Stereochemistry of Pyrrolizidine Alkaloid Biosynthesis: Incorporation of Chiral [2-^{2}H]Putrescines into Retrorsine

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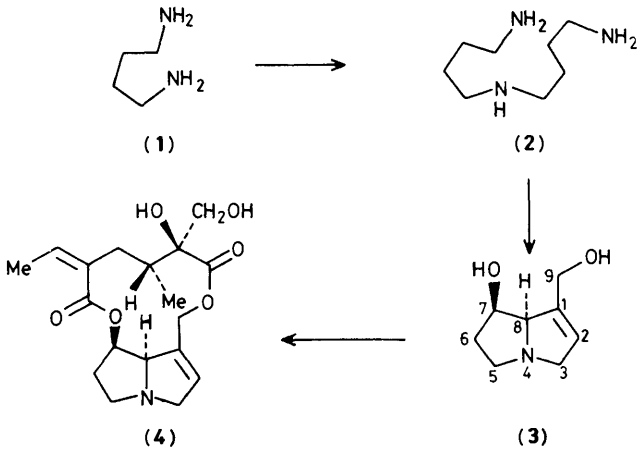
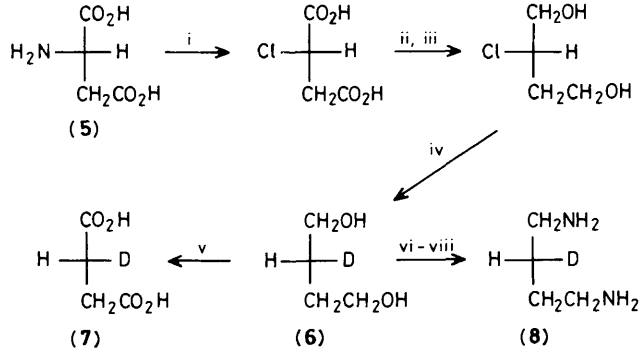
Feeding experiments with (2*R*)- and (2*S*)-[2-^{2}H]putrescine on *Senecio isatideus* plants have shown that hydroxylation at C-7 of retronecine proceeds with retention of configuration, and formation of the 1,2-double bond involves removal of the *pro-S* hydrogen and retention of the *pro-R* hydrogen at C-2 of retronecine.

Retrorsine (**4**) is one of many pyrrolizidine alkaloids that contain retronecine (**3**) as the base portion.[1,2] The C_8 skeleton of retronecine is derived biosynthetically from L-ornithine or L-arginine[3] *via* putrescine (**1**).[4—7] A later intermediate in the biosynthetic pathway is homospermidine (**2**), formed from two molecules of putrescine (Scheme 1).[6,8] We have shown that ^{2}H n.m.r. spectroscopy can be used to establish complete labelling patterns in retrorsine derived biosynthetically from [1,4-$^2H_4$]- and [2,3-$^2H_4$]-putrescine in *S. isatideus* plants.[9] With the latter precursor, ^{2}H is retained at C-2, C-6α, C-6β, and C-7α of retronecine (**3**). The stereochemical course of a number of enzymic processes involving removal of hydrogen



Scheme 1



Scheme 2. *Reagents:* i, HCl, HNO_3, urea; ii, $SOCl_2$, MeOH; iii, di-isobutylaluminium hydride; iv, $LiAlD_4$; v, chromic acid; vi, HBr; vii, NaN_3; viii, $LiAlH_4$.

from the 1- and 4-positions of putrescine has been established using chiral [1-^{2}H]putrescine.[10,11] We have now prepared samples of chiral [2-^{2}H]putrescine and used them to obtain ^{2}H-labelled retrorsine. The stereochemical course of further enzymic processes in retronecine biosynthesis has been established.

(2*R*)-[2-^{2}H]Succinic acid (**7**) was prepared from (2*S*)-aspartic acid (**5**) as outlined by Arigoni and Eliel (Scheme 2).[12] An o.r.d. curve of this material (**7**) was in accord with literature values,[12,13] and mass spectral data on the deuteriated succinic anhydride indicated *ca.* 98% $^2H_1$ species. The immediate precursor of the deuteriated succinic acid (**7**), (2*R*)-[2-^{2}H]butane-1,4-diol (**6**) was converted into (2*R*)-[2-^{2}H]putrescine (**8**) dihydrochloride (Scheme 2) [^{2}H{^{1}H} n.m.r. (H_2O) δ 1.76 p.p.m. (s)]. Analogous treatment of (2*R*)-aspartic acid yielded samples of (2*S*)-[2-^{2}H]succinic acid and (2*S*)-[2-^{2}H]putrescine dihydrochloride. The latter sample contained an impurity of *ca.* 15% [1-^{2}H]putrescine dihydrochloride [from ^{2}H{^{1}H} n.m.r. (H_2O) δ 3.08 p.p.m. (s)]. This impurity may have arisen by lithium aluminium deuteride reduction of a small amount of incompletely reduced diester, left after the reduction with di-isobutylaluminium hydride (Scheme 2). Each precursor (200 mg) together with [1,4-^{14}C]putrescine dihydrochloride (5 μCi) was pulse fed into the xylems of four *S. isatideus* plants over a period of two weeks. After a further two weeks, retrorsine (**4**) was isolated and recrystallised to constant specific radioactivity.

The ^{2}H{^{1}H} n.m.r. spectrum[9] of retrorsine derived from (2*R*)-[2-^{2}H]putrescine (**8**) was obtained in chloroform at 60 °C

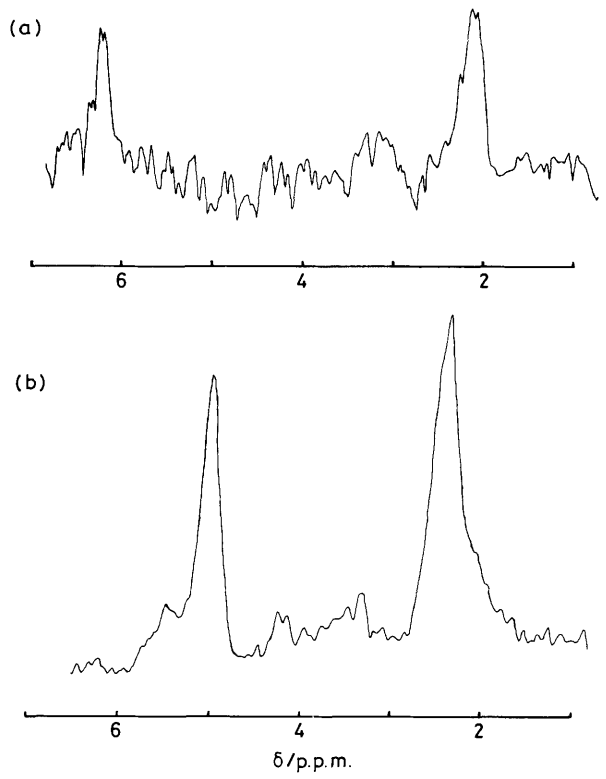


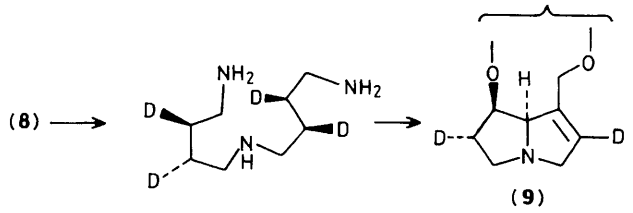
Figure 1. 30.72 MHz ^{2}H{^{1}H} N.m.r. spectra of retrorsine in chloroform at 60 °C: (a) sample of (**9**) derived from (2*R*)-[2-^{2}H]-putrescine (**8**); (b) sample of (**11**) derived from (2*S*)-[2-^{2}H]putrescine (**10**). Natural abundance ^{2}H in $CHCl_3$ was the internal reference at δ 7.15.

[Figure 1(a)]. This showed two major signals at δ 2.15 and 6.20 p.p.m. corresponding to retrorsine (**9**) labelled with ^{2}H at C-6α and C-2, respectively (Scheme 3). The enrichment factors† for the two labelled sites are nearly equal at *ca.* 0.18% ^{2}H. The specific incorporation of ^{2}H per C_4 unit is therefore 0.18% [0.18/2 × 2/98 × 100%, where 98/2 atom % ^{2}H is the average enrichment of ^{2}H at each labelled site of putrescine (**8**)]. This value is about half the ^{14}C specific incorporation of 0.30% per C_4 unit. Removal of ^{2}H from the carbon atoms destined to become C-1 and C-7 in retronecine accounts for this loss of about half of the ^{2}H from (2*R*)-[2-^{2}H]putrescine (**8**) on its conversion into retrorsine (**9**).‡

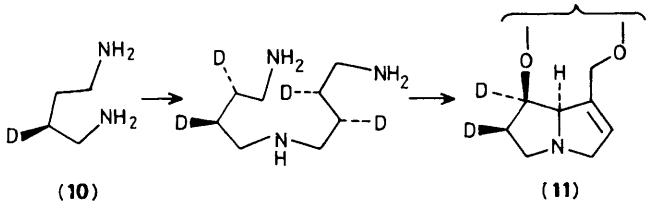
Using (2*S*)-[2-^{2}H]putrescine (**10**) as a precursor, a sample of retrorsine was obtained which showed two major signals in the ^{2}H{^{1}H} n.m.r. spectrum, obtained in chloroform at 60 °C, at δ 2.38 and 5.00 p.p.m. [Figure 1(b)]. This corresponds to retrorsine (**11**) labelled with ^{2}H at C-6β and C-7α, respectively (Scheme 4). Enrichment factors† of 0.34% ^{2}H for C-6β and 0.20% for C-7α were estimated, leading to a specific incorporation of ^{2}H of 0.65% (0.54/2 × 2/83 × 100%) for the C_4 unit in which most of the ^{2}H appears. This value is similar to the ^{14}C specific incorporation of 0.73% per C_4 unit.‡ About half of the

† The enrichment factor for a labelled site in retrorsine is calculated from [(integral of labelled site in retