symmetry.

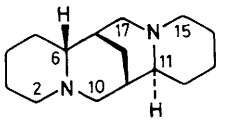
The carbon skeleton of lupinine (**1**), the major alkaloid of *Lupinus luteus*,[1,2] is derived from two C_5-units related to lysine.[3–5] The label from DL-[2-^{14}C]lysine[3] (**2**) and from its decarboxylation product, [1,5-^{14}C]cadaverine[4,5] (**3**) was incorporated nonrandomly into lupinine. The distribution of label, determined by partial chemical degradation, was consistent with the intermediacy, on the route from these substrates into the alkaloid, either of the 'dimer' (**5**),[3,6,7] a compound with C_{2v} symmetry (route A, Scheme 1), or of the dimer (**4**),[3,8] a compound which is dissymmetric (C_1) (route B, Scheme 1). Biogenetically modelled chemical syntheses of the lupinine skeleton have been reported, starting from (**4**)[8] and from (**5**).[9,10] Tracer experiments[11,12] designed to distinguish between these alternatives were inconclusive.

To show whether or not a non-dissymmetric intermediate such as (**5**) lies on the pathway, we have determined the mode of incorporation of intramolecularly ^{13}C,^{15}N-labelled cadaverine ($NH_2[CH_2]_4{}^{13}CH_2{}^{15}NH_2$)† into lupinine by ^{13}C n.m.r. spectroscopy. Entry of this substrate into lupinine by the dissymmetric route B (Scheme 1) should yield a single species of intramolecularly ^{13}C, ^{15}N-doubly labelled product, in which the ^{13}C, ^{15}N moiety, which has been transferred intact, is located at the C-6,N position of lupinine. Entry *via* the 'symmetrical' (*i.e.* non-dissymmetric) route A (Scheme 1) must yield, as a necessary consequence of the C_{2v} symmetry of the intermediate (**5**), an equimolar mixture of two species of intramolecularly ^{13}C, ^{15}N-doubly labelled product, one showing enrichment at C-6,N, the other at C-4,N. Non-equimolar enrichment at these two sites is not consistent with route A.

The results show that a 'symmetrical' dimeric species C_5–N–C_5 such as (**5**) is not involved in lupinine biosynthesis.‡



(**1**)

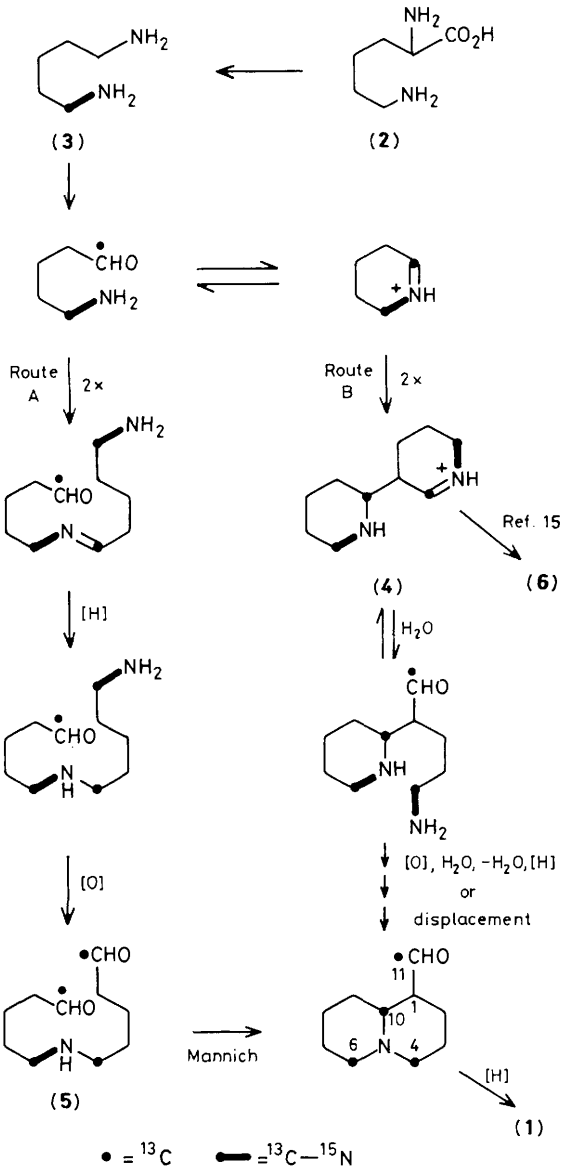


(**6**)

