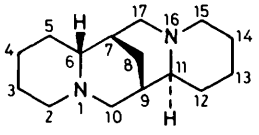
Incorporation of [1,2-$^{13}C_2$]Cadaverine and the Enantiomeric [1-^{2}H]Cadaverines into the Quinolizidine Alkaloids in *Baptisia australis* [1]

Alison M. Fraser, David J. Robins,* and Gary N. Sheldrake
Department of Chemistry, University of Glasgow, Glasgow G12 8QQ

The mode of incorporation of [1,2-$^{13}C_2$]cadaverine (**2**) dihydrochloride into (−)-*N*-methylcytisine (**14**) in *Baptisia australis* was established by ^{13}C n.m.r. spectroscopy. The tricyclic alkaloid (−)-*N*-methylcytisine displayed a labelling pattern (**16**) which was consistent with degradation of a tetracyclic intermediate. The (*R*)-[1-^{2}H]- (**11**) and (*S*)-[1-^{2}H]-cadaverine (**12**) dihydrochlorides were prepared and fed to *Baptisia australis*, and labelling patterns in the alkaloids were determined by ^{2}H n.m.r. spectroscopy. After feeding the (*R*)-precursor (**11**), ^{2}H was retained in (+)-sparteine (**13**) at C-2α, -6α, -11β, -15α, and -17β, whereas C-2β, -10β, and -15β were labelled from the (*S*)-precursor (**12**). The presence of ^{2}H at C-17β in (+)-sparteine after feeding the (R)-isomer (**11**) shows that 17-oxosparteine [enantiomer of (**10**)] cannot be an intermediate in the formation of (+)-sparteine. With (−)-*N*-methylcytisine (**14**), ^{2}H was retained after feeding the (*R*)-isomer (**11**) at C-10β and C-11β; the (*S*)-isomer (**12**) labelled C-13β. Comparison of these labelling patterns (**19**) with those of (+)-sparteine (**17**) establishes which outer ring of a tetracyclic intermediate is cleaved and which is converted into a pyridone during the formation of (−)-*N*-methylcytisine (**14**).

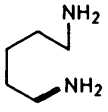
Quinolizidine alkaloids such as (−)-sparteine (**1**) are accumulated in many species of the plant family Leguminosae.[2] The biosynthesis of these alkaloids is known to take place from



(**1**)

L-lysine *via* cadaverine. Complete labelling patterns were observed by ^{13}C n.m.r. spectroscopy in (−)-lupinine (**3**), (−)-sparteine (**4**), (+)-lupanine (**5**), (+)-13-hydroxylupanine (**6**), and (+)-angustifoline (**7**) after synthesizing and feeding [1,2-$^{13}C_2$]cadaverine (**2**) dihydrochloride to two *Lupinus* species.[3] Two units of cadaverine are required to form lupinine and three cadaverine units combine to form the tetracyclic alkaloids. The labelling pattern determined for (+)-angustifoline (**7**) is consistent with formation and cleavage of a tetracyclic intermediate to produce the allyl group without rearrangement (Scheme 1).

Furthermore, [1-*amino*-^{15}N, 1-^{13}C]cadaverine (**8**) dihydrochloride was fed to the same two *Lupinus* species, and the labelling patterns of the quinolizidine alkaloids were determined by ^{13}C n.m.r. spectroscopy. This experiment demonstrated which C–N bonds remain intact in the biosynthesis of the alkaloids (**3**)—(**7**). Thus, in lupinine (**3**), the C-6/N-5 bond is retained,[4,5] while in the tetracyclic alkaloids (**4**)—(**6**), the N-1/C-2 and C-15/N-16 bonds in the outer rings remain intact.[5–7] A similar situation holds true for angustifoline except that the C-15/N-16 bond has undergone cleavage.[7]



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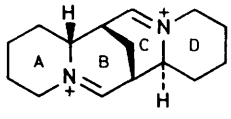

