Application of ^{13}C N.M.R. Spectroscopy to Study the Biosynthesis of the Quinolizidine Alkaloids Lupinine and Sparteine[1]

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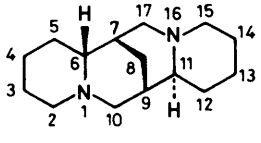
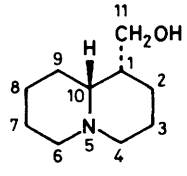
The labelling patterns in (−)-sparteine (**14**) and (−)-lupinine (**15**) derived biosynthetically from [1-*amino*-^{15}N,1-^{13}C]cadaverine dihydrochloride (**13**) have been established by ^{13}C n.m.r. spectroscopy. Three units of (**13**) are incorporated to about the same extent into sparteine, and two ^{13}C-^{15}N doublets are observed in the $^{13}C\{^1H\}$ n.m.r. spectrum of sparteine, demonstrating that two of these cadaverine units are converted into the outer rings of sparteine in a specific fashion. Two cadaverine (**13**) units are incorporated into lupinine (**15**) and one ^{13}C-^{15}N doublet is observed. These results, and ^{14}C-labelling experiments with 1,7,13-triazatridecane (**10**) indicate that a later C_5–N–C_5 intermediate with C_{2v} symmetry, such as (**10**), is not involved in lupinine or sparteine biosynthesis.

Quinolizidine alkaloids are present in many species of the plant family Leguminosae.[2] One type of quinolizidine alkaloid is exemplified by (−)-sparteine (**1**). Experiments with radio-

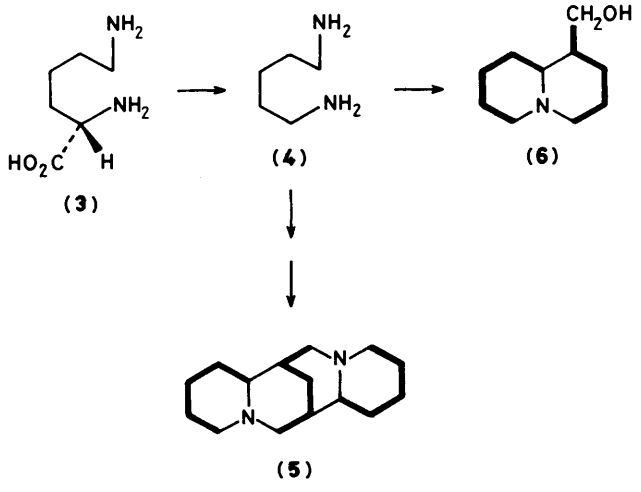


(**1**)



(**2**)

labelled precursors have shown that this common C_{15} tetracyclic alkaloid is derived biosynthetically from L-lysine (**3**) *via*

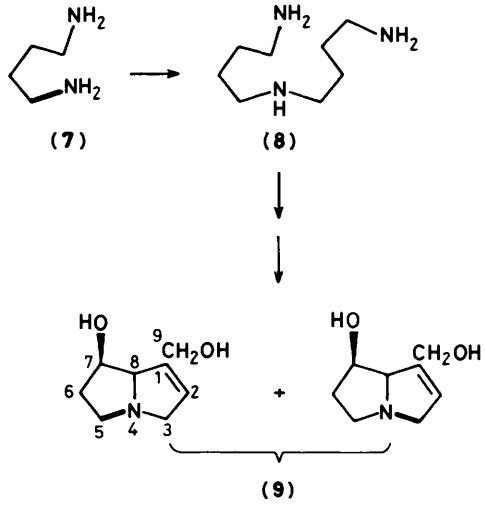


Scheme 1.

cadaverine (**4**) (Scheme 1). Thus, Schütte *et al.* demonstrated that [1,5-$^{14}C_2$]cadaverine is incorporated into sparteine.[3] Partial degradation of the labelled sparteine showed that a sixth of the radioactivity was at each of the three positions C-2, C-15, and C-17. The rest of the radioactivity was assumed to be equally spread between C-6, C-10, and C-11, leading to the conclusion that three molecules of cadaverine are required to form sparteine, as indicated in structure (**5**).

Lupinine (**2**) represents a different type of quinolizidine alkaloid. The biosynthesis of lupinine is also known to proceed from L-lysine *via* cadaverine (Scheme 1) from the results of experiments with ^{14}C-labelled precursors. Partial degradation of lupinine labelled after feeding [1,5-$^{14}C_2$]cadaverine established that about a quarter of the radioactivity was at the exocyclic methylene C-11, and about a half of the activity was at two of the carbons (C-4 and C-6) adjacent to nitrogen.[4] This activity was assumed to be equally spread between these two methylene carbons, and the remainder of the radioactivity was assumed to be at the third carbon adjacent to nitrogen (C-10). The proposed mode of incorporation of the two cadaverine units, shown in structure (**6**), has been confirmed by ^{13}C n.m.r. spectroscopy on lupinine after feeding DL-[4,5-$^{13}C_2$]lysine to *Lupinus luteus* plants.[5]

The labelling pattern obtained for lupinine appears to be analogous to that determined for the pyrrolizidine base (+)-retronecine (**9**) using ^{14}C-labelled precursors.[6] Complete



(**9**)

labelling patterns in retronecine were also established by ^{13}C n.m.r. spectroscopy after carrying out feeding experiments with ^{13}C-labelled precursors.[7] For example, incorporation of [1-^{13}C]putrescine into retronecine produced a sample of the base which was equally enriched with ^{13}C at four positions, C-3, C-5, C-8, and C-9. The observation of this labelling pattern supports the theory that retronecine is derived from two molecules of putrescine, and is consistent with the formation of a later intermediate in the biosynthetic pathway with C_{2v} symmetry.

Evidence for the existence of this intermediate was obtained by using a [^{13}C-^{15}N] doubly labelled putrescine (**7**) in feeding experiments.[8,9] When this material was fed to *Senecio isatideus* plants, a sample of retronecine was produced which showed two ^{13}C-^{15}N doublets of equal intensity associated with C-3 and C-5 of retronecine in the $^{13}C\{^1H\}$ n.m.r. spectrum. The presence of equal amounts of the two labelled species (**9**) is evidence for a later C_4–N–C_4 intermediate with C_{2v} symmetry in the biosynthetic pathway. This intermediate was shown to be 1,6,11-triazaundecane (homospermidine) (**8**) by carrying out feeding experiments with homospermidine labelled with ^{14}C[10] and with ^{13}C.[11] The present paper presents evidence that the corresponding triamine (**10**) with C_{2v} symmetry is not an intermediate

$$RNH(CH_2)_5NR(CH_2)_5NHR$$

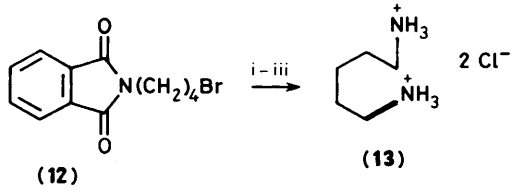
(**10**) R = H

(**11**) R = CSNHPh

in lupinine or sparteine biosynthesis.

Results and Discussion

The doubly labelled precursor, [1-*amino*-^{15}N,1-^{13}C]cadaverine dihydrochloride (**13**) was prepared in 34% overall yield by



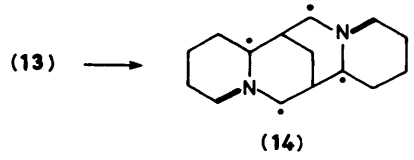
(**12**) (**13**)

Scheme 2. *Reagents:* i, $K^{13}C^{15}N$; ii, H_2–PtO_2–AcOH; iii, HCl.

treatment of 4-bromo-1-phthalimidobutane (**12**) with $K^{13}C^{15}N$ followed by catalytic hydrogenation and hydrolysis (Scheme 2).[12] Pulsed feeding of this ^{13}C–^{15}N doubly labelled precursor (**13**) together with [1,5-$^{14}C_2$]cadaverine dihydrochloride was carried out by introduction of a sterile aqueous solution of the precursor mixture into the xylems of eight *Lupinus luteus* plants through stem punctures during one week. Two weeks after the feeding was completed, the plants were harvested, and the alkaloid mixture was isolated. Purification by column chromatography gave (−)-lupinine (**2**) and (−)-sparteine (**1**).

