Incorporation of Chiral [1-^{2}H]Cadaverines into the Quinolizidine Alkaloids Sparteine, Lupanine, and Angustifoline

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^{2}H N.m.r. spectroscopy has been used to establish the labelling patterns in sparteine (**3**), lupanine (**5**), and angustifoline (**7**), derived biosynthetically from (*R*)-[1-^{2}H]- (**8**) and (*S*)-[1-^{2}H]-cadaverine (**9**); the presence of ^{2}H at C-17 in these three alkaloids from the former precursor (**8**) demonstrates that 17-oxosparteine cannot be an intermediate in quinolizidine alkaloid biosynthesis.

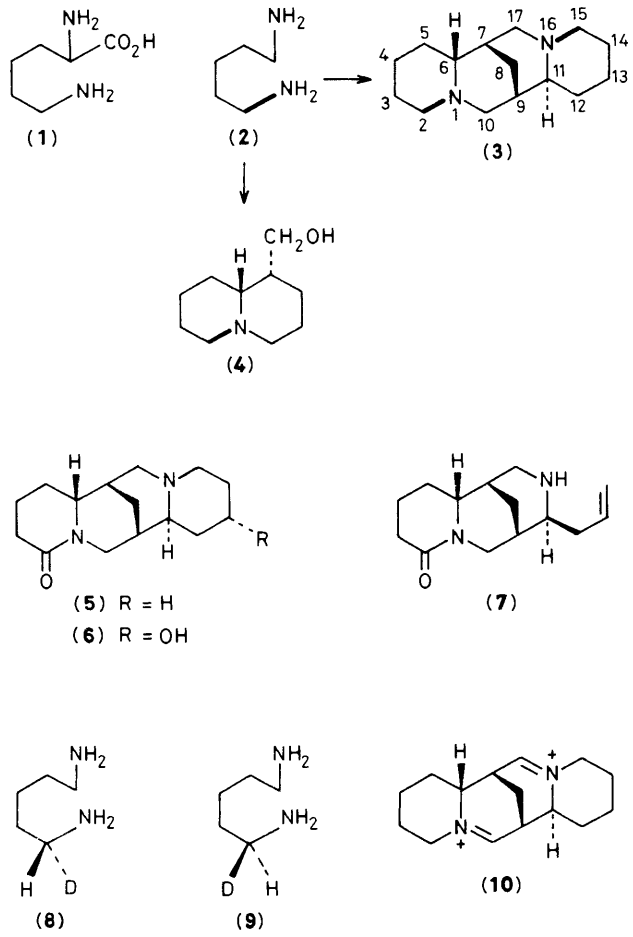
Quinolizidine alkaloids are present in a number of species of the plant family Leguminosae.[1] The biosynthesis of the most common of these alkaloids, sparteine (**3**), proceeds from lysine (**1**) *via* cadaverine.[2] The use of ^{13}C-^{15}N-doubly labelled cadaverine (**2**) has shown that three cadaverine units are incorporated into sparteine (**3**).[3,4] Two of the C–N bonds from two of the cadaverine units remain intact in sparteine (**3**). Two cadaverine units are also incorporated into the bicyclic alkaloid lupinine (**4**), and one of the C–N bonds in cadaverine (**2**) remains intact.[4,5] Little is known about the biosynthetic pathway from cadaverine to the tetracyclic quinolizidine alkaloids such as sparteine, but it has been demonstrated that crude enzyme preparations from cell suspension cultures of *Lupinus polyphyllus* are able to catalyse the conversion of cadaverine into 17-oxosparteine in the presence of pyruvic acid.[6] It was therefore postulated that 17-oxosparteine is a key intermediate in quinolizidine alkaloid biosynthesis, and that other quinolizidine alkaloids such as sparteine and lupanine are derived from 17-oxosparteine. We report the use of chiral [1-^{2}H]cadaverines to produce samples of ^{2}H-labelled quinolizidine alkaloids. ^{2}H N.m.r. spectroscopy has been utilised to demonstrate that 17-oxosparteine cannot be an intermediate in the biosynthesis of sparteine (**3**), lupanine (**5**), and angustifoline (**7**).

(*R*)-[1-^{2}H]Cadaverine (**8**) (98% $^2H_1$ species) was prepared by decarboxylation of L-lysine in ^2H_2O using lysine decarboxylase.[7] Similar decarboxylation of [2-^{2}H]-DL-lysine in H_2O afforded (*S*)-[1-^{2}H]cadaverine (**9**) (91% $^2H_1$ species).[7] Both samples of cadaverine were converted into their dihydrochlorides, ^{2}H n.m.r. (H_2O) δ 2.9 p.p.m. (s). Each precursor (175 mg) together with [1,5-^{14}C]cadaverine dihydrochloride (1.67 μCi) was pulse fed[8] into the xylems of ten *Lupinus polyphyllus*

Table 1. Incorporation of (*R*)- (**8**) and (*S*)-[1-^{2}H]cadaverine (**9**) dihydrochloride into sparteine (**3**), lupanine (**5**), and angustifoline (**7**) (^{1}H[a] and ^{2}H[b] chemical shifts in p.p.m.).

	(−)-Sparteine (**3**)			(+)-Lupanine (**5**)			(+)-Angustifoline (**7**)		
	^{1}H	^{2}H, after feeding		^{1}H	^{2}H, after feeding		^{1}H	^{2}H, after feeding	
H atom		(**8**)	(**9**)		(**8**)	(**9**)		(**8**)	(**9**)
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.



plants over a period of five days. After a further ten days, the alkaloid mixture was isolated. Column chromatography on basic alumina gave (+)-lupanine (**5**), (+)-angustifoline (**7**), and a small amount of (+)-13-hydroxylupanine (**6**).†[9] Similar experiments were carried out by feeding the cadaverine precursors (**8**) and (**9**) to nine *L. luteus* plants. (−)-Sparteine (**3**) and (−)-lupinine (**4**) were isolated and separated by column chromatography on basic alumina. The ^{14}C specific incorporations‡ for each alkaloid in these experiments were 2.5—6.0% per C_5 unit.

The ^{1}H n.m.r. chemical shifts in $C_6{}^2H_6$ for hydrogen on the carbon atoms adjacent to nitrogen in the three alkaloids (**3**), (**5**), and (**7**) are shown in Table 1. These were assigned from literature values,[10] together with extensive decoupling experiments. These assignments were confirmed by homonuclear (^{1}H) and by heteronuclear (^{13}C–^{1}H) chemical shift correlation spectroscopy.

The ^{2}H n.m.r. spectra of sparteine (**3**) obtained from the feeding experiments with (*R*)- (**8**) and with (*S*)-[1-^{2}H]-cadaverine (**9**) are shown in Figure 1. The use of line-narrowed spectra of sparteine and the other alkaloids helped to establish the number of ^{2}H n.m.r. signals and their exact chemical shifts. The assignment of the ^{2}H n.m.r. chemical shifts for each alkaloid was made by comparison with the ^{1}H n.m.r. chemical shifts, and with the aid of the following assumptions:[11] (a) ^{2}H will remain on the carbon atoms adjacent to nitrogen; (b) the 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 the opposite enantiomers of cadaverine cannot have ^{2}H at the same site. These assumptions can be made because the incorporation of ^{2}H is stereospecific [different ^{2}H n.m.r. spectra are obtained for each alkaloid from the two precursors (**8**) and (**9**)]. The ^{2}H n.m.r. spectral assignments for the three alkaloids (**3**), (**5**), and (**7**), are shown in Table 1. All the ^{2}H signals within the same sample were of approximately the same intensity. Samples of lupinine (**4**) displayed ^{2}H n.m.r. signals at δ 1.68, 2.42, and 4.13 p.p.m. from (*R*)-[1-^{2}H]-cadaverine (**8**), and signals were observed at δ 1.48 and 1.66 p.p.m. from the (*S*)-isomer (**9**), in agreement with recent findings.[11]

The number of ^{2}H n.m.r. signals observed for the three alkaloids (**3**), (**5**), and (**7**) is consistent with the differences in their structures. Thus, the additional signal in sparteine (**3**) compared with lupanine (**5**) from the precursors (**8**) and (**9**) is

† The amounts of (**3**), (**5**), and (**7**) isolated were 55, 127, and 30 mg after feeding (**8**), and 59, 33, and 53 mg after feeding (**9**).

‡ The % ^{14}C specific incorporation is calculated as [(molar activity of alkaloid × ⅓; × ½ for lupinine)/(molar activity of precursor)] × 100.

