Incorporation of the Enantiomeric [1-^{2}H]Cadaverines into the Quinolizidine Alkaloid (−)-Anagyrine in *Anagyris foetida*

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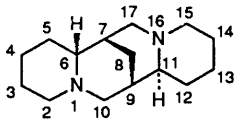
The labelling patterns in (−)-anagyrine **13** derived biosynthetically from (*R*)- and (*S*)-[1-^{2}H]-cadaverine **5** and **6**, respectively in *Anagyris foetida* have been established by ^{2}H NMR spectroscopy. Comparison of the composite labelling pattern **14** with that **11** published for (+)-sparteine has distinguished between two possible pathways for conversion of a tetracyclic intermediate into (−)-anagyrine.

Quinolizidine alkaloids are produced by many species in the plant family Leguminosae.[1] Most of the tetracyclic quinolizidine alkaloids have an 8β-bridge as in (−)-sparteine **1**. The



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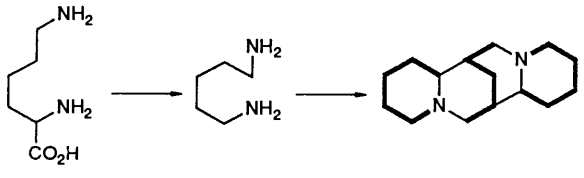
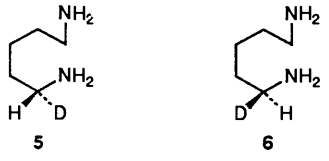
biosynthesis of (−)-sparteine has been shown to proceed from three units of L-lysine **2** *via* cadaverine **3** according to results from experiments using precursors doubly labelled with ^{13}C–^{13}C,[2] and ^{13}C–^{15}N.[3,4] The three cadaverine units are incorporated into (−)-sparteine **4** as shown by heavy lines (Scheme 1) and two of the C–N bonds remain intact.



2 **3** **4**

Scheme 1

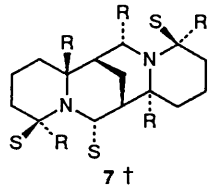
Information about the stereochemistry of the enzymic processes involved in the construction of (−)-sparteine **1** was provided when (*R*)- and (*S*)-[1-^{2}H]cadaverine (**5** and **6**) were prepared as



5 **6**

dihydrochlorides and fed to *Lupinus luteus*.[5,6] From the composite labelling pattern **7** for (−)-sparteine it was clear that where the C–N bonds remain intact, the ^{2}H labels are retained with their original stereochemistry on C-2 and C-15 adjacent to the nitrogen atoms. At the other end of these two cadaverine units oxidation had occurred with removal of the *pro-S* hydrogens. In the cadaverine unit providing the central portion of (−)-sparteine **1** the *pro-R* hydrogen is retained at one end (C-17) and the *pro-S* hydrogen is kept at the other (C-10). The mechanism involved in joining the three units are still not known. However, the appearance of ^{2}H at C-17 in (−)-sparteine **7** after feeding the (*R*)-isomer **5** served to dismiss the theory[7] that 17-oxosparteine is a key intermediate in sparteine biosynthesis.

The plant *Baptisia australis* (Leguminosae) produces (+)-sparteine **8**, (−)-*N*-methylcytisine **9** and (−)-cytisine **10**,



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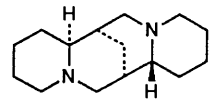

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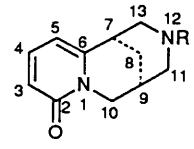
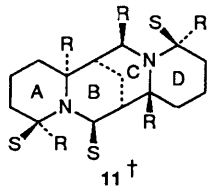
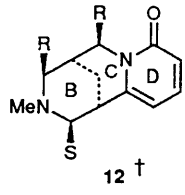


9 R = Me
10 R = H

and there is evidence that tricyclic alkaloids are formed from tetracyclic intermediates in the biosynthetic pathway.[8] Samples of (*R*)- and (*S*)-[1-^{2}H]cadaverine (**5** and **6**) as dihydrochlorides were fed to *B. australis*, and the labelling patterns in the quinolizidine alkaloids **8**–**10** were determined by ^{2}H NMR spectroscopy.[2] Comparison of the labelling patterns for (+)-sparteine **11** and (−)-sparteine **7** showed that the stereochemistry of the deuterium atoms at C-2 and C-15 was preserved as these C–N bonds remain intact, whereas the rest of the labels are in mirror image positions. Further comparison of the labelling patterns in (+)-sparteine **11** and (−)-*N*-methylcytisine **12**



11 †



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suggested that if (+)-sparteine and (−)-*N*-methylcytisine are formed from the same tetracyclic intermediate then ring A (as in **11**) must be degraded and ring D must be converted into a

† Representation of composite labelling pattern: R denotes ^{2}H present after feeding (*R*)-[1-^{2}H]cadaverine **5** dihydrochloride and S denotes ^{2}H present after feeding (*S*)-[1-^{2}H]cadaverine **6** dihydrochloride in separate experiments

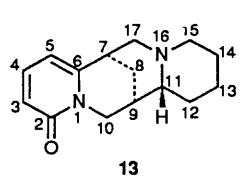
Table 1 Hydrogen NMR parameters[a] of anagyrine

Position	Sample[b] 1	2	3	4	
14β	0.90	1.06			
12β	0.92	1.08			
13β	1.23	1.40[c]			
14α	1.35	1.53[c]			
8*pro-R*	1.43	1.57			
13α	1.64	1.78			
12α	1.65	1.79			
8*pro-S*	1.77	1.92	1.97	1.99	
9	1.91	2.06	2.08	2.10	
17β	2.23	2.37	2.39	2.41	2.41*
15α	2.39	2.55	2.63*		
15β	2.49	2.64			2.67*
11	2.65	2.79	2.80	2.79* 2.82	
7	2.74	2.88	2.89	2.91	
17α	3.15	3.29	3.33	3.36	
10α	3.64	3.79	3.82	3.84	
10β	3.82	3.97	4.03	4.02* 4.04	
5	5.75	5.90	5.88	5.89	
3	6.17	6.33	6.35	6.37	
4	7.03	7.18			

[a] Obtained from 4.7 T spectra unless stated otherwise; chemical shifts δ_H in ppm relative to the solvent signal $CHCl_3$ or $CDCl_3$ at 7.25. Values marked * are deuterium chemical shifts. [b] Sample 1 was used for the 2D COSY and direct δ_C–δ_H correlation experiments:[10] solvent, $CDCl_3$; temperature, 298 K. Sample 2 was sealed up in the NMR tube and also used to obtain the 600 MHz spectrum:[10] solvent, $CDCl_3$; temperature, 298 K. Sample 3 was derived from (*R*)-[1-^{2}H]cadaverine **5**: solvent, $CHCl_3$; temperature, 333 K. Sample 4 was derived from (*S*)-[1-^{2}H]-cadaverine **6**: solvent, $CHCl_3$; temperature, 333 K. [c] Parameters obtained from the 600 MHz spectrum.

pyridone in order to product the observed labels at C-10 and C-13 in (−)-*N*-methylcytisine **12**.

(−)-Anagyrine **13** is a tetracyclic quinolizidine alkaloid found in several species of *Lupinus* and *Genista*.[1] No biosynthetic experiments have been reported on (−)-anagyrine, but comparison of its structure with (+)-sparteine **8**, in particular the stereochemistry at C-11, would suggest that if (−)-anagyrine and (+)-sparteine are formed from a tetracyclic intermediate with the same absolute configuration at C-6 and C-11, then it is likely to be ring A of a tetracyclic intermediate (as in **11**) that would be converted into a pyridone to form (−)-anagyrine **13**.



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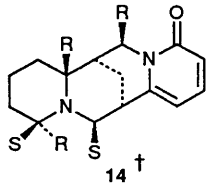

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Results and Discussion

The plant *Anagyris foetida* produces (−)-anagyrine **13** as the major alkaloidal constituent together with (−)-*N*-methylcytisine **9**.[1] The absolute configuration of (−)-anagyrine was established by degradation to (+)-epilupinine.[9]

Complete assignment of the 200 and 600 MHz ^{1}H NMR spectra of (−)-anagyrine **13** was carried out previously.[10] The conformation of the alkaloid was established by consideration of the proton–proton spin–spin coupling constants and from nuclear Overhauser enhancement measurements.[10] Ring B has an envelope conformation, and rings C and D are in chair conformations with a *cis*-decalin type of ring fusion. Some

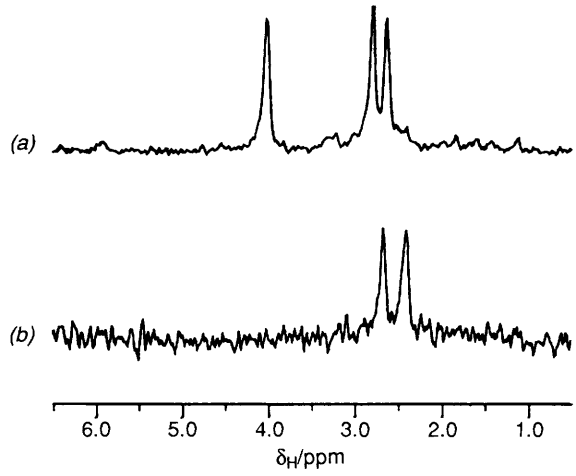

Fig. 1 30.72 MHz ^{2}H{^{1}H} NMR spectrum of (−)-anagyrine in chloroform at 60 °C: (*a*), sample of (−)-anagyrine derived from (*R*)-[1-^{2}H]cadaverine **5**; (*b*), sample of (−)-anagyrine derived from (*S*)-[1-^{2}H]cadaverine **6**

sample dependence of the NMR chemical shifts relative to the solvent reference was observed for (−)-anagyrine, but the shifts relative to each other were virtually constant. This did mean that it was important to run the ^{1}H NMR spectrum of the solution used to obtain the ^{2}H NMR spectrum. Hydrogen NMR chemical shifts are shown in Table 1 for four different samples of (−)-anagyrine. The first sample was used to obtain the 2D COSY and direct δ_C–δ_H correlation spectra.[10] The second sample was sealed in an NMR tube and was also used to measure the 600 MHz ^{1}H NMR spectrum; the parameters obtained from the 200 and 600 MHz NMR spectra were essentially the same. Samples three and four were (−)-anagyrine biosynthetically derived from (*R*)- and (*S*)-[1-^{2}H]cadaverine (**5** and **6**), respectively.

Samples of (*R*)- and (*S*)-[1-^{2}H]cadaverine (**5** and **6**) were prepared as described previously and isolated as their dihydrochlorides.[6] The ^{2}H content of the two samples was estimated to be 90 and 85% $^2H_1$ species, respectively, by analysis of their ^{1}H NMR and mass spectra. Each ^{2}H-labelled precursor was mixed with [1,5-^{14}C]cadaverine dihydrochloride prior to feeding to eight *A. foetida* plants by the wick method. After harvesting the plants, the alkaloids were isolated and separated by preparative TLC.

The (−)-anagyrine isolated after feeding (*R*)-[1-^{2}H]cadaverine **5** had a ^{14}C specific incorporation of 0.79% per C_5 unit. The ^{2}H NMR spectrum taken in chloroform showed three peaks at δ 2.63, 2.79 and 4.02 [Fig. 1(*a*)] corresponding to (−)-anagyrine **14** labelled with ^{2}H at C-10β, C-11 and C-15α. The (−)-anagyrine obtained after feeding the (*S*)-[1-^{2}H]cadaverine **6** afforded a ^{14}C specific incorporation of 1.39% per C_5 unit, and the ^{2}H NMR spectrum in chloroform contained two signals at δ 2.41 and 2.67 [Fig. 1(*b*)]. This indicates that the (−)-anagyrine **14** is labelled with ^{2}H at C-15β and C-17β. (Although the chemical shifts for C-15α and β are similar, it is reasonable to assume that the deuterium which appears at these positions has stayed intact during the biosynthesis with its original stereochemistry as observed with other quinolizidine alkaloids.[5,6])

It is immediately clear from comparison of the composite labelling patterns for (−)-anagyrine **14** and (+)-sparteine **11**, that if they are formed from a similar tetracyclic intermediate, then it must be ring D (as in **11**) that is converted into a pyridone. This is the same orientation as required for the formation of the pyridone in (−)-*N*-methylcytisine **9**. *N*-Methylcytisine was also isolated from *Anagyris foetida* during this work, and samples from each feeding experiment were

shown to have the same labelling pattern **12** as previously established.

The result observed with the feeding experiments to give ^{2}H-labelled anagyrine is the opposite to that expected. Conversion of ring A of the tetracylic intermediate required to form (+)-sparteine **11** into a pyridone would preserve the β-configuration at C-11. This result suggests that the tetracyclic intermediate involved in (−)-anagyrine formation has different stereochemistry to (+)-sparteine at C-6. It is not clear if (−)-anagyrine and (−)-*N*-methylcytisine are both formed from the same tetracyclic intermediate [different to that used in the formation of (+)-sparteine], or if the formation of (−)-anagyrine from a tetracyclic intermediate with the stereochemistry of the A–B ring junction opposite to that shown in **11** prevents further metabolism of (−)-anagyrine to (−)-*N*-methylcytisine.

Experimental

General.—Organic solutions were dried with anhydrous magnesium sulphate and solvents were evaporated under reduced pressure below 40 °C. ^{2}H NMR spectra were obtained on a Bruker WP-200SY spectrometer operating at 30.72 MHz. Radiochemicals were obtained from Amersham International. Radioactivity was measured with a Philips liquid scintillation analyser using Ecoscint (National Diagnostics) solutions. Sufficient counts were accumulated to give a standard error of less than 1% for each determination. Radioactive solutions were counted in duplicate. A Panax thin-layer scanner RTLS-1A was used for the radioscanning of TLC plates. TLC 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.[11]

Samples of (*R*)- and (*S*)-[1-^{2}H]cadaverine (**5** and **6**) dihydrochloride were prepared as described.[6]

Feeding Experiments.—Plants of *Anagyris foetida* were grown in pots in a greenhouse from seeds supplied by Professor M. Wink. Eight plants were used for each experiment. Samples of [1,5-^{14}C]cadaverine dihydrochloride (2 μCi, <1 mg) were added to the precursors **5** (191 mg) and **6** (211 mg). The precursor mixtures were dissolved in sterile water and pulse fed by the wick method on alternate days over a period of 12 d. The plants were harvested after a further 12 d and the alkaloid mixtures were extracted as described.[2] Analytical TLC revealed two radioactive bands for the mixtures from each feeding experiment which were coincident with unlabelled (−)-anagyrine **13** (R_F 0.80) and (−)-*N*-methylcytisine **9** (R_F 0.75). The alkaloid mixtures were carefully separated by preparative TLC. Anagyrine was obtained as an oil: 368 mg from feeding the (*R*)-precursor **5**, and 230 mg after feeding the (*S*)-isomer **6**; $[\alpha]_D$ $-164\ 10^{-1}$ deg cm^2 g^{-1} (*c* 1 in EtOH) (lit.,[12] $[\alpha]_D$ $-165.5\ 10^{-1}$ deg cm^2 g^{-1} in EtOH) (Found: M^+ 244.1565. $C_{15}H_{20}N_2O$ requires *M*, 244.1576). (−)-*N*-Methylcytisine was crystallised from benzene, m.p. 135–136 °C (lit.,[2] 135–137 °C). The ^{1}H and ^{2}H NMR spectral details for (−)-anagyrine are given in Table 1.

Acknowledgements

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References

1 *Chemotaxonomy of the Leguminosae*, ed. J. B. Harborne, D. Boulter and B. L. Turner, Academic Press, London and New York, 1971.
2 D. J. Robins and G. N. Sheldrake, *J. Chem. Res.* (*S*), 1987, 256; (*M*), 1987, 2101; A. M. Fraser, D. J. Robins and G. N. Sheldrake, *J. Chem. Soc., Perkin Trans. 1*, 1988, 3275.
3 W. M. Golebiewski and I. D. Spenser, *J. Am. Chem. Soc.*, 1984, **106**, 7925.
4 J. Rana and D. J. Robins, *J. Chem. Soc., Perkin Trans. 1*, 1986, 1133; *J. Chem. Res.* (*S*), 1985, 196.
5 W. M. Golebiewski and I. D. Spenser, *Can. J. Chem.*, 1985, **63**, 2707.
6 A. M. Fraser and D. J. Robins, *J. Chem. Soc., Perkin Trans. 1*, 1987, 105.
7 M. Wink, T. Hartmann and H. M. Schiebel, *Z. Naturforsch., Teil C*, 1979, **34**, 704.
8 Y. D. Cho, R. O. Martin and J. N. Anderson, *J. Am. Chem. Soc.*, 1971, **93**, 2087.
9 S. Okuda, K. Tsuda and H. Kataoka, *Chem. Ind.*, 1961, 1751.
10 D. S. Rycroft, D. J. Robins and I. H. Sadler, *Magn. Reson. Chem.*, in the press.
11 R. Munier and M. Macheboef, *Bull. Soc. Chim. Biol.*, 1951, **33**, 846.
12 S. Okuda, H. Kataoka and K. Tsuda, *Chem. Pharm. Bull.*, 1965, **13**, 491.

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