The ^{13}C n.m.r. spectrum of sparteine was first assigned by Bohlmann and Zeisberg,[13] with the aid of specifically deuteriated samples of sparteine, and by comparisons with ^{13}C n.m.r. spectra of related compounds. These assignments were largely confirmed by us, and by Shaka and Freeman who used a new technique of selective excitation of proton spectra by polarisation transfer from an adjacent carbon site.[14] (The pairs of assignments for C-4/C-13 and for C-7/C-9 have been reversed.)

Comparison of the 50 MHz $^{13}C\{^1H\}$ n.m.r. spectrum of ^{13}C-labelled sparteine with that of unlabelled material run under the same conditions showed approximately equal enhancements of six of the signals due to C-2, C-6, C-10, C-11, C-15, and C-17, with enrichment factors* of 1.6, 1.7, 1.7, 1.9, 1.9, and 1.7% ^{13}C, respectively (all values ±0.1%). The average enrichment factor is therefore 1.75% ^{13}C for each labelled site, which corresponds to a specific ^{13}C incorporation per C_5 unit of sparteine of (1.75 × 2/90.6) × 100% = 3.9% (where 90.6/2 atom % ^{13}C is the average enrichment at each labelled position of cadaverine). This value compares well with the observed ^{14}C specific incorporation† of 3.8% per C_5 unit of cadaverine. The approximately equal levels of enhancement of ^{13}C at each of the six labelled positions in sparteine support the theory that it is formed from three units of cadaverine [as in (**5**)]. Moreover, the resolution enhanced $^{13}C\{^1H\}$ n.m.r. spectrum of sparteine (Figure 1) showed the presence of doublets at δ 56.5 (*J* 3.7 Hz) and 55.7 (*J* 3.4 Hz). The different values observed for these two coupling constants supports the conclusion that ^{13}C–^{15}N species are present associated with C-2 and C-15 in the sparteine sample. These doublet signals flanked signals for C-2 and C-15 at natural abundance intensity. The lack of enhancement of these central signals demonstrates that there has been no detectable breakdown of the ^{13}C-^{15}N bonds in the cadaverine (**13**) molecules forming the C(2)–N(1) and C(15)– N(16) bonds



(**14**)

Scheme 3.

of sparteine. Two of the cadaverine (**13**) units are therefore transformed into the outer rings of sparteine in a specific fashion as shown in (**14**) (Scheme 3).

It should be pointed out that further couplings are visible in the $^{13}C\{^1H\}$ n.m.r. spectrum of sparteine, probably as a consequence of the technique of pulsed feeding. The chances of the combination of two molecules of the labelled cadaverine (**13**) are increased by feeding relatively large quantities of labelled material to the plants over a short period. The resulting dilution by endogenous unlabelled cadaverine is likely to be quite small. Thus the signals for C-6 and C-11 each display geminal coupling to both C-10 and C-17. The signal for C-10 shows further coupling to ^{15}N-1 and C-2, and C-17 is coupled to ^{15}N-16 and C-15. From consideration of the labelling pattern shown in (**14**) it follows that no later C_5–N–C_5 intermediate with C_{2v} symmetry can be involved in the biosynthetic pathway to sparteine.

In the ^{13}C n.m.r. spectrum of lupinine (**2**) taken in deuteriochloroform the signals for two of the key carbon atoms, C-4 and C-6, have the same chemical shift of 57.2 p.p.m.[13] We found that these signals could be resolved by taking the spectrum in deuteriobenzene, but the assignments for these two signals required interpretation of the resolution enhanced $^{13}C\{^1H\}$ n.m.r. spectrum of the ^{13}C-labelled lupinine (*vide infra*). The signals at δ 65.2 and 65.5 were assigned to C-10 and C-11 of lupinine, respectively, after carrying out a distortionless enhancement by polarisation transfer experiment.

The ^{13}C n.m.r. spectrum of ^{13}C-labelled lupinine showed that the four downfield carbon atoms (C-4, C-6, C-10, and C-11) are enriched with ^{13}C to approximately the same extent, and all the other carbons are at natural abundance intensity. Enrichment factors of 1.3, 1.6, 1.4, and 1.5% ^{13}C (all values ±0.1%) were estimated for the signals at δ 57.3, 57.2, 65.2, and 65.5, respectively. The average enrichment factor for each labelled site is therefore 1.5% ^{13}C. This corresponds to a specific ^{13}C

* The enrichment factor for a specific site in lupinine or sparteine is the excess of ^{13}C label above natural abundance and is calculated from [integral of the labelled site − natural abundance integral/(natural abundance integral)] × 1.1%.

† The % specific ^{14}C incorporation of cadaverine per C_5 unit is calculated as [(molar activity of alkaloid × ⅓ for sparteine or × ½ for lupinine)/(molar activity of cadaverine)] × 100%.

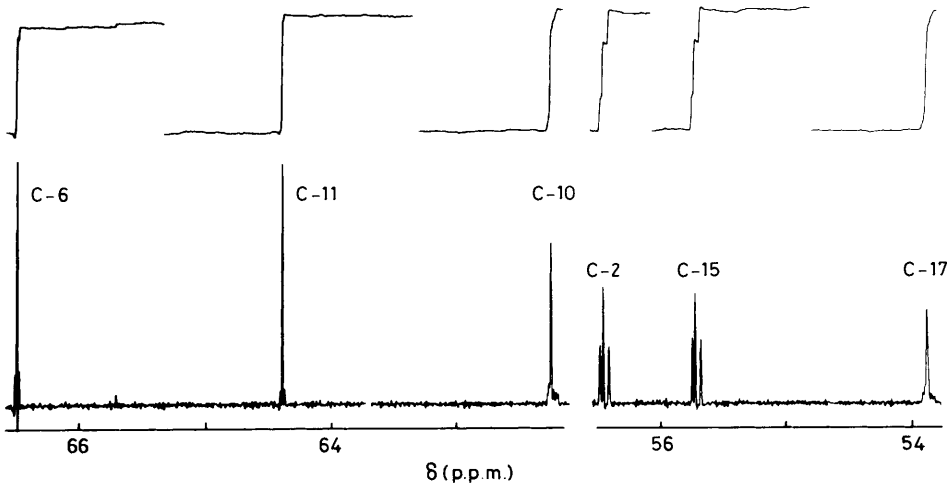


Figure 1. 50.32 MHz $^{13}C\{^1H\}$ N.m.r. spectrum of sparteine (**14**) (15 mg) in [$^2H_6$]benzene derived from [1-*amino*-^{15}N,1-^{13}C]cadaverine dihydrochloride (**13**).

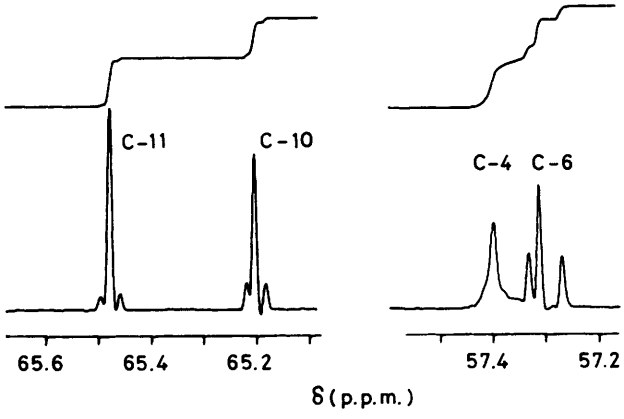
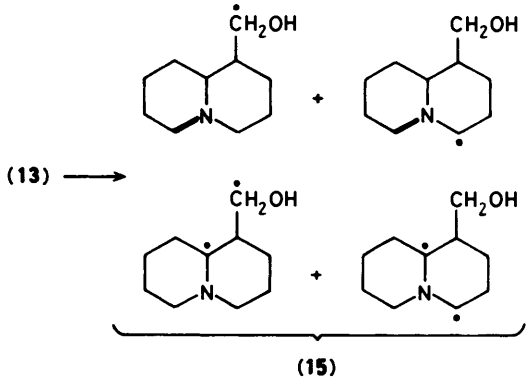


Figure 2. 50.32 MHz $^{13}C\{^1H\}$ N.m.r. spectrum of lupinine (**15**) (24 mg) in [$^2H_6$]benzene derived from [1-*amino*-^{15}N,1-^{13}C]cadaverine dihydrochloride (**13**).



Scheme 4.

incorporation per C_5 unit of lupinine of (1.5 × 2/90.6) × 100% = 3.3%, which is in fair agreement with the observed ^{14}C specific incorporation of 3.9% per C_5 unit.

The approximately equal levels of enhancement of ^{13}C at the four labelled carbon atoms in lupinine are in accord with the theory that it is formed from two molecules of cadaverine [as in (**6**)]. Part of the resolution enhanced $^{13}C\{^1H\}$ n.m.r. spectrum of lupinine is shown in Figure 2. The doublet at δ 57.3 with J 3.2 Hz is ascribed to ^{13}C–^{15}N coupling because no other doublet with the same intensity or coupling constant is visible in the spectrum. It is important to note that this doublet flanks a signal at natural abundance intensity. The other couplings visible in this part of the $^{13}C\{^1H\}$ n.m.r. spectrum of lupinine arise as a consequence of the incorporation of two cadaverine (**13**) units into the same molecule of lupinine, and can again be ascribed to the technique of pulsed feeding. These couplings facilitate the assignment of C-4 and C-6 of lupinine. The intensity of the doublet associated with the bridgehead carbon, C-10, at δ 65.2, is approximately double that of the doublet around the signal for C-11 at δ 65.5 This indicates that C-10 is coupling to both C-11 and the carbon with δ 57.4. This latter carbon is therefore identified as C-4, because it is impossible for any molecules of lupinine to be labelled with ^{13}C at both C-6 and C-10 [see structures (**15**) for the species formed from the combination of two labelled cadaverine (**13**) units (Scheme 4)]. The ^{13}C–^{15}N doublet at δ 57.3 is therefore associated with a signal for C-6 of lupinine at natural abundance intensity. The lack of enhancement of this central signal for C-6 indicates that there is no detectable breakdown of the ^{13}C–^{15}N bond in cadaverine forming the C(6)–N(5) bond in lupinine. One of the cadaverine (**13**) units is thus transformed in a specific fashion into the N(5), C(6) to C(10) portion of lupinine (**15**). This labelling pattern demonstrates that no later C_5–N–C_5 intermediate with C_{2v} symmetry is involved in lupinine biosynthesis. Similar results have been obtained by Golebiewski and Spenser for the incorporation of [^{13}C-^{15}N]cadaverine (**13**) into lupinine and sparteine.[15] Doubly-labelled cadaverine (**13**) has also been shown to be incorporated into the outer rings of other tetracyclic quinolizidine alkaloids in the same specific fashion.[12,16]

The results reported here for lupinine are quite different from those obtained for the pyrrolizidine base retronecine (**9**), where homospermidine (**8**) has been identified as an intermediate in the biosynthetic pathway. The corresponding triamine (**10**), labelled with ^{14}C, has been shown to be poorly incorporated into lupinine (0.03%) and sparteine (0.01%),[17] but we felt that the status of (**10**) as an intermediate in quinolizidine alkaloid biosynthesis should be assessed further. Accordingly, the triamine (**10**) was prepared with ^{14}C labels in the terminal carbons. This was achieved by reaction of benzylamine with two equivalents of 5-chloro-[1-^{14}C]pentanenitrile followed by catalytic reduction.[18] The labelled triamine, as its tri-

hydrochloride, was pulse fed to nine *Lupinus luteus* plants as usual, and lupinine (**2**) and sparteine (**1**) were isolated and separated. Lupinine was recrystallised to a constant specific ^{14}C incorporation of 0.04%, and the incorporation into sparteine (purified as the picrate) was less than 0.02%, indicating that the triamine (**10**) is unlikely to be an intermediate in quinolizidine alkaloid biosynthesis.

