Application of ^{2}H N.M.R. Spectroscopy to Study the Incorporation of Enantiomeric ^{2}H-Labelled Cadaverines into Quinolizidine Alkaloids[1]

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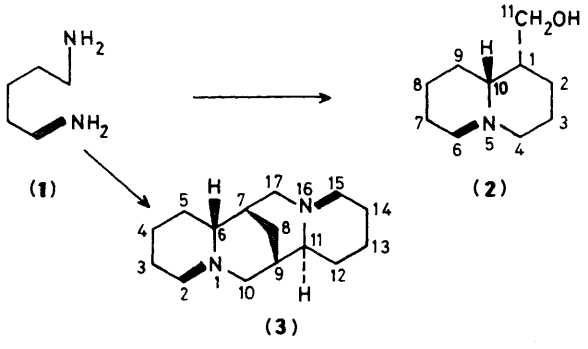
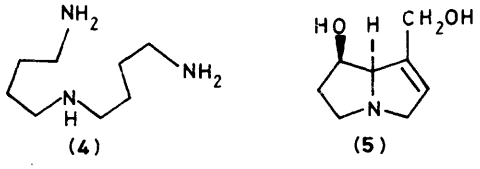
The biosynthesis of a number of quinolizidine alkaloids has been studied in two *Lupinus* species using cadaverine precursors chirally labelled with deuterium. The labelling patterns in lupinine (**2**), sparteine (**3**), lupanine (**6**), and angustifoline (**8**), derived biosynthetically from (*R*)-[1-^{2}H]- (**11**) and (*S*)-[1-^{2}H]cadaverine (**12**) have been established by ^{2}H n.m.r. spectroscopy. The stereochemistry of a number of the steps involved in the conversion of cadaverine into lupinine (**2**) and the tetracyclic quinolizidine alkaloids has been established. The presence of ^{2}H at C-17 in samples of sparteine (**3**), lupanine (**6**), and angustifoline (**8**) derived from the (*R*)-isomer (**11**) demonstrates that 17-oxosparteine (**9**) cannot be an intermediate in the biosynthesis of tetracyclic quinolizidine alkaloids. The presence of ^{2}H at C-2 in both samples of sparteine (**3**) after feeding the precursors (**11**) and (**12**) disproves the theory that lupanine (**6**) is a precursor of sparteine (**3**).

Many species of the plant family Leguminosae produce quinolizidine alkaloids.[2] The biosynthesis of lupinine (**2**) takes place from two molecules of L-lysine *via* cadaverine (**1**). A complete labelling pattern was established in lupinine by ^{13}C n.m.r. spectroscopy after feeding DL-[4,5-$^{13}C_2$,6-^{14}C]-lysine to *Lupinus luteus* plants.[3] Further information about the mode of incorporation of cadaverine was obtained by feeding [1-*amino*-^{15}N,1-^{13}C]cadaverine (**1**) to *L. luteus* (Scheme). Two of these

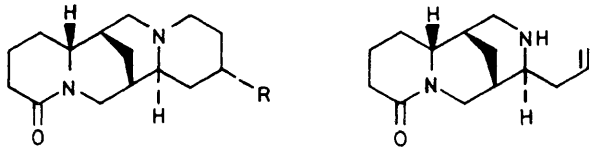


Scheme.

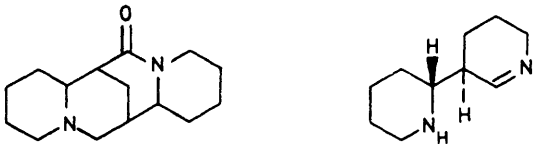
cadaverine (**1**) units were incorporated into lupinine (**2**), but only one ^{13}C–^{15}N doublet, associated with C-6, was observed in the ^{13}C-{^{1}H} n.m.r. spectrum of lupinine (**2**). This finding showed that a later C_5–N–C_5 intermediate with C_{2v} symmetry formed from two molecules of cadaverine is not involved in lupinine biosynthesis.[4,5] This result is in marked contrast to pyrrolizidine alkaloid biosynthesis where a later C_4–N–C_4 intermediate with C_{2v} symmetry is involved in the biosynthetic pathway to retronecine (**5**).[6,7] This C_4–N–C_4 intermediate was shown to be 1,6,11-triazaundecane (homospermidine) (**4**) by carrying out feeding experiments with homospermidine labelled with ^{14}C[8] and with ^{13}C.[9]



