# Stereochemistry of Quinolizidine Alkaloid Biosynthesis: Incorporation of the Enantiomeric [2-$^2$H]Cadaverines into Lupinine

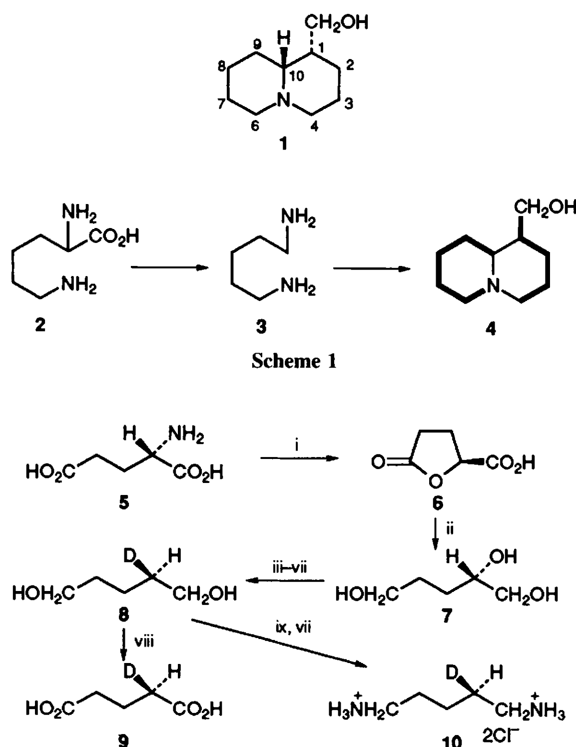
**David J. Robins* and Gary N. Sheldrake**

*Department of Chemistry, University of Glasgow, Glasgow UK G12 8QQ*

Samples of (*R*)- and (*S*)-[2-$^2$H]cadaverines **10** and **12** prepared from L and D-glutamic acid, respectively, were fed to *Lupinus luteus* plants and the labelling patterns in lupinine **1** determined by $^2$H NMR spectroscopy demonstrated that the quinolizidine ring system is formed by removal of the *pro-S* hydrogen and retention of the *pro-R* hydrogen at C-1 of lupinine.

Lupinine **1** is the simplest of the quinolizidine alkaloids produced by many species of the plant family Fabaceae (formerly Leguminosae).[1] Experiments with $^{13}C_2$-lysine,[2] [$^{13}$C–$^{15}$N]cadaverine,[3] and [1,2-$^{13}C_2$]cadaverine[4] have shown that lupinine is derived from two molecules of lysine **2** *via* cadaverine **3** and only one of the C–N bonds remains intact as in **4** (Scheme 1). The stereochemistry of the enzymic processes involved in lupinine **1** biosynthesis concerning the fate of the hydrogen atoms at the 1- and 5-positions of cadaverine **3** has been established using (*R*)- and (*S*)-[1-$^2$H]cadaverine.[5] We now report the synthesis of (*R*)- and (*S*)-[2-$^2$H]cadaverines **10** and **12** and their use to detemine the stereochemistry of the formation of the quinolizidine ring system.

L-Glutamic acid **5** was converted into the lactonic acid **6**[6] (63%) which was reduced to give the optically active triol **7** (66%).[7] The primary hydroxy groups of **7** were selectively protected (95%) and the secondary hydroxy group was converted into the mesylate (86%). Displacement of the mesylate with deuteride with inversion of configuration afforded disilylated deuteriated diol (37%) together with the labelled diol **8** (30%) probably arising by deprotection during the work up. The disilylated material was converted into the diol **8** *via* the diacetate[8] (82%). The overall yield of **8** was 60%. A portion of the diol **8** was oxidized to (*R*)-[2-$^2$H]glutaric acid **9**.[9] The remaining diol **8** was converted by the method of Fabiano *et al.*[10] into (*R*)-[2-$^2$H]cadaverine dihydrochloride **10**