† (1-^{13}C, 1-^{15}N)Cadaverine was synthesised in three steps from $Na^{13}C^{15}N$ (0.15 g, 99 atom % ^{13}C, 99 atom % ^{15}N) (MSD Isotopes, Montreal, Canada) and 1-bromo-4-phthalimidobutane, followed by reduction and acid hydrolysis (*cf.*, ref. 13). ^1H N.m.r. (recorded at 90 MHz on a Varian EM 390 spectrometer) (^2H_2O) δ 3.76 (t, 1H), 2.97 (t, 2H), 2.18 (t, 1H), 1.45—1.75 (m, 6H); *J* (^{13}C, H-1) 142 Hz (the signals at δ 3.76 and 2.18 are a doublet of triplets, owing to ^{13}C, H-1 coupling of the $^{13}CH_2$ group next to ^{15}N; the signal at δ 2.97 is due to the hydrogen atoms on the carbon atom α to ^{14}N). ^{13}C N.m.r. [recorded at 20.15 MHz in the Fourier mode on a Bruker WP 80 spectrometer with proton noise decoupling. Spectra were determined in ^2H_2O with 1,4-dioxane as external reference (δ 67.4 p.p.m.)] (^2H_2O) δ 40.0 [d, *J* (^{13}C-1, ^{15}N) 4.4 Hz, C-1, C-5 not observable], 27.0 [s, d, *J* (^{13}C-2, ^{13}C-1) 18.4 Hz, C-2, C-4], 23.4 (s, C-3) p.p.m.

‡ This is in marked contrast with the biosynthetic route from ornithine to retronecine, which does indeed pass through a 'dimeric' intermediate with C_{2v} symmetry (refs. 13, 14). The biosynthesis of lupinine and retronecine had hitherto been assumed to proceed by analogous routes (ref. 7).

(1-^{13}C,1-^{15}N)-1,5-Diaminopentane (cadaverine) dihydrochloride,† (99 atom % ^{13}C, 99 atom % ^{15}N, 140 mg) in admixture with [1,5-^{14}C]cadaverine was administered by the



Scheme 1. Two routes from lysine into lupinine. Route A, *via* a nondissymmetric (C_{2v}) 'dimeric' intermediate (**5**); route B, *via* dissymmetric (C_1) dimeric intermediates [*e.g.*, (**4**)]. Formulae of labelled 'monomeric' intermediates (5-aminopentanal and Δ^1-piperideine) represent a 50/50 mixture of two different labelled species, one doubly labelled with ^{15}N and with ^{13}C at the adjacent methylene group, the other singly labelled with ^{13}C at the sp^2 carbon atom. Formulae of all 'dimeric' intermediates represent mixtures of eight labelled species, whose relative contribution is determined by the relative quantities of labelled and unlabelled cadaverine available to the plant during biosynthesis.

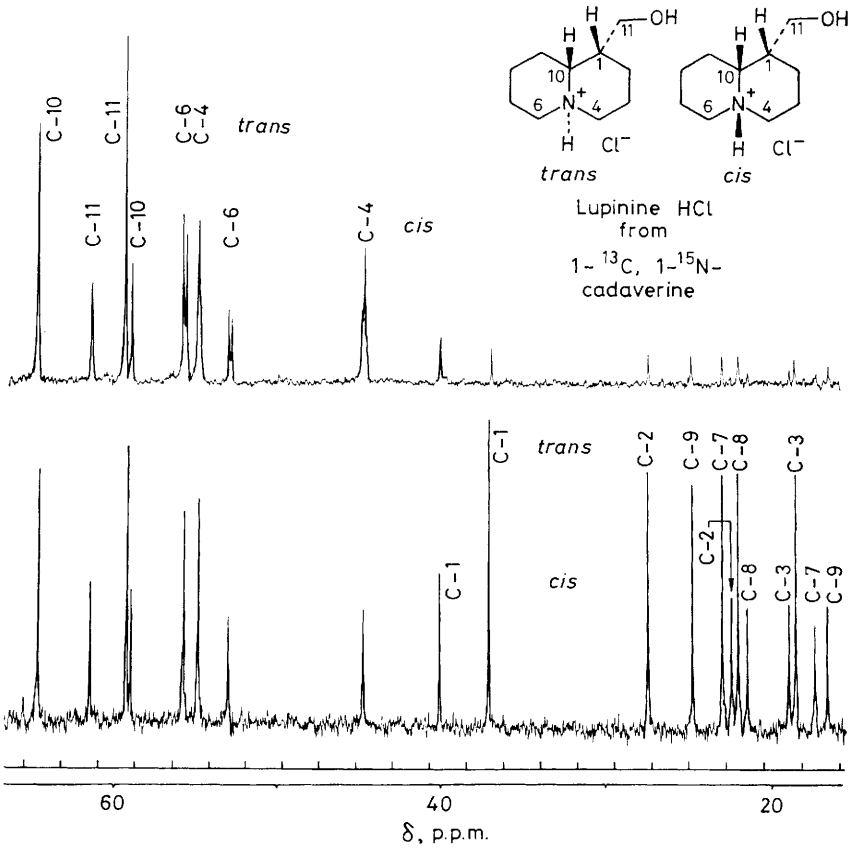


Figure 1. 20.15 MHz ^{13}C N.m.r. spectra of lupinine hydrochloride. Top: Proton-noise decoupled (P.N.D.) spectrum (20000 scans) of ^{13}C, ^{15}N-enriched sample (10 mg in 10 μl ^2H_2O) derived from (1-^{13}C, 1-^{15}N)cadaverine. Bottom: P.N.D. spectrum (18112 scans) of natural abundance sample (17 mg in 20 μl ^2H_2O). The spectra were recorded in the Fourier mode on a Bruker WP 80 spectrometer. The natural abundance ^{13}C methyl signal of acetone was employed as internal reference. The signals in the ^{13}C spectrum of lupinine hydrochloride were assigned on the basis of the off resonance spectrum (C-1, C-10) and by comparison with spectra of standard compounds (*cf.* refs. 16, 17).

wick method to 50 plants of *Lupinus luteus* (yellow lupin) over a period of 5 days (August 1982). The alkaloids were isolated by conventional methods. After purification to constant specific radioactivity lupinine (**1**) hydrochloride (specific incorporation 28% per C_5 unit) and sparteine (**6**) mono-(hydrogen sulphate) (specific incorporation 47% per C_5 unit) were obtained.

The distribution of label in the two alkaloids is evident from their ^{13}C n.m.r. spectra. The proton noise decoupled ^{13}C n.m.r. spectrum of the sample of lupinine hydrochloride (a mixture of the *trans* and the *cis* isomers, in the ratio 1.7:1) (Figure 1) shows that, as expected, only four of the ten C-atoms (C-4, C-6, C-10, C-11) are enriched in ^{13}C, to an equal extent. The average specific incorporation of label per C_5 unit (C_5 unit A: C-6,-7,-8,-9,-10, ^{13}C enrichment in either C-6 or C-10 but not in both within the same molecule; C_5 unit B: C-11,-1,-2,-3,-4, ^{13}C enrichment in either C-11 or in C-4, but not in both), based on the ^{13}C n.m.r. data, was *ca.* 30 atom % ^{13}C, in agreement with the ^{14}C incorporation.