Finally, an intermediate trapping experiment was carried out. (2*S*)-[U-^{14}C]Lysine was fed to a single *Lupinus luteus* plant. After one day, the plant was blended in aqueous trichloracetic acid, and the extract was divided into three equal portions. Inactive cadaverine (**4**) dihydrochloride was added to one portion; no addition was made to the second; and inactive triamine (**10**) trihydrochloride was added to the third portion. The *N*-phenylaminothiocarbonyl derivatives were then prepared by adding isothiocyanatobenzene to each portion as described for other polyamines by Golding and Nassereddin.[19] The derivatives were extracted and the total ^{14}C incorporation into each of the three extracts was *ca.* 3%. The *N*-phenylaminothiocarbonyl derivative of cadaverine was recrystallised to constant specific activity, corresponding to a total incorporation of *ca.* 2%. The major radioactive component of the second portion was also the *N*-phenylaminothiocarbonyl derivative of cadaverine. Recrystallisation of the derivative (**11**) of the triamine produced material with very low specific activity (total ^{14}C incorporation was less than 0.03%). These results confirm that the triamine (**10**) is unlikely to be an intermediate in the biosynthesis of lupinine or sparteine.

The biosynthetic pathways to the various types of quinolizidine alkaloids from cadaverine are still poorly understood. The conversion of cadaverine into 17-oxosparteine by crude enzyme preparations from cell suspension cultures of *Lupinus polyphyllus* has been demonstrated by Wink *et al.*[20] These workers therefore proposed that 17-oxosparteine is a key intermediate in quinolizidine alkaloid biosynthesis, and that other quinolizidine alkaloids are derived from 17-oxosparteine. Recently, (1*R*)-[1-$^{2}H_1$]cadaverine was fed to several *Lupinus* species, and a number of ^{2}H-labelled quinolizidine alkaloids were isolated. Each tetracyclic alkaloid was shown to contain ^{2}H at C-17, proving that 17-oxosparteine cannot be an intermediate in quinolizidine alkaloid biosynthesis.[16,21]

Experimental

M.p.s were measured with a Kofler hot-stage apparatus. Organic solutions were dried with anhydrous Na_2SO_4, and solvents were evaporated off under reduced pressure below 40 °C. ^{13}C N.m.r. spectra were obtained on a Bruker WP-200SY spectrometer operating at 50.32 MHz. Mass spectra were obtained with A.E.I. MS 12 or MS 902 spectrometers. All ^{13}C-labelled compounds were purchased from B.O.C. Prochem Ltd., London, and radiochemicals were obtained from the New England Nuclear Corporation or Amersham International. Radioactivity was measured with a Philips liquid scintillation analyser using toluene–methanol solutions. Sufficient counts were accumulated to give a standard error of less than 1% for each determination. Radioactive samples were counted in duplicate. A Panax thin-layer scanner RTLS-1A was used for the radioscanning of t.l.c. plates. T.l.c. of the alkaloids was carried out on silica gel G plates of 0.25 mm thickness developed with chloroform–methanol–conc. ammonia (85:14:1), and the alkaloids were visualised with the modified Dragendorff reagent.[22]

[1-*amino*-^{15}N,1-^{13}C]*Cadaverine Dihydrochloride* (**13**).—This was prepared as described.[12]

[2,12-$^{14}C_2$]-1,7,13-*Triazatridecane Trihydrochloride*.—A solution of K^{14}CN (1 mCi, 56.28 mCi mmol^{-1}) and unlabelled KCN (500 mg, 8 mmol) in aqueous ethanol (2 ml) was added to 1-bromo-4-chlorobutane (2.75 g, 16 mmol) in ethanol (10 ml). The mixture was heated at reflux for 4 h. The cooled solution was concentrated and the residue was taken up in water (10 ml) and extracted with chloroform (4 × 10 ml). The chloroform extracts were dried, filtered, and concentrated to an oil. Column chromatography on silica gel developed in chloroform–light petroleum (b.p. 40—60 °C) (7:3) gave 5-chloro-[1-^{14}C]pentanenitrile (0.6 g, 46 μCi mmol^{-1}). Treatment of this material (2 equiv.) with benzylamine followed by catalytic hydrogenation as described by Bergeron *et al.*[18] gave [2,12-$^{14}C_2$]-1,7,13-triazatridecane, which was isolated as the trihydrochloride and recrystallised to constant specific activity from aqueous ethanol (39% yield); 88.5 μCi mmol^{-1}; m.p. 288—290 °C (decomp.) [lit.,[23] m.p. 290 °C (decomp.)]; undepressed mixed m.p. with an authentic unlabelled sample. Radioscans of a t.l.c. cellulose-coated plate developed in propan-2-ol–conc. ammonia (7:3) showed one radioactive band coincident with authentic unlabelled 1,7,13-triazatridecane trihydrochloride.

1,7,13-*Tris*[*phenylamino*(*thiocarbonyl*)]-1,7,13-*triazatridecane* (**11**).—This was prepared from 1,7,13-triazatridecane (**10**) trihydrochloride by treatment with isothiocyanatobenzene in aqueous ethanol using the method of Golding and Nassereddin.[19] The *derivative* had m.p. 197—199 °C (EtOH); R_F 0.38 [CH_2Cl_2–MeCN, (9:1)]; $\nu_{max.}$(KBr) 3 400, 1 600, 1 500, and 1 450 cm^{-1}; δ{[$^{2}H_6$]Me_2SO} 1.4—1.9 (12 H, complex), 3.5—3.8 (8 H, complex), 7.1—7.3 (15 H, m), 7.5—7.7 (2 H, m, NH), and 9.0—9.4 (3 H, NH) (Found: C, 63.0; H, 6.8; N, 14.4; S, 16.0. $C_{31}H_{40}N_6S_3$ requires C, 62.80; H, 6.80; N, 14.18; S, 16.22%).

The corresponding derivative of cadaverine was also prepared for reference purposes: 1,5-*bis*[*phenylamino*(*thiocarbonylamino*)]*pentane* had m.p. 148—150 °C; R_F 0.40 [CH_2Cl_2–MeCN, (9:1)]; $\nu_{max.}$(KBr) 3 240, 1 600, 1 540, 1 500, and 1 450 cm^{-1}; δ{[$^{2}H_6$]Me_2SO} 1.3—1.7 (6 H, complex), 3.4—3.7 (4 H, complex), 7.1—7.6 (10 H, complex), 7.65 (2 H, m, NH), and 9.3 (2 H, br s, NH) (Found: C, 61.5; H, 6.3; N, 14.9; S, 16.9. $C_{19}H_{24}N_4S_2$ requires C, 61.25; H, 6.49; N, 15.04; S, 17.21%).

Feeding Experiment with [1-amino-^{15}N,1-^{13}C]*Cadaverine Dihydrochloride* (**13**).—Seeds of *Lupinus luteus* were germinated and the plants were grown in a standard compost. Eight plants were used in this experiment. [1,5-$^{14}C_2$]Cadaverine dihydrochloride (5 μCi) was added to [1-*amino*-^{15}N,1-^{13}C]cadaverine dihydrochloride (**13**) (60 mg) to give an initial ^{14}C specific radioactivity of 14.7 μCi mmol^{-1}. The precursor mixture was dissolved in sterile water, and drops of the solution were introduced into the xylems of the eight plants through stem punctures made with a sterile needle.[6] The precursor mixture was pulse fed on alternate days for a period of one week. Two weeks after administration of the precursor mixture had ceased, the plants were removed from the compost, and the roots were washed well with water. The plant material was macerated repeatedly with methanol in a Waring blender until the filtered methanolic extracts were colourless. These extracts were concentrated, and the residue was dissolved in 1M-H_2SO_4 (50 ml). The acidic solution was washed with dichloromethane (5 × 50 ml), and it was then stirred with zinc dust for 1 h to reduce any *N*-oxides present. The mixture was filtered through Celite, and the filtrate was basified with conc. ammonia and extracted with dichloromethane (4 × 50 ml). The aqueous layer was basified more strongly with saturated NaOH (to pH > 12) and extracted with further dichloromethane (4 × 50 ml). The combined organic extracts were dried, filtered, and concentrated to afford the alkaloid mixture (120 mg). This mixture was separated by column chromatography on basic alumina and elution with dichloromethane.