Scheme 1

**Scheme 2** *Reagents and conditions*: i, $NaNO_2/H_2SO_4$; ii, $LiAlH_4$, THF, 3 d; iii, $Bu^tMe_2SiCl$, $Et_3N$, DMAP, $CH_2Cl_2$–MeCN; iv, MsCl, $Et_3N$, $CH_2Cl_2$; v, $LiAlD_4$, $Et_2O$; vi, $FeCl_3$, $Ac_2O$; vii, HCl; viii, chromic acid; ix, $HN_3$, $Pr^iOCON_2CO_2Pr^i$, $Ph_3P$, THF

in 75% yield. Samples of (*S*)-[2-$^2$H]glutaric acid **11** and (*S*)-[2-$^2$H]cadaverine dihydrochloride **12** were made in the same way from D-glutamic acid. The CD curves of **9** and **11** were analogous to those obtained previously for (*R*)- and (*S*)-[2-$^2$H]succinic acid,[9] and mass spectral data on the corresponding glutaric anhydrides indicated about 96% $^2H_1$ species.

An initial feeding experiment was carried out with [2,2,4,4-$^2H_4$]cadaverine dihydrochloride (200 mg) with a $^2H_4$-content of 92% (prepared as described previously[11]) with [1,5-$^{14}$C]cadaverine dihydrochloride (1.9 μCi). This mixture was fed to ten *Lupinus luteus* plants by the wick method over a nine day period and after a further 14 days lupinine **1** (79 mg) and sparteine (28 mg) were isolated and separated by preparative TLC[2] with specific incorporations of 0.73 and 0.68%, respectively. The labelling pattern **13** of the (−)-sparteine, established by $^2$H NMR spectroscopy, was similar to that reported previously for (+)-sparteine after feeding the same precursor mixture to *Baptisia australis* and confirms that deuterium is lost from the carbons (C-7 and C-9) destined to become bridgehead positions during the biosynthesis.[11] A full assignment of the $^1$H NMR spectrum of lupinine **1** was recently reported[12] using homonuclear $^1$H NOE difference, 2D $\delta_H/\delta_H$ COSY and direct $\delta_C/\delta_H$ correlation experiments. Some of the literature assignments[13] were reversed. The $^2$H NMR spectrum of lupinine from the feeding experiment with [2,2,4,4-$^2H_4$]cadaverine dihydrochloride showed seven labelled sites consistent with the labelling pattern **14**, although

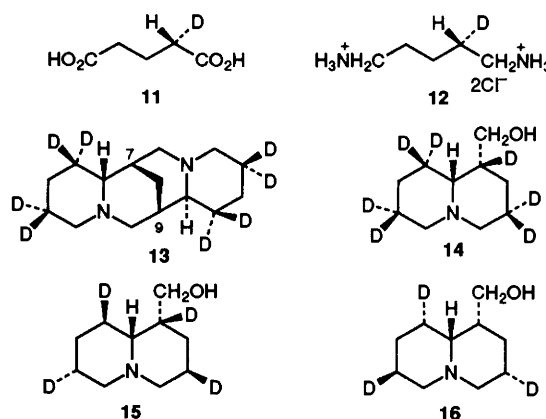


**Table 1** Chemical shift data ($^1H^a$ and $^2H^b$ chemical shifts in ppm) for the incorporation of $^2$H-labelled precursors **10** and **12** into lupinine **1**

| Site | $^1$H for **1**[12] | $^2$H for **15** | $^2$H for **16** |
|---|---|---|---|
| 1 | 1.24 | 1.24 | |
| 3α | 2.35 | | 2.33 |
| 3β | 1.36 | 1.39 | |
| 7α | 1.42 | 1.39 | |
| 7β | 1.34 | | 1.31 |
| 9α | 1.73 | | 1.68 |
| 9β | 1.26 | 1.30 | |

$^a$ 600 MHz $^1$H NMR spectrum of lupinine in $C_6{}^2H_6$ with $C_6{}^2H_5{}^1H_1$ as internal reference at δ 7.20. $^b$ 30.72 or 55.28 MHz $^2$H NMR spectra in $C_6H_6$ with $C_6{}^2H_1{}^1H_5$ as internal reference at δ 7.20.

the closeness of some of the signals (Table 1) made a complete assignment difficult. The presence of seven signals indicates that only one $^2$H is lost during the conversion of two molecules of cadaverine into lupinine.

Each $^2$H-labelled precursor **10** and **12** (85 and 10 mg, respectively) was fed with [1,5-$^{14}$C]cadaverine dihydrochloride (*ca.* 1 μCi) to eight *Lupinus luteus* plants over a seven day period. After a further six days the plants were harvested and lupinine and sparteine were isolated and purified. Lupinine (38 mg) isolated after feeding the (*R*)-isomer had a $^{14}$C specific incorporation of 0.12%. The $^2$H NMR spectrum of lupinine taken in benzene at 60 °C corresponded to lupinine labelled as shown **15** (Table 1). Lupinine (43 mg) obtained after feeding the (*S*)-isomer **12** possessed a $^{14}$C specific incorporation of 0.13% and the $^2$H NMR spectrum is consistent with the labelling pattern **16** (Table 1). Although the chemical shifts for C-7α, C-7β and C-3β of lupinine are similar, it is reasonable to assume that the deuterium atoms which appear at these positions have stayed intact during the biosynthesis with their original stereochemistry as observed with other quinolizidine alkaloids.[5] Low specific incorporations (0.03 and 0.04%, respectively) were observed for the samples of sparteine (14 and 19 mg, respectively) in these experiments and their $^2$H NMR spectra were not recorded.

It is clear that the quinolizidine ring system is formed by the loss of the *pro-S* hydrogen and retention of the *pro-R* hydrogen at the carbon which becomes C-1 of lupinine. It is of interest to note that the opposite stereochemical outcome is observed in the formation of the pyrrolizidine ring system in rosmarinine.[14] The presence of $^2$H at C-1 and C-3 of lupinine indicates that no imine-enamine equilibria are involved in the biosynthetic pathway to remove $^2$H from these positions in lupinine as happens at the corresponding positions (C-7 and C-9) in sparteine **13** and other quinolizidine alkaloids.[11] Further information about the stereochemical course of the enzymic reactions involved in the biosynthesis of other quinolizidine alkaloids should be obtained by using the enantiomeric precursors **10** and **12**.

We thank Dr D. S. Rycroft (University of Glasgow) and Dr I. H. Sadler (University of Edinburgh) for recording the NMR spectra and the SERC for use of the highfield NMR service and for a Research Assistantship (to G. N. S.). We are grateful to Dr A. F. Drake (University of London) for running the CD spectra.

*Received, 16th March 1994; Com. 4/01583E*

## References

1 *Chemotaxonomy of the Leguminosae*, ed. J. B. Harborne, D. Boulter and B. L. Turner, Academic Press, London and New York, 1971.
2 J. Rana and D. J. Robins, *J. Chem. Res. (S)*, 1984, 164.
3 W. M. Golebiewski and I. D. Spenser, *Can. J. Chem.*, 1985, **63**, 2707; J. Rana and D. J. Robins, *J. Chem. Soc., Perkin Trans. 1*, 1986, 1133.
4 D. J. Robins and G. N. Sheldrake, *J. Chem. Res.*, 1987 (*S*), 256; (*M*), 2101; A. M. Fraser, D. J. Robins and G. N. Sheldrake, *J. Chem. Soc., Perkin Trans 1*, 1988, 3275.
5 W. M. Golebiewski and I. D. Spenser, *J. Am. Chem. Soc.*, 1984, **106**, 1441; A. M. Fraser and D. J. Robins, *J. Chem. Soc., Perkin Tans. 1*, 1987, 105.
6 O. H. Gringore and F. P. Rouessac, *Org. Synth.*, 1985, **63**, 121.
7 O. Cervinka and L. Hub, *Collect. Czech. Chem. Commun.*, 1968, **33**, 2927.
8 B. Ganem and V. R. Small, *J. Org. Chem.*, 1974, **39**, 3728.
9 E. K. Kunec and D. J. Robins, *J. Chem. Soc., Perkin Trans. 1*, 1987, 1089.
10 E. Fabiano, B. T. Golding and M. M. Sadeghi, *Synthesis*, 1987, 190.
11 D. J. Robins and G. N. Sheldrake, *J. Chem. Res. (S)*, 1988, 230.
12 D. S. Rycroft, D. J. Robins and I. H. Sadler, *Magn. Reson. Chem.*, 1992, **30**, S15.
13 W. M. Golebiewski, *Bull. Pol. Acad. Sci., Chem.*, 1986, **34**, 191.
14 H. A. Kelly and D. J. Robins, *J. Chem. Soc., Perkin Trans. 1*, 1987, 2195.