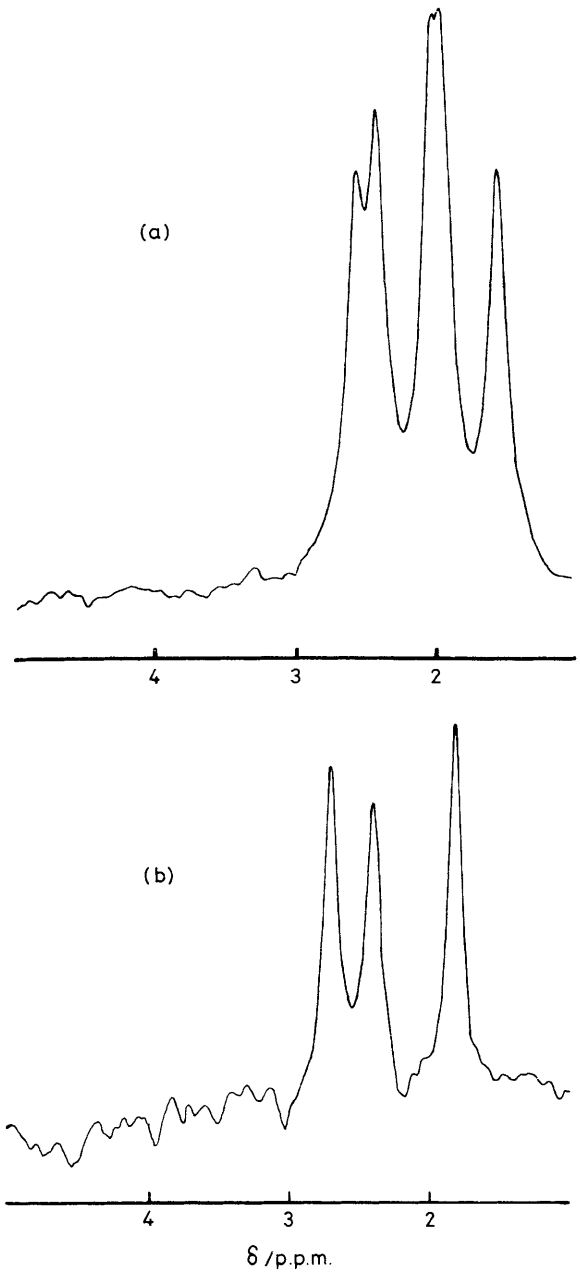


Figure 1. 30.72 MHz ^2H N.m.r. spectra of sparteine (**3**) (50 mg) in benzene with natural abundance $C_6{}^2H_1{}^1H_5$ as internal reference at δ 7.15 p.p.m. (a) Sparteine derived from (*R*)-[1-^{2}H]cadaverine (**8**). (b) Sparteine derived from (*S*)-[1-^{2}H]cadaverine (**9**).

due to the appearance of ^2H label at C-2α and C-2β, respectively. Angustifoline (**7**) displays one less ^2H n.m.r. signal from each precursor, owing to the absence of C-15. It is clear that ^2H enters at C-2 and C-15 of sparteine and at C-15 of lupanine with retention of configuration. This is in accord with the finding that the corresponding C–N bonds remain intact in the biosynthetic pathway to sparteine[3,4] and to lupanine.§

§ Three [1-^{15}N,1-^{13}C]cadaverine (**2**) units are incorporated to about the same extent into lupanine (**5**), 13-hydroxylupanine (**6**), and angustifoline (**7**). The presence of two ^{13}C–^{15}N doublets in the $^{13}C\{^1H\}$ n.m.r. spectra associated with C-2 and C-15 of lupanine and 13-hydroxylupanine, and one ^{13}C–^{15}N doublet for C-2 of angustifoline is analogous to the labelling pattern observed for sparteine.[3,4,12]

The presence of ^2H at C-17α in all three alkaloids (**3**), (**5**), and (**7**) after feeding (*R*)-[1-^{2}H]cadaverine (**8**) clearly demonstrates that 17-oxosparteine cannot be an intermediate in the biosynthetic pathway to any of these alkaloids. It has also been postulated that lupanine (**5**) is a precursor for sparteine (**3**) in quinolizidine alkaloid biosynthesis.[13] This hypothesis is no longer tenable because ^2H appears at C-2α and C-2β in sparteine (**3**) from the two feeding experiments with the precursors (**8**) and (**9**), respectively.

It has been suggested that the C_{15} quinolizidine alkaloids are modified trimers of Δ^1-piperideine.[14] Our results could be accommodated by this theory, which implies the presence of (**10**) as an intermediate in the biosynthetic pathway. From our results, formation of this intermediate (**10**) would require the loss of ^2H from the position destined to become C-10 in sparteine after feeding (*R*)-[1-^{2}H]cadaverine (**8**), and loss of ^2H from the carbon which becomes C-17 after feeding the (*S*)-isomer (**9**). Stereospecific attack of a hydride donor on the C-*re* (β) faces of both iminium ions in (**10**) would then lead to the three alkaloids (**3**), (**5**), and (**7**) labelled with ^2H at C-17α from precursor (**8**), and with ^2H at C-10α from the precursor (**9**). Further work is required to establish the detailed biosynthetic pathway from cadaverine to the tetracyclic quinolizidine alkaloids.

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