The signals in the proton noise decoupled ^{13}C n.m.r. spectrum due to two of the ^{13}C-enriched carbon atoms, C-10 and C-11, appear as singlets, the signals due to the two others, C-6 and C-4, as multiplets. Since the coupling constants of the doublet component of the multiplets are different from one another [J(^{13}C-4, ^{15}N): *trans* isomer 4.1 Hz, *cis* isomer 4.1 Hz; J(^{13}C-6, ^{15}N): *trans* isomer 7.0 Hz, *cis* isomer 6.4 Hz; isotope shift C-4, *cis* +0.02, *trans* +0.02; C-6, *cis* −0.04, *trans*, −0.04], the multiplets do not arise from ^{13}C-6, ^{13}C-4 coupling, but are due to ^{13}C, ^{15}N coupling. Each of the two sets of multiplets consists of a doublet superimposed on a singlet. The doublet (87 ± 11% of signal area) (^{13}C-6, ^{15}N) in the signals due to C-6 (*trans*, δ 55.7, *cis*, δ 52.9 p.p.m.) is much more intense than the singlet (13 ± 2%) (^{13}C-6, ^{14}N) so that the latter is not apparent in the unexpanded spectrum (Figure 1). The intense ^{13}C-6, ^{15}N doublet indicates intact incorporation of the ^{13}C–^{15}N unit of the administered cadaverine into C-6,N of lupinine. In the signals due to C-4 (*trans*, δ 54.8, *cis*, δ 44.6 p.p.m.) the doublet (36 ± 5%) (^{13}C-4, ^{15}N) appears as a shoulder straddling the singlet (64 ± 8%) (^{13}C-4, ^{14}N). The low intensity ^{13}C-4, ^{15}N doublet arises as a consequence of the remarkably high efficiency of incorporation, into the alkaloid, of the administered (1-^{13}C,1-^{15}N)cadaverine. It can be calculated from the ^{13}C n.m.r. data that the administered (1-^{13}C, 1-^{15}N)cadaverine was diluted by no more than 25% of endogenous natural abundance cadaverine, prior to conversion into lupinine. The intensity of the ^{13}C-4, ^{15}N doublet is fully accounted for by intermolecular ^{13}C, ^{15}N coupling between two monomer units, derived from this highly enriched cadaverine, which are incorporated into lupinine after dimerization. The observed difference in the doublet/singlet ratio of the signals due to C-6 [doublet/singlet (87 ± 11)/(13 ± 2) = 6.7 ± 1.3] and C-4 [doublet/singlet (36 ± 5)/(64 ± 8) = 0.6 ± 0.1] shows that an intermediate with C_{2v} symmetry, such as (**5**), cannot be implicated and that lupinine biosynthesis cannot occur *via* route A (Scheme 1).

The ^{13}C n.m.r. spectrum (Figure 2) of the sample of sparteine (**6**) mono(hydrogen sulphate) derived from (1-^{13}C,1-^{15}N)-cadaverine, showed that only six of the fifteen C-atoms, those adjacent to nitrogen (C-2,-6,-10,-11,-15,-17) were enriched (*ca.* 50 atom % ^{13}C per C_5 unit) (C_5 unit A: C-2,-3,-4,-5,-6, ^{13}C-enrichment in either C-2 or C-6 but not in both within the same molecule; C_5 unit B: C-17,-7,-8,-9,-10, ^{13}C-enrichment in