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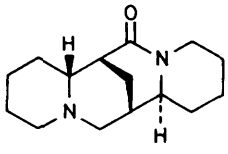

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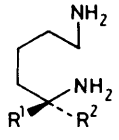
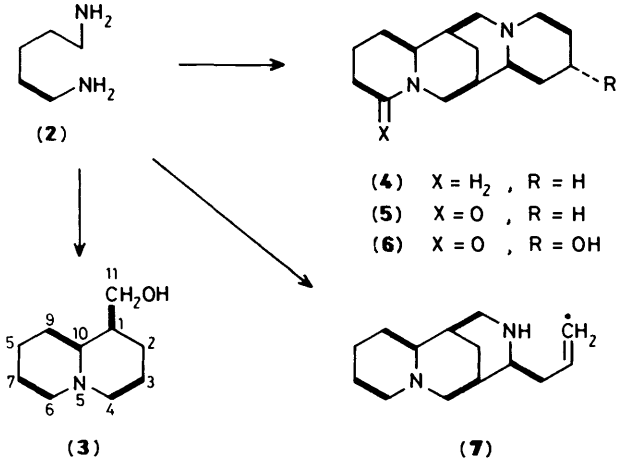


(**11**) R^1 = H, R^2 = ^{2}H
(**12**) R^1 = ^{2}H, R^2 = H



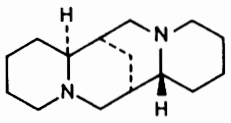
(**2**) (**4**) X = H_2, R = H; (**5**) X = O, R = H; (**6**) X = O, R = OH; (**3**); (**7**)

Scheme 1.

From the results of these feeding experiments, it seems likely that (−)-sparteine (**1**) and (+)-angustifoline (**7**) are formed from a common tetracyclic intermediate such as (**9**) by cleavage of ring D. However, the first evidence for the identity of this intermediate as 17-oxosparteine (**10**) was supplied by Wink *et al.*[8] They found that crude enzyme preparations from cell suspension cultures of *Lupinus polyphyllus* were able to catalyse the conversion of cadaverine into 17-oxosparteine in the presence of pyruvic acid.

Important information about the stereochemistry of some of the enzymic processes involved in lupinine (**3**) biosynthesis became available when the enantiomeric [1-^{2}H]cadaverines (**11**) and (**12**) were prepared and fed to *Lupinus luteus*.[4,9] Labelling patterns were determined by ^{2}H n.m.r. spectroscopy. These studies were extended to the tetracyclic quinolizidine alkaloids in *Lupinus polyphyllus*[9] and *L. angustifolius*.[6] In particular, (−)-sparteine (**1**), (+)-lupanine (**5**), and (+)-angustifoline (**7**) all retained ^{2}H at C-17 after feeding the (*R*)-isomer (**11**). These results demonstrate that 17-oxosparteine (**10**) cannot be an intermediate in the biosynthesis of the quinolizidine alkaloids (**4**)—(**7**).

The plant *Baptisia australis* produces (+)-sparteine (**13**), (−)-*N*-methylcytisine (**14**), and (−)-cytisine (**15**).[10] It is not often possible to study the biosynthesis of both enantiomers of a



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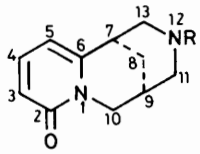
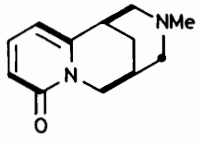


(**14**) R = Me
(**15**) R = H



(**16**)

natural product, but the occurrence of (+)-sparteine provides this opportunity. Furthermore, the co-occurrence of tricyclic and tetracyclic alkaloid structural types in *B. australis* enables a study of the biosynthetic pathways to these alkaloids to be made. The results of experiments with ^{14}C-labelled cadaverine[11] and with $^{14}CO_2$[12] indicate that the tricyclic alkaloids (**14**) and (**15**) are formed from tetracyclic intermediates. In addition, the co-occurrence of lupanine (**5**) and angustifoline (**7**) in *Lupinus polyphyllus* provided circumstantial evidence that it is ring D of a tetracyclic precursor [such as (**9**)] that is cleaved and ring A that is oxidised to the lactam group. This idea was supported by feeding experiments with the enantiomeric [1-^{2}H] cadaverines.[9] By analogy, it has been suggested that it is ring D of a tetracyclic precursor that is cleaved and ring A that is converted into a pyridone in order to form (−)-*N*-methylcytisine (**14**).[13]

Results and Discussion

The dihydrochloride of [1,2-$^{13}C_2$]cadaverine (**2**) was prepared by sequential introduction of the ^{13}C labels starting with the reaction of Na^{13}CN and a C_3-precursor, and the final product (**2**) was shown to contain *ca.* 85% $^{13}C_2$ species.[3] This precursor, together with [1,5-$^{14}C_2$]cadaverine dihydrochloride was pulse fed by the wick method to one well-established *Baptisia australis* plant over two weeks. Two weeks after administration of the precursor had ceased, the plant was harvested and the three major alkaloids, (−)-*N*-methylcytisine (**14**), (−)-cytisine (**15**), and (+)-sparteine (**13**), were isolated and separated by column chromatography on alumina. The amounts of each alkaloid isolated and their ^{14}C specific incorporations† are shown in Table 1.

Table 1. Incorporation of [1,2-$^{13}C_2$]cadaverine (**2**) dihydrochloride and (*R*)-[1-^{2}H]- (**11**) and (*S*)-[1-^{2}H]-cadaverine (**12**) dihydrochloride into quinolizidine alkaloids in *Baptisia australis*

Alkaloid	Precursor	Quantity isolated/mg	% ^{14}C Specific Incorporation
(+)-Sparteine (**1**)	(**2**)	28	0.10
(**1**)	(**11**)	110	0.26
(**1**)	(**12**)	46	0.77
(−)-*N*-Methylcytisine (**14**)	(**2**)	72	0.22
(**14**)	(**11**)	160	0.22
(**14**)	(**12**)	230	0.76
(−)-Cytisine (**15**)	(**2**)	11	0.10
(**15**)	(**11**)	64	0.14
(**15**)	(**12**)	70	0.30

Table 2. Incorporation of [1,2-$^{13}C_2$]cadaverine dihydrochloride (**2**) into (−)-*N*-methylcytisine (**14**)

	(−)-*N*-Methylcytisine (**14**)	
C-atom	δ_C[a]	$J(^{13}C\text{-}^{13}C)$[b]
2	163.1	69.5
3	117.1	69.0
4	138.0	—
5	103.0	68.0
6	151.6	67.5
13	62.6	34.0
7	35.5	34.0
8	25.3	—
9	28.1	35.5
10	50.0	35.0
11	62.1	—
N-Me	46.0	—

[a] $^{13}C\{^1H\}$ chemical shifts for (−)-*N*-methylcytisine (70 mg, 328 000 scans) in [$^2H_6$]benzene with the natural abundance ^{13}C signal of [$^2H_6$]benzene as internal reference at 128.0 p.p.m. [b] Coupling constants $J(^{13}C\text{-}^{13}C)$ of satellites in Hz ± 0.5.

It was essential to assign unambiguously n.m.r. chemical shifts for ^{13}C and for ^{1}H atoms on C atoms adjacent to N in the three alkaloids (**13**)—(**15**). This was achieved for the tricyclic alkaloids (**14**) and (**15**) with the help of homonuclear (^{1}H) and heteronuclear ($^{13}C\text{-}^1H$) chemical shift correlation spectroscopy aided by extensive decoupling experiments. The previous assignment of the ^{13}C n.m.r. spectrum of (−)-cytisine (**15**) was confirmed.[14] The ^{1}H n.m.r. spectroscopic assignments for (−)-sparteine (**1**) have already been made.[6,9]

In the $^{13}C\{^1H\}$ n.m.r. spectra of (−)-*N*-methylcytisine (**14**) taken in [$^2H_6$]benzene, satellites were observed around eight of the signals (Table 2), and slight enrichment of C-11 was observed. Comparison of the normalised signal integrals in this ^{13}C n.m.r spectrum with those of unlabelled material obtained under the same conditions gave estimated enrichment factors of 0.10 ± 0.01% ^{13}C for each of the eight doublets. The estimated ^{13}C specific incorporation per C_5 unit is therefore 0.24% [(0.10 × 2/85) × 100%] (where 85/2 is the average enrichment due to $^{13}C_2$ species at each labelled position of cadaverine (**2**). The labelling pattern (**16**) for (−)-*N*-methylcytisine is consistent with the cleavage of a tetracyclic intermediate.

A sample of (*R*)-[1-^{2}H]cadaverine (**11**) was prepared by decarboxylation of L-lysine in ^2H_2O using lysine decarboxylase. Similar decarboxylation of the L-enantiomer of [2-^{2}H]-DL-lysine in H_2O afforded (*S*)-[1-^{2}H]cadaverine (**12**).[6,9] Both ^{2}H-

† The ^{14}C specific incorporation per C_5 unit is calculated as [(molar activity of alkaloids × 1/3)/(molar activity of precursor)] × 100%

Table 3. Chemical shift data for incorporation of (*R*)-[1-^{2}H]- (**11**) and (*S*)-[1-^{2}H]-cadaverine (**12**) dihydrochloride into (−)-sparteine (**1**) and (+)-sparteine (**13**) (^{1}H[a] and ^{2}H[b] chemical shifts in p.p.m.)

		(−)-Sparteine (**1**) ^{2}H, after feeding			(+)-Sparteine (**13**) ^{2}H, after feeding	
H-atom	^{1}H	(**11**)	(**12**)	^{1}H	(**11**)	(**12**)
2-α	2.60	2.58		1.84	1.82	
2-β	1.84		1.83	2.60		2.58
6-α	—			1.59	1.57	
6-β	1.59	1.57		—		
10-α	2.44		2.42	1.93		
10-β	1.93			2.44		2.42
11-α	2.08	2.05		—		
11-β	—			2.08	2.05	
15-α	1.99	1.97		2.75	2.73	
15-β	2.75		2.72	1.99		1.97
17-α	2.46	2.44		2.66		
17-β	2.66			2.46	2.44	

[a] 200 or 360 MHz ^{1}H N.m.r. spectra in $C_6{}^2H_6$ with $C_6{}^2H_5{}^1H_1$ as internal reference at δ 7.15. [b] 30.72 MHz ^{2}H N.m.r. spectra in C_6H_6 with $C_6{}^2H_1{}^1H_5$ as internal reference at δ 7.15.

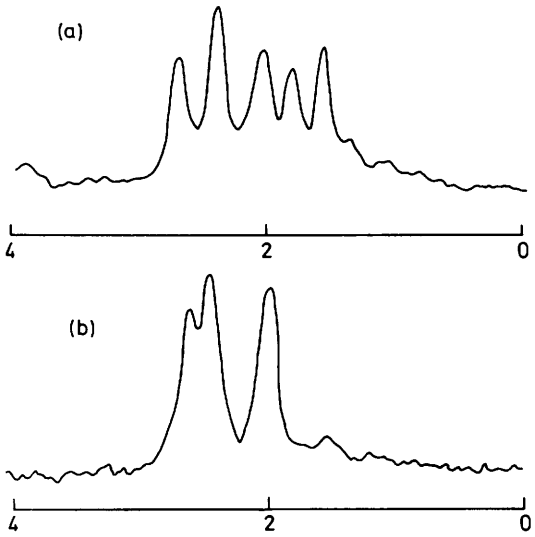
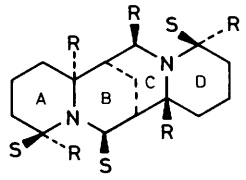