(—)-*Lupinine* (**2**) was recrystallised to constant specific radioactivity, 26 mg, 1.15 μCi mmol^{-1}, m.p. 69.5—70 °C (lit.,[24] 68—69 °C); $[\alpha]_D^{18}$ −20.1° (*c* 1.5, in EtOH) (lit.,[24] $[\alpha]_D^{28}$ −20.9°); δ_C {[$^2H_6$]benzene} 23.0 (C-3), 25.1 (C-8), 25.9 (C-7), 29.9 (C-9), 31.1 (C-2), 39.3 (C-1), 57.3 (C-6), 57.4 (C-4), 65.0 (C-10), and 65.2 (C-11). Radioscans of t.l.c. plates showed one radioactive band, coincident with authentic unlabelled (−)-lupinine at R_F 0.56.

(−)-*Sparteine* (**1**) was obtained as an oil, 25 mg, 1.66 μCi mmol^{-1}, $[\alpha]_D^{18}$ −16° (*c* 1.2, in EtOH) (lit.,[25] −17°); δ_C {[$^2H_6$]benzene} 25.1 (C-4), 25.4 (C-13), 26.2 (C-3), 26.4 (C-14), 28.0 (C-8), 29.5 (C-5), 33.7 (C-7), 35.0 (C-12), 36.9 (C-9), 53.9 (C-17), 55.7 (C-15), 56.5 (C-2), 62.2 (C-10), 64.4 (C-11), and 66.5 (C-6). Radioscans of t.l.c. plates showed one radioactive band, coincident with authentic unlabelled (−)-sparteine at R_F 0.35.

Feeding Experiment with [2,12-$^{14}C_2$]-1,7,13-*Triazatridecane Trihydrochloride*.—A sterile aqueous solution of [2,12-$^{14}C_2$]-1,7,13-triazatridecane trihydrochloride (50 mg, 15 μCi) was pulse fed in small doses to nine *Lupinus luteus* plants over a ten day period. Ten days later the plants were harvested and lupinine (**2**) and sparteine (**1**) were isolated as described above. Lupinine was recrystallised to a constant specific radioactivity of 0.035 μCi mmol^{-1}. Sparteine was converted into the picrate, m.p. 205—206 °C (lit.,[26] m.p. 208 °C), and recrystallised (methanol) to a constant specific radioactivity of 0.024 μCi mmol^{-1}.

Intermediate Trapping Experiment.—A solution of L-[U-^{14}C]lysine (25 μCi) was fed to one *Lupinus luteus* plant in flower. After 24 h, the plant material was blended in 0.4M-trichloroacetic acid (3 × 75 ml). Each extract was also sonicated for 20 min. The aqueous extracts were filtered through Celite, washed with ether (2 × 200 ml), and divided into three equal portions (*a*)—(*c*). Inlabelled cadaverine dihydrochloride (70 mg) was added to (*a*); no addition was made to (*b*); and unlabelled 1,7,13-triazatridecane (**10**) trihydrochloride (50 mg) was added to (*c*). Each aqueous solution was basified to pH 9 with KOH. A solution of isothiocyanatobenzene (0.5 ml) in ethanol (5 ml) was added to each solution. The solutions were then stirred at room temperature for 4 days. Each aqueous solution was extracted with dichloromethane (3 × 100 ml), and the organic extracts were dried, filtered, and concentrated to afford white solids: (*a*) 81 mg, 0.8 μCi (3.2%); (*b*) 20 mg, 0.65 μCi (2.6%); (*c*) 62 mg, 0.79 μCi (3.18%). Radioscans of sample (*b*) on a t.l.c. plate developed in CH_2Cl_2–MeCN (9:1) showed one major radioactive band at R_F 0.40 corresponding to the *N*-phenylaminothiocarbonyl derivative of cadaverine. Samples (*a*) and (*c*) were recrystallised (EtOH) to constant specific radioactivity: (*a*) 2.6 μCi mmol^{-1}; (*c*) 0.02 μCi mmol^{-1}.

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