Tetracyclic quinolizidine alkaloids such as sparteine (**3**) are formed from three [^{13}C–^{15}N]cadaverine (**1**) units. The ^{13}C-{^{1}H} n.m.r. spectrum of sparteine (**3**) obtained after feeding the doubly labelled cadaverine (**1**) to *Lupinus polyphyllus* plants (Scheme) showed two ^{13}C–^{15}N doublets associated with C-2 and C-15, indicating that two of the cadaverine (**1**) units are incorporated into the outer rings of sparteine in a specific fashion.[5,10] Analogous labelling patterns have been observed after carrying out feeding experiments with [^{13}C–^{15}N]-cadaverine (**1**) to obtain labelled samples of lupanine (**6**),[11,12] 13-hydroxylupanine (**7**),[12] and angustifoline (**8**).[12]



Little is known about the biosynthetic pathway from cadaverine to the quinolizidine alkaloids. Wink *et al.*, have shown that crude enzyme preparations from cell suspension cultures of *Lupinus polyphyllus* can catalyse the conversion of cadaverine into 17-oxosparteine (**9**) in the presence of pyruvic acid.[13] This finding suggests that transamination reactions are occurring with pyruvic acid acting as a receptor for the amino groups in cadaverine that are undergoing transamination. No intermediates were detected during the enzymic processes and a series of enzyme-linked intermediates on an enzyme complex was postulated. A key role was assigned to 17-oxosparteine (**9**) in the formation of tetracyclic quinolizidine alkaloids, and it was proposed that other quinolizidine alkaloids such as sparteine (**3**) and lupanine (**6**) are derived from 17-oxosparteine (**9**).[13]



We have used the enantiomeric [1-^{2}H]cadaverines to produce samples of a range of quinolizidine alkaloids. Labelling patterns in these alkaloids have been determined by ^{2}H n.m.r. spectroscopy. The stereochemistry of a number of the enzymic

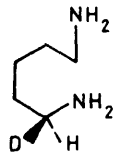
Table 1. Incorporation of (*R*)- (**11**) and (*S*)-[1-^{2}H]cadaverine (**12**) dihydrochloride into quinolizidine alkaloids in *Lupinus* species

Alkaloid	Precursor	Quantity isolated/mg	%^{14}C Specific Incorporation*
Lupinine (**2**)	(**11**)	35	3.7
(**2**)	(**12**)	93	3.5
Sparteine (**3**)	(**11**)	55	5.8
(**3**)	(**12**)	59	5.5
Lupanine (**6**)	(**11**)	127	4.7
(**6**)	(**12**)	33	6.0
Angustifoline (**8**)	(**11**)	30	2.5
(**8**)	(**12**)	53	3.3
13-Hydroxylupanine (**7**)	(**11**)	11	3.2
(**7**)	(**12**)	18	2.7

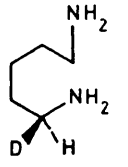
processes involved in the formation of these alkaloids has been established, and it has been demonstrated that 17-oxosparteine (**9**) cannot be an intermediate in the biosynthesis of sparteine (**3**), lupanine (**6**), or angustifoline (**8**).

Results and Discussion

Treatment of L-lysine in ^2H_2O with lysine decarboxylase (EC 4.1.1.18) yielded (*R*)-[1-^{2}H]cadaverine (**11**), because this enzymic decarboxylation is known to proceed with retention of configuration.[14] Similar decarboxylation of the L-component of [2-^{2}H]-DL-lysine in H_2O gave (*S*)-[1-^{2}H]cadaverine (**12**), after removal of the unchanged [2-^{2}H]-D-lysine. Both labelled cadaverine samples (**11**) and (**12**) were isolated as their dihydrochlorides, and the ^{2}H content of each sample was estimated to be 98% and 91% $^2H_1$ species, respectively, by analysis of their



(**11**)



(**12**)

^{1}H n.m.r. and mass spectra. Radioactive [1,5-^{14}C]cadaverine dihydrochloride was added to each sample of ^{2}H-labelled precursor prior to the feeding experiments. The precursors were pulse fed[15] into the xylems of *Lupinus luteus* and *L. polyphyllus* plants. Ten days after each feeding was completed, the plants were harvested and the alkaloid mixture was isolated. Purification of each mixture was achieved by column chromatography to give (−)-lupinine (**2**) and (−)-sparteine (**3**) from *L. luteus*,[3] and (+)-lupanine (**6**), (+)-angustifoline (**8**), and (+)-13-hydroxylupanine (**7**) from *L. polyphyllus*.[12,16] The amount of each alkaloid isolated after feeding the (*R*)- and (*S*)-isomers (**11**) and (**12**), together with the ^{14}C specific incorporation* of each alkaloid are listed in Table 1.

The ^{1}H n.m.r. chemical shifts in $C_6{}^2H_6$ for hydrogen atoms on the carbon atoms adjacent to nitrogen in lupinine (**2**) are given in Table 2, and values for sparteine (**3**), lupanine (**6**), and angustifoline (**8**) are listed in Table 3. The literature values[11,17,18] were checked by carrying out extensive decoupling experiments, and they were confirmed by homonuclear (^{1}H) and heteronuclear (^{13}C–^{1}H) chemical shift correlation spectroscopy. The assignments for the 11-*pro-R* and 11-*pro-S* hydrogen atoms in lupinine (**2**) at δ 3.75 and 4.16 p.p.m., respectively, were established by Podkowinska.[19]

The ^{2}H n.m.r. spectra of the two labelled samples of each of the four quinolizidine alkaloids [(**2**), (**3**), (**6**), and (**8**)] were obtained in C_6H_6. The use of line-narrowed spectra helped to establish the number of ^{2}H n.m.r. signals and their exact

* The ^{14}C specific incorporation per C_5 unit is calculated as: {[molar activity of alkaloids $\times \frac{1}{3}(\times \frac{1}{2}$ for lupinine)]/(molar activity of precursor)} × 100%.

Table 2. Incorporation of (*R*)- (**11**) and (*S*)-[1-^{2}H]cadaverine (**12**) dihydrochloride into lupinine (**2**) (^{1}H[a] and ^{2}H[b] chemical shifts in p.p.m.)

H-atom	^{1}H	(−)-Lupinine (**2**), ^{2}H, after feeding (**11**)	(**12**)
4α	2.51		
4β	1.68		1.66
6α	2.43	2.42	
6β	1.50		1.48
10	1.71	1.68	
11 *pro-R*	3.69		
11 *pro-S*	4.14	4.13	

[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 p.p.m. [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 p.p.m.

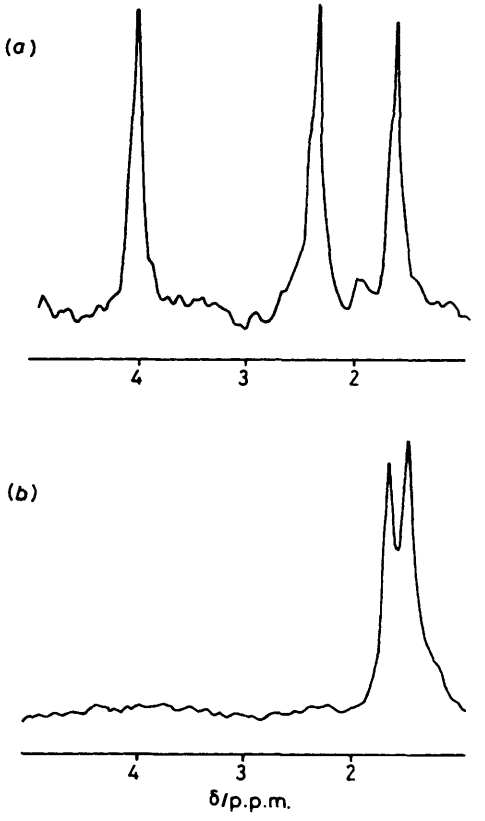


Figure 1. 30.72 MHz ^{2}H n.m.r. spectra of lupinine (**2**) in C_6H_6 with natural abundance $C_6{}^2H_1{}^1H_5$ as internal reference at δ 7.15 p.p.m. (*a*) Lupinine derived from (*R*)-[1-^{2}H]cadaverine (**11**) (*b*) Lupinine derived from (*S*)-[1-^{2}H]cadaverine (**12**)

Table 3. Incorporation of (*R*)- (**11**) and (*S*)-[1-^{2}H]cadaverine (**12**) dihydrochloride into sparteine (**3**), lupanine (**6**), and angustifoline (**8**) (^{1}H[a] and ^{2}H[b] chemical shifts in p.p.m.)

	(−)-Sparteine (**3**)			(+)-Lupanine (**6**)			(+)-Angustifoline (**8**)[d]		
		^{2}H, after feeding			^{2}H, after feeding			^{2}H, after feeding	
H-atom	^{1}H	(**11**)	(**12**)	^{1}H	(**11**)	(**12**)	^{1}H	(**11**)	(**12**)
2α	2.60	2.58		—			—		
2β	1.84		1.83	—			—		
6β	1.59	1.57		2.69	2.65		2.82	2.80	
10α	2.44		2.42	4.75		4.72	4.82		4.79
10β	1.93			2.25			2.56		
11α	2.08	2.05		1.65	1.62		2.72	2.70	
15α	1.99	1.97		1.79	1.78[c]		—		
15β	2.75		2.72	2.62		2.58	—		
17α	2.46	2.44		1.76	1.74[c]		2.68	2.65	
17β	2.66			2.51			2.68		

[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 p.p.m. [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 p.p.m. [c] Signals resolved at 55.28 MHz. [d] The same numbering scheme is used for angustifoline (**8**) as the tetracyclic quinolizidine alkaloids for convenience.

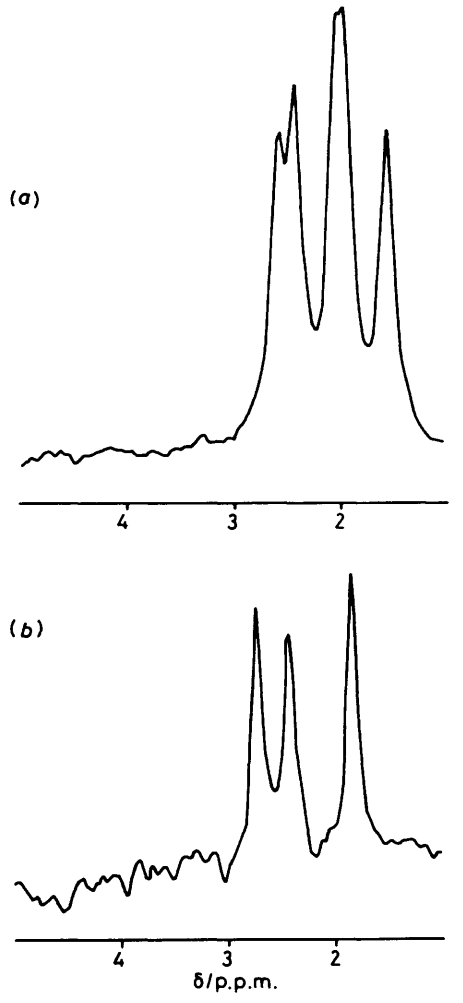


Figure 2. 30.72 MHz ^{2}H n.m.r. spectra of sparteine (**3**) in C_6H_6 with natural abundance $C_6{}^2H_1{}^1H_5$ as internal reference at δ 7.15 p.p.m. (*a*) Sparteine derived from (*R*)-[1-^{2}H]cadaverine (**11**) (*b*) Sparteine derived from (*S*-[1-^{2}H]cadaverine (**12**)