Figure 1. 30.72 MHz ^{2}H N.m.r. spectra of (+)-sparteine (**13**) in C_6H_6 with natural abundance $C_6{}^2H_1H_5$ as internal reference at δ 7.15 p.p.m. (Spectral width 1 000 Hz. Block size 4 K zero-filled to 8 K. Exponential line broadening 1.5 Hz). (a) (+)-Sparteine derived from (*R*)-[1-^{2}H]-cadaverine (**11**) (28 000 scans). (b) (+)-Sparteine derived from (*S*)-[1-^{2}H]cadaverine (**12**) (78 000 scans)



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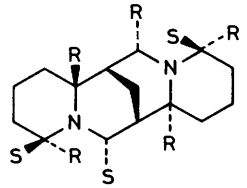

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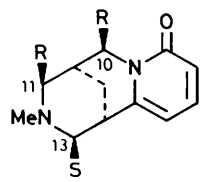

(**19**)

Figure 2. Representation of composite labelling patterns in quinolizidine alkaloids (**17**)—(**19**). R Denotes ^{2}H present after feeding (*R*)-[1-^{2}H]cadaverine (**11**) dihydrochloride. S Denotes ^{2}H present after feeding (*S*)-[1-^{2}H]cadaverine (**12**) dihydrochloride

labelled cadaverines (**11**) and (**12**) were isolated as their dihydrochlorides and the ^{2}H content of the samples was estimated to be 98% and 91% $^2H_1$ species, respectively, by analysis of their ^{1}H n.m.r. and mass spectra. Aliquots of [1,5-^{14}C]cadaverine dihydrochloride were added to each enantiomer (**11**) and (**12**) prior to feeding to six *Baptisia australis* plants over 10 days. Three weeks later the plants were harvested and the alkaloid mixture was separated as before. The amounts of (−)-*N*-methylcytisine (**14**), (−)-cytisine (**15**), and (+)-sparteine (**13**) isolated together with the ^{14}C specific incorporations are listed in Table 1.

The ^{2}H n.m.r. spectra of the labelled alkaloids were obtained in benzene. The line narrowed spectra helped to establish the number of ^{2}H n.m.r. signals and their exact chemical shifts. The assumption was made that ^{2}N will remain on the carbon atoms adjacent to nitrogen. The ^{2}H n.m.r. spectrum for (+)-sparteine (**13**) is shown in Figure 1, and the assignments are shown in Table 3. The composite labelling pattern (**17**) determined for (+)-sparteine can be compared with that (**18**) obtained previously for (−)-sparteine (Figure 2). The retention of ^{2}H at C-17 in (+)-sparteine after feeding the (*R*)-isomer (**11**) establishes that 17-oxosparteine [enantiomer of (**10**)] is not an intermediate in the biosynthesis of (+)-sparteine. The labelling patterns in (+)- and (−)-sparteine [(**17**) and (**18**)] are mirror images in the central portion of the molecules, but not at C-2 and C-15. At these positions the stereochemistry of the ^{2}H in the precursors is retained, in agreement with the finding that the corresponding C–N bonds are preserved during the biosynthesis of (−)-sparteine (**1**).[5,6]

Consideration of the homonuclear 2D ^{1}H n.m.r. spectrum of (−)-*N*-methylcytisine (**14**) established the coupling relationships among all the protons. The key features are that the C-10 protons occur at δ 3.65 and 4.05. The signal at lower field is assigned to 10β-H because of the additional deshielding due to the proximity of the lone pair of electrons on N-12. Furthermore, the signals for the more deshielded protons at C-11 and C-13 at δ 2.29 and 2.42 are assigned to the equatorial C-

