Quinolizidine Alkaloid Biosynthesis: Incorporation of [1-*amino*-^{15}N,1-^{13}C]Cadaverine into Lupinine

Jatinder Rana and David J. Robins*

Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, U.K.

Two [1-*amino*-^{15}N,1-^{13}C]cadaverine (**4**) units are incorporated into lupinine (**5**), but only one ^{13}C–^{15}N doublet is observed in the ^{13}C{^{1}H} n.m.r. spectrum, demonstrating that a later C_5–N–C_5 symmetrical intermediate such as the triamine (**7**) is not involved in lupinine biosynthesis.

Lupinine (**1**) is the simplest bicyclic quinolizidine alkaloid.[1] It is known to be formed from lysine (**2**) *via* cadaverine (**3**). Thus, two molecules of [1,5-$^{14}C_2$]cadaverine are incorporated into lupinine (**1**) with about ¼ of the radioactivity at C-11, and about ½ at C-(4 + 6).[2] This labelling pattern is analogous to that obtained for the pyrrolizidine alkaloid retronecine with [1,4-$^{14}C_2$]putrescine[3] and, in more detail, with ^{13}C-labelled putrescines.[4] The use of [^{13}C–^{15}N] doubly labelled putrescine indicated that retronecine is derived from a later C_4–N–C_4 symmetrical intermediate,[5,6] and this intermediate was shown to be homospermidine (**6**).[5,7] We now present evidence that the analogous *N*-(5-aminopentyl)-1,5-diaminopentane (**7**) is not an intermediate in lupinine biosynthesis.

[1-*amino*-^{15}N,1-^{13}C]Cadaverine (**4**) was prepared by treatment of 1-phthalimido-4-bromobutane with K^{13}C^{15}N followed by catalytic hydrogenation and hydrolysis.[8] This material as the dihydrochloride (60 mg) together with [1,5-$^{14}C_2$]-cadaverine hydrochloride (5 μCi) was pulse fed[3,4] to eight *Lupinus luteus* plants. Lupinine was isolated and purified by column chromatography on basic alumina, 25 mg, m.p. 69.5—70 °C (lit.,[9] 68—69 °C). The 50 MHz ^{13}C{^{1}H} n.m.r. spectrum [10] of labelled lupinine taken in [$^2H_6$]benzene showed four signals with approximately equal enhancement due to the four downfield carbon atoms. The enrichment factors of 1.3, 1.6, 1.4, and 1.5% ^{13}C for these signals at δ 57.3, 57.4, 65.2, and 65.5 p.p.m. respectively, were estimated by comparison of the normalised signal integrals in the ^{13}C{^{1}H} n.m.r. spectrum of lupinine with those of unlabelled material run under the same conditions. The average enrichment factor is 3.0% per C_5 unit of cadaverine, corresponding to a specific ^{13}C incorporation of 3.3% (the observed ^{14}C specific incorporation was 3.9% per C_5 unit).

The ^{13}C n.m.r. assignment for the four downfield carbons was made as follows. The signals at δ 65.2 and 65.5 p.p.m. are assigned to C-10 and C-11 respectively on the basis of a distortionless enhancement by polarisation transfer experiment. The resolution enhanced ^{13}C{^{1}H} n.m.r. spectrum of labelled lupinine (Figure 1) contains a doublet at δ 57.3 p.p.m. (*J* 3.2 Hz) due to ^{13}C–^{15}N species and flanks a signal at natural abundance intensity. The assignment of this signal to C-6 follows from consideration of the ^{13}C–^{13}C couplings visible in the spectrum due to the presence of some lupinine molecules formed by incorporation of two cadaverine (**4**) units into the same molecule of lupinine. The intensity of the doublet at the bridgehead carbon, C-10 (δ 65.2 p.p.m.), is approximately double that of C-11. This indicates that C-10 experiences coupling to both C-11 and the carbon at δ 57.4 p.p.m., which is therefore identified as C-4, since it is impossible for a molecule of lupinine to be labelled with ^{13}C at both C-6 and C-10 [see structures (**5**)].