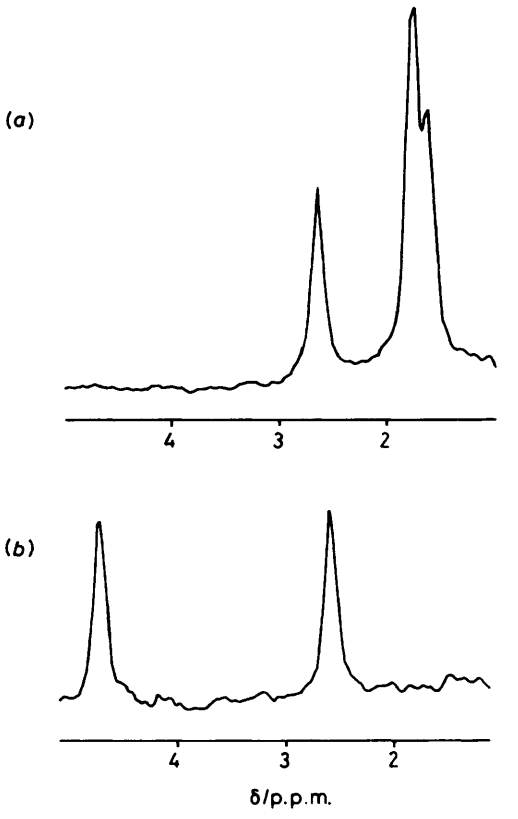


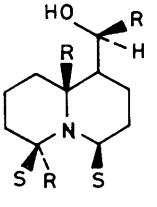
Figure 3. 30.72 MHz ^{2}H n.m.r. spectra of lupanine (**6**) in C_6H_6 with natural abundance $C_6{}^2H_1{}^1H_5$ as internal reference at δ 7.15 p.p.m. (*a*) Lupanine derived from (*R*)-[1-^{2}H]cadaverine (**11**) (*b*) Lupanine derived from (*S*)-[1-^{1}H]cadaverine (**12**)

chemical shifts. The assignment of the ^{2}H n.m.r. chemical shifts for each labelled sample was made by comparison with the ^{1}H n.m.r. chemical shifts together with the aid of the following assumptions: (a) that the ^{2}H will remain on the carbon atoms adjacent to nitrogen, and where the C–N bond is known to be incorporated intact, the stereochemistry of the ^{2}H atom attached to this carbon will be preserved; (b) and α- and β-hydrogens at one carbon atom cannot both be deuteriated in an individual experiment and; (c) two samples of the same alkaloid obtained from feeding experiments with the precursors (**11**) and (**12**) cannot have ^{2}H at the same site. These assumptions are valid because the incorporation of ^{2}H is stereospecific, and different ^{2}H n.m.r. spectra are obtained for each alkaloid from the two precursors (**11**) and (**12**).

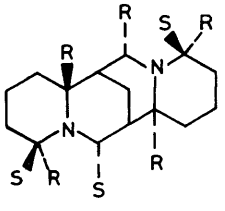
The ^{2}H n.m.r. spectra of lupinine (**2**) obtained from the

feeding experiments with (*R*)- and (*S*)-[1-^{2}H]cadaverines (**11**) and (**12**) are shown in Figure 1. The ^{2}H n.m.r. chemical shifts and assignments for lupinine are listed in Table 2. The ^{2}H n.m.r. signals within each sample were of about the same intensity. A composite labelling pattern for lupinine (**13**) is illustrated in Figure 4. The ^{2}H is retained when the C–N bond in cadaverine remains intact in the formation of the N-5, C-6 bond in lupinine. This leads to the appearance of ^{2}H at C-6α from precursor (**11**) and C-6β form (**12**). With reference to the composite labelling pattern of lupinine (**13**), it is clear that a total of three ^{2}H atoms are lost from the two molecules of the cadaverine precursors (**11**) and (**12**) as they form lupinine probably as a result of transamination processes on the primary amino groups. These reactions occur with stereospecific removal of the *pro-S* hydrogen at the carbon atoms destined to become C-10 and C-11 of lupinine, whereas the *pro-R* hydrogen is removed from the carbon atom which becomes C-4 of lupinine. The net inversion of stereochemistry of ^{2}H attached to C-4 of lupinine, obtained from the (*S*)-precursor (**12**), may be due to formation of an intermediate N-4, C-5 iminium ion, which then undergoes reduction from the C-*si* face of the iminium ion to afford lupinine labelled with ^{2}H at C-4β. Finally, the formation of (11*S*)-[11-^{2}H]lupinine (**13**) is consistent with reduction of an intermediate aldehyde by attack of a hydride donor on the C-*re* face of the carbonyl group. The same stereospecificity was observed in the formation of the primary alcohol in retronecine (**5**) biosynthesis.[20,21] Normal coupled dehydrogenase enzyme systems also exhibit the same stereospecificity.[22] Similar results were obtained by Golebiewski and Spenser for the incorporation of (*R*)- and (*S*)-[1-^{2}H]cadaverines (**11**) and (**12**) into lupinine (**13**).[17] These authors postulate a biosynthetic pathway to lupinine involving dimerisation of two 2,3,4,5-tetrahydropyridine molecules to give tetrahydroanabasine (**10**) as an intermediate.