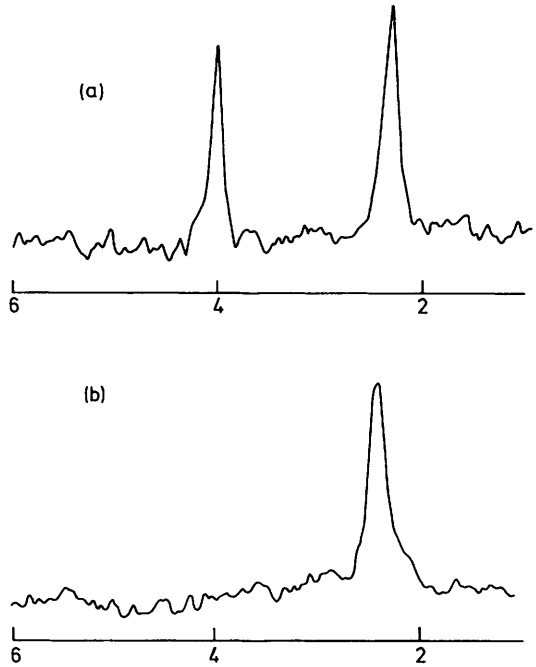

Figure 3. 30.72 MHz ^2H N.m.r. spectra of (−)-*N*-methylcytisine (**14**) in C_6H_6 with natural abundance $C_6{}^2H_1{}^1H_5$ as internal reference at δ 7.15 p.p.m. (Spectral width 1 000 Hz. Block size 4 K zero fitted to 8 K. Exponential line broadening 1.5 Hz). (a) (−)-*N*-Methylcytisine derived from (*R*)-[1-^{2}H]cadaverine (**11**) (60 000 scans). (b) (−)-*N*-Methylcytisine derived from (*S*)-[1-^{2}H]cadaverine (**12**) (30 000 scans)

11β and 13β-H, respectively, and the more shielded signals at δ 1.62 and 1.70 are due to the axial protons at 11α-H and 13α-H, respectively. The assignment of a chair conformation in benzene solution for the ring of (−)-*N*-methylcytisine containing C-11 and C-13 is supported by the observation of long range couplings between both 11β-H and 13β-H with 8-H *pro-R*, and between 10α-H and 8-H *pro-S* (W effect). *X*-Ray crystal structure analyses have recently shown that in the solid state both (−)-*N*-methylcytisine and (−)-cytisine have similar conformations.[15] The pyridone is essentially planar, the central ring is in an envelope conformation, and the third ring exists in a chair conformation. The ^1H n.m.r. spectrum of cytisine has been analysed in detail.[16]

The ^2H n.m.r. spectra for (−)-*N*-methylcytisine (**14**) obtained after carrying out feeding experiments with the (*R*)-[1-^{2}H]- (**11**) and (*S*)-[1-^{2}H]-cadaverines (**12**) are shown in Figure 3 and the assignments are listed in Table 4. Similar spectra were obtained for (−)-cytisine (**15**) with signals at δ 2.37 and 3.95 after feeding the (*R*)-isomer (**11**) and one signal at δ 2.42 after feeding the (*S*)-isomer (**12**). A composite labelling pattern (**18**) is shown for (−)-*N*-methylcytisine in the arrangement which illustrates a relationship with the composite labelling pattern (**17**) of (+)-sparteine. It follows that if (+)-sparteine and (−)-*N*-methylcytisine are formed from the same tetracyclic intermediate, then ring A [as in (**17**)] must be degraded and ring D must be converted into a pyridone in order to produce the observed labels at C-10 and C-13. The ^2H label at C-11 of (−)-*N*-methylcytisine (**14**) derived from the (*R*)-precursor (**11**) is retained on cleavage of ring A but with inversion of stereochemistry. A possible mechanism to account for this observation would involve reduction of an intermediate C-11;N-12 immonium ion stereospecifically from the *re*-face. Finally, no ^2H labels were detected in the *N*-methyl group of (−)-*N*-methylcytisine (**14**) after feeding the (*R*)- and (*S*)- precursors (**11**) and (**12**). This suggests that the *N*-methyl group is not derived from C-2 of a tetracyclic precursor. In accord with this finding, it has been demonstrated that (−)-*N*-methylcytisine (**14**) is formed from (−)-cytisine (**15**) by an *N*-methyltransferase enzyme.[17]