The crucial observation of a signal due to C-6 at natural abundance intensity (δ 57.3 p.p.m.) flanked by a doublet arising from ^{13}C–^{15}N coupling shows that there is no detectable breakdown of the C–N bond in the cadaverine molecule forming the C-6,N-5 bond in lupinine (**5**). This result, together with the lack of a similar doublet due to ^{13}C–^{15}N coupling associated with C-4, indicate that no later symmetrical intermediate of the type C_5–N–C_5 is involved in lupinine biosynthesis. To examine this point further, *N*-(5-aminopentyl)-1,5-diaminopentane (**7**)[11] labelled with ^{14}C in the terminal carbons was prepared by reaction of benzylamine with two equivalents of [1-^{14}C]-5-chloropentanenitrile followed by catalytic reduction.[12] The triamine (15 μCi) was pulse fed to nine *L. luteus* plants over 10 days. After a further 10 days, lupinine was extracted and recrystallised to a constant

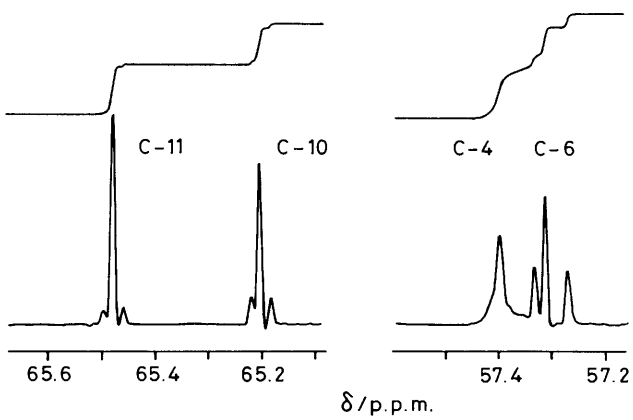
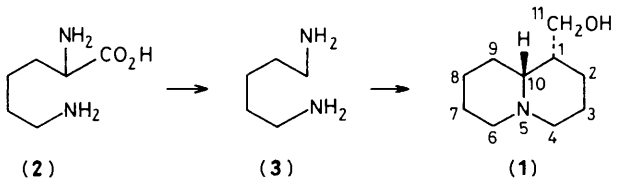


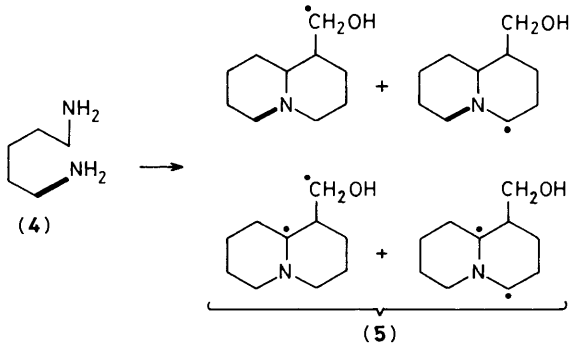
Figure 1. 50.32 MHz ^{13}C{^{1}H} n.m.r. spectrum of lupinine (**5**) (24 mg) in [$^2H_6$]benzene derived from [1-*amino*-^{15}N,1-^{13}C]cadaverine (**4**) dihydrochloride.

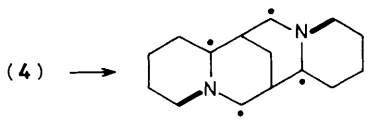


$H_2N[CH_2]_nNH[CH_2]_nNH_2$

(**6**) *n* = 4

(**7**) *n* = 5





(8)

specific ^{14}C incorporation of 0.04%, suggesting that the triamine (**7**) is unlikely to be an intermediate in the biosynthesis of lupinine. Furthermore, an intermediate trapping experiment was carried out. (2*S*)-[U-^{14}C]Lysine (25 μCi) was fed to one *L. luteus* plant. After 24 h, the plant was macerated in 0.4 M aqueous trichloroacetic acid containing inactive triamine (**7**) trihydrochloride (50 mg). The *N*-phenylaminothiocarbonyl derivative was formed with isothiocyanatobenzene, and extracted and purified as described for other polyamines.[13] Recrystallisation to constant activity gave material containing less than 0.03% of the original radioactivity, indicating that the triamine (**7**) is not a normal intermediate in lupinine biosynthesis.

We recently showed[8] that three [^{13}C–^{15}N]cadaverine (**4**) units are incorporated into the tetracyclic quinolizidine alkaloid sparteine. The presence of two ^{13}C–^{15}N doublets in the $^{13}C\{^1H\}$ n.m.r. spectrum of sparteine demonstrated that two of these units are transformed into the outer rings of sparteine in a specific fashion (**8**). The pattern of incorporation of cadaverine (**4**) into sparteine (**8**) and lupinine (**5**), and the absence of the intermediate (**7**) in the biosynthesis of lupinine, is consistent with work reported on the conversion of cadaverine into 17-oxosparteine by enzyme preparations from cell suspension cultures of *L. polyphyllus*.[14] During this enzymic conversion, no intermediates were detected, and so a series of enzyme-linked intermediates was proposed. Lupinine (or a close precursor) is presumably liberated from the enzyme complex prior to addition of the third cadaverine unit required for the formation of sparteine.

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