The ^{2}H n.m.r. spectra for sparteine (**3**) and lupanine (**6**) obtained from the feeding experiments with (*R*)- (**11**) and with (*S*)-[1-^{2}H]-cadaverine (**12**) are shown in Figures 2 and 3, respectively. All of the ^{2}H n.m.r. signals within the same sample were of about the same intensity. The two overlapping signals at δ 1.76 p.p.m. in Figure 3*a* were resolved at 55.28 MHz. The ^{2}H n.m.r. spectral assignments for these two alkaloids and angustifoline (**8**) are shown in Table 3.



(**13**)



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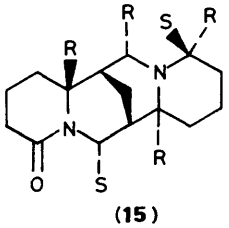

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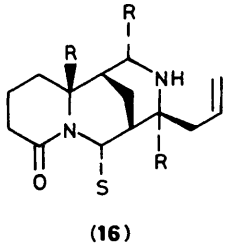


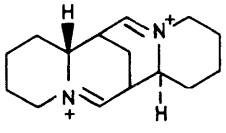
(**16**)

Figure 4. Representation of composite labelling patterns in quinolizidine alkaloids (**2**), (**3**), (**6**), and (**8**). 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.

A composite labelling pattern for sparteine (**14**) is given in Figure 4. During the conversion of three molecules of cadaverine into sparteine, ^{2}H is retained on the carbons of the two C–N bonds that remain intact. This leads to the appearance of ^{2}H at C-2α and C-15α after feeding the (*R*)-precursor (**11**), and at C-2β and C-15β from the experiment with (*S*)-precursor (**12**). Four ^{2}H atoms are lost in total from the three molecules of the enantiomeric cadaverine precursors (**11**) and (**12**) as they are transformed into sparteine (**14**). This demonstrates that the *pro-S* hydrogens are removed stereospecifically from the carbon atoms which become C-6, C-11, and C-17, while the *pro-R* hydrogen is removed from the carbon atom which becomes C-10 of sparteine. By contrast with lupinine, the ^{2}H at C-10 in sparteine (**14**) is retained with retention of stereochemistry. This would require attack by a hydride donor on an intermediate N-1, C-10 iminium ion from the C-*re* face.

Analogous ^{2}H labelling patterns were observed for the other two quinolizidine alkaloids lupanine and angustifoline when due account was taken of the structural differences from sparteine. The composite labelling patterns for lupanine (**15**) and angustifoline (**16**) are illustrated in Figure 4. The same labelling patterns for sparteine (**14**) and lupanine (**15**) were reported by Golebiewski and Spenser after feeding experiments were carried out on *Lupinus* species with the (*R*)- and (*S*)-precursors (**11**) and (**12**).[17]

The presence of ^{2}H at C-17α in the three alkaloids sparteine (**14**), lupanine (**15**), and angustifoline (**16**)* after feeding (*R*)-[1-^{2}H]cadaverine (**11**) clearly shows that 17-oxosparteine (**9**) cannot be an intermediate as claimed[13] in the biosynthetic pathway from cadaverine to any of these quinolizidine alkaloids. Several groups[23] have postulated that lupanine (**6**) is a precursor of sparteine (**3**). This has also been disproved, because ^{2}H appears at C-2α and C-2β in sparteine (**14**) from the feeding experiments with the precursors (**11**) and (**12**), respectively.