Table 4. Chemical shift data for incorporation of (*R*)-[1-^{2}H]- (**11**) and (*S*)-[1-^{2}H]-cadaverine (**12**) dihydrochloride into (−)-*N*-methylcytisine ($^1H^a$ and $^2H^b$ chemical shifts in p.p.m.)

		(−)-*N*-Methylcytisine (**14**) ^2H, after feeding	
H-atom	^1H	(**11**)	(**12**)
10α	3.65		
10β	4.05	4.03	
11α	1.62		
11β	2.29	2.28	
13α	1.70		
13β	2.42		2.40

[a] 200 or 360 MHz ^1H N.m.r. spectra in $C_6{}^2H_6$ with $C_6{}^2H_5{}^1H_1$ as internal reference at δ 7.15. [b] 30.72 MHz ^2H N.m.r. spectra in C_6H_6 with $C_6{}^2H_1{}^1H_5$ as internal reference at δ 7.15.

Experimental

General.—M.p.s were measured with a Kofler hot-stage apparatus and are uncorrected. Organic solutions were dried with anhydrous magnesium or sodium sulphate 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. ^2H N.m.r. spectra were obtained on a Bruker WP-200SY spectrometer operating at 30.72 MHz. Radiochemicals were obtained from the New England Nuclear Corporation or from Amersham International. Radioactivity was measured with a Philips liquid scintillation analyser using toluene–methanol or Ecoscint (National Diagnostics) 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–saturated aqueous ammonia (85:14:1) and the alkaloids were visualised with the modified Dragendorff reagent.[18]

Samples of [1,2-$^{13}C_2$]cadaverine (**2**) dihydrochloride[3] and the (*R*)-[1-^{2}H]- (**11**) and (*S*)-[1-^{2}H]-cadaverine (**12**) dihydrochlorides were prepared as described.[6,9]

Feeding Experiment with [1,2-$^{13}C_2$]*Cadaverine* (**2**) *Dihydrochloride.*—Plants of the perennial *Baptisia australis* were maintained in open ground. [1,5-$^{14}C_2$]Cadaverine dihydrochloride (4.2 μCi; ≪1 mg) was added to [1,2-$^{13}C_2$]cadaverine dihydrochloride (**2**) (57 mg) and the precursor mixture was dissolved in sterile water and was pulse fed to one healthy plant by the wick method over a period of 14 days. After a further 14 days, the plant was harvested and the roots were washed to remove soil. The plant was cut into small pieces and macerated with methanol in a Waring blender. The solid was removed by filtration at the pump and washed repeatedly with methanol until the filtrate was colourless. The combined extracts were concentrated under reduced pressure and the residue was partitioned between 2M sulphuric acid (100 ml) and chloroform (100 ml). The aqueous phase was washed with chloroform (3 × 50 ml) and was then stirred with zinc dust (0.5 g) for 1 h to reduce any *N*-oxides present. The mixture was filtered through

a small pad of Celite and the orange-red filtrate was cooled in ice and basified with conc. ammonia. The deep red aqueous solution was extracted with dichloromethane (4 × 50 ml). The aqueous layer was then further basified (to pH > 12) with KOH pellets and extracted again with dichloromethane (2 × 50 ml). The combined organic extracts were dried, filtered, and concentrated to afford the crude alkaloid mixture (309 mg). Analytical t.l.c. revealed three radioactive bands which were coincident with unlabelled *N*-methylcytisine (**14**) (R_F 0.59), cytisine (**15**) (R_F 0.43), and sparteine (**13**) (R_F 0.17). The alkaloid mixture was separated by column chromatography on basic alumina (activity III) and elution with dichloromethane (to obtain *N*-methylcytisine and cytisine) and then with dichloromethane-methanol (96:4) (to obtain sparteine). (−)-N-*Methylcytisine* (**14**) was recrystallised from benzene to constant specific radioactivity, m.p. 135—137 °C (lit.,[19] 138 °C); $[\alpha]_D^{19}$ −220° (*c* 1.0 in EtOH) [lit.,[19] $[\alpha]_D$ −221° (H_2O)]; δ_H([$^2H_6$]benzene) 1.02 (1 H, m, J_{gem} 12.5 Hz, 8-H *pro-S*), 1.13 (1 H, m, J_{gem} 12.5 Hz, 8-H *pro-R*), 1.62 (1 H, m, J_{gem} 10 Hz, 11α-H), 1.70 (1 H, m, J_{gem} 10.5 Hz, 13α-H), 1.75 (1 H, m, 9-H), 1.76 (3 H, s, NMe), 2.20 (1 H, m, 7-H), 2.29 (1 H, m, J_{gem} 10 Hz, 11β-H), 2.42 (1 H, m, J_{gem} 10.5 Hz, 13β-H), 3.65 (1 H, m, J_{gem} 15.5 Hz, 10α-H), 4.05 (1 H, part of AB system, *J* 15.5 Hz, 10β-H), 5.44 (1 H, dd, *J* 1.5 and 6.7 Hz, 5-H), 6.50 (1 H, dd, *J* 1.5 and 8.7 Hz, 3-H), and 6.79 (1 H, dd, *J* 6.7 and 8.7 Hz, 4-H) (Found: M^+, 204.1258. $C_{12}H_{16}N_2O$ requires *M*, 204.1263). (−)-*Cytisine* (**15**) was obtained as a solid, m.p. 155 °C (lit.,[19] 155 °C), $[\alpha]_D^{19}$ −118° (*c* 1.0 in H_2O) [Lit.,[19] $[\alpha]_D^{17}$ −119.6° (H_2O)] (Found: M^+, 190.1098. $C_{11}H_{14}N_2O$ requires *M*, 190.1106). (+)-Sparteine (**13**) was obtained as a yellow oil, $[\alpha]_D^{20}$ +17° (*c* 1.0 in EtOH) [lit.,[19] $[\alpha]_D$ +17.1° (EtOH)] (Found: M^+, 234.2096. $C_{15}H_{26}N_2$ requires *M*, 234.2096). The quantities of the alkaloids (**13**)—(**15**) isolated together with their ^{14}C specific incorporations are shown in Table 1. The ^{13}C n.m.r. spectral data for (−)-*N*-methylcytisine (**14**) are listed in Table 2.

Feeding Experiments with (R)-[1-^{2}H]- (**11**) *and* (S)-[1-^{2}H]-*Cadaverine* (**12**) *Dihydrochloride.*—(*R*)-[1-^{2}H]Cadaverine (**11**) dihydrochloride (465 mg) was mixed with [1,5-$^{14}C_2$]cadaverine dihydrochloride (2.4 μCi, ≪1 mg) and (*S*)-[1-^{2}H]cadaverine (**12**) dihydrochloride (500 mg) was also mixed with [1,5-$^{14}C_2$]cadaverine dihydrochloride (2.4 μCi, ≪ 1 mg). Each mixture was pulse fed to 6 well established plants over 10 days. After a further 21 days, the plants were harvested and the alkaloids were isolated as described above. The quantities of alkaloids (**13**)—(**15**) isolated and their ^{14}C specific incorporations are shown in Table 1. The ^2H n.m.r. spectra for (+)-sparteine (**13**) are given in Table 3 and Figure 1, while those for (−)-*N*-methylcytisine (**14**) are shown in Table 4 and Figure 3.

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