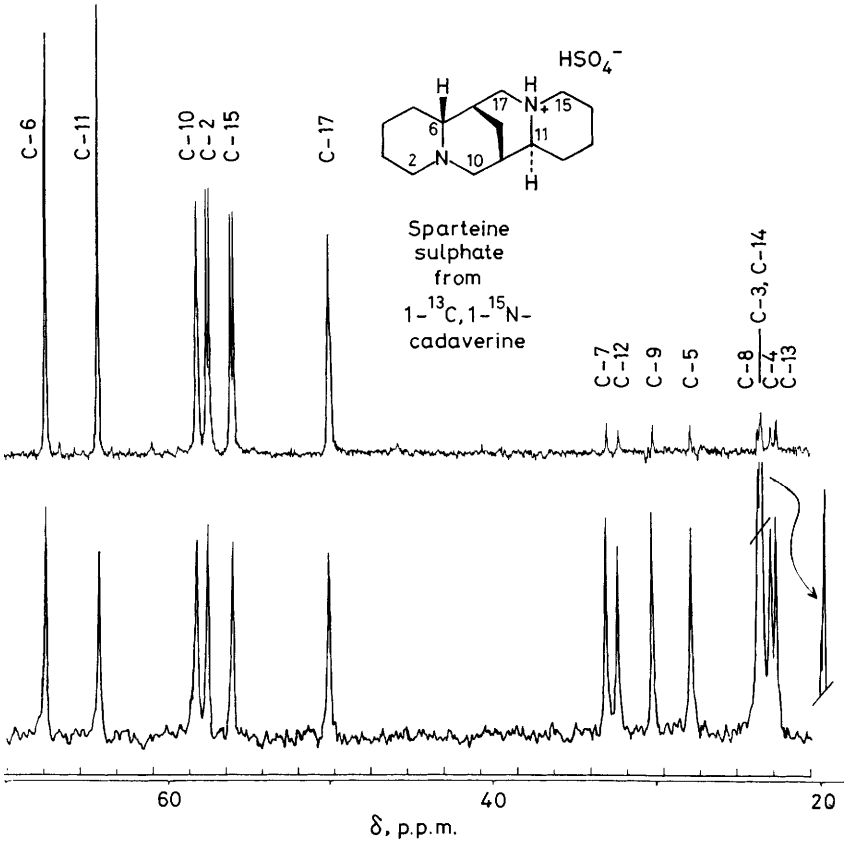


Figure 2. 20.15 MHz ^{13}C N.m.r. spectra of sparteine mono(hydrogen sulphate). Top: P.N.D. spectrum (22000 scans) of ^{13}C, ^{15}N-enriched sample (4.5 mg in 10 μl ^2H_2O) derived from (1-^{13}C, 1-^{15}N)cadaverine. Bottom: P.N.D. spectrum (23720 scans) of natural abundance sample (10 mg in 10 μl ^2H_2O). The spectra were recorded in the Fourier mode on a Bruker WP 80 spectrometer. The natural abundance ^{13}C signal of 1,4-dioxane was employed as internal reference. The signals for enriched carbon atoms in the spectrum of sparteine mono(hydrogen sulphate) were assigned on the basis of deuteriation experiments (C-2, -6, -10, -15, -17) and the off resonance spectrum (C-11). Assignment of the other signals is tentative (*cf.* ref. 18).

either C-10 or C-17 but not in both; C_5 unit C: C-11,-12,-13, -14,-15, ^{13}C-enrichment in either C-11 or C-15 but not in both). This result complements and completes the partial evidence on the distribution within sparteine of label from cadaverine, provided by earlier investigations with radioactive tracers.[15] Two of the six enriched signals, those due to C-6 (δ 67.4 p.p.m.) and C-11 (δ 64.1 p.p.m.), are singlets. Two others, those due to C-10 (δ 58.0 p.p.m.) and C-17 (δ 49.9 p.p.m.) appear as a singlet (^{13}C-10, ^{14}N-1; ^{13}C-17, ^{14}N-16) with a doublet (^{13}C-10, ^{15}N-1; ^{13}C-17, ^{15}N-16) superimposed on it (doublet/singlet ratio 0.7 ± 0.1; 0.7 ± 0.1). As in the case of lupinine, these doublets are fully accounted for by the high efficiency of incorporation of (1-^{13}C, 1-^{15}N)cadaverine. The two signals due to C-2 and C-15 appear as doublets,§ as a result of ^{13}C, ^{15}N coupling [J(^{13}C-2, ^{15}N-1) 3.6 Hz, J(^{13}C-15, ^{15}N-16) 3.8 Hz]. 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 into sparteine from the precursor, and constitutes new evidence in support of the biogenetic hypothesis of the origin of sparteine and related $C_{15}N_2$ lupine alkaloids which we put forward in 1976 on the basis of radioactive tracer investigations.[15]

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§ The corresponding singlets (^{13}C-2, ^{14}N-1; ^{13}C-15, ^{14}N-16) are masked by the doublets. The doublet/singlet ratio (>7) can therefore not be accurately determined.

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