(**17**)

It has been suggested that the tetracyclic quinolizidine alkaloids are formed from a trimer of 2,3,4,5-tetrahydropyridine.[11] The labelling patterns (**14**)—(**16**) obtained with the enantiomeric ^{2}H-precursors (**11**) and (**12**) are consistent with this theory. A possible intermediate in the pathway is (**17**) formed by rearrangement of a 2,3,4,5-tetrahydropyridine trimer.[11] Stereospecific attack of a hydride donor on the C-*re* faces of both iminium ions in the intermediate (**17**) would lead to the three quinolizidine alkaloids (**14**)—(**16**) labelled with ^{2}H at C-17α from the (*R*)-precursor (**11**), and with ^{2}H at C-10α from the (*S*)-precursor (**12**). Further work is required to identify the intermediates in the biosynthetic pathways from cadaverine to lupinine and the tetracyclic quinolizidine alkaloids.

Experimental

General.—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. N.m.r. spectra were obtained on a Bruker

* The same numbering scheme is used for angustifoline (**8**) as the tetracyclic quinolizidine alkaloids for convenience.

WH-360 spectrometer at 360 MHz for ^1H and 55.28 MHz for ^2H, or on a Bruker WP-200SY spectrometer operating at 200 MHz for ^1H and 30.72 MHz for ^2H. Radiochemicals were purchased 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.[24]

(*R*)-[1-^{2}H]Cadaverine (**11**) dihydrochloride (96% $^2H_1$ species) and (*S*)-[1-^{2}H]cadaverine (**12**) dihydrochloride (91% $^2H_1$ species) were prepared as described.[13] Both compounds gave similar spectra: $\delta_H(D_2O)$ 1.6 (6 H, m), and 2.9 (3 H, t, *J* 7 Hz); δ_D-{^{1}H} (H_2O) 2.9 (s); *m/z* 89 (10%), 88 (100), and 87 (15).

Feeding Experiments.—Plants were grown from seed in a standard compost. Feeding experiments were commenced at the onset of flowering. [1,5-^{14}C]Cadaverine dihydrochloride (1.67 μCi) was added to each sample of ^{2}H-labelled precursor (175 mg). Aqueous solutions of the two precursors were introduced into the xylems of the plants (9 or 10 per experiment) through stem punctures made with a sterile needle. Each precursor was pulse fed daily for 5 days. After a further 10 days, the alkaloid mixture was isolated as described.[25]

(a) Lupinus luteus. Column chromatography of the alkaloid mixture from these plants gave (−)-lupinine (**2**) and (−)-sparteine (**3**).[3,23] Radioscans of the t.l.c. plates showed one radioactive band for each sample of alkaloid (**2**) and (**3**), coincident with authentic unlabelled material at R_F 0.56 and 0.32, respectively.

(b) Lupinus polyphyllus. The alkaloid mixture was separated by column chromatography on basic alumina to give (+)-lupanine (**6**), (+)-angustifoline (**8**), and (+)-13-hydroxylupanine (**7**).[12] Each sample of these alkaloids gave one radioactive band when radioscans of the t.l.c. plates were carried out, coincident with authentic unlabelled material at R_F 0.80, 0.65, and 0.48, respectively.

The amounts of the five alkaloids (**2**), (**3**), (**6**)—(**8**) isolated in the feeding experiments with the (*R*)- and (*S*)-precursors (**11**) and (**12**), together with their ^{14}C specific incorporations are shown in Table 1. The i.r. and ^{1}H n.m.r. spectra for each sample of alkaloid isolated were closely similar to those of the authentic alkaloids. The ^{2}H n.m.r. spectra for lupinine (**2**) obtained after feeding ^{2}H-labelled precursors (**11**) and (**12**) are shown in Figure 1, and the ^{2}H n.m.r. spectral data are listed in Table 2. The ^{2}H n.m.r. spectra for sparteine (**3**) and lupanine (**6**) are shown in Figures 2 and 3, respectively. The ^{2}H n.m.r. spectral data for sparteine (**3**), lupanine (**6**), and angustifoline (**8**), are displayed in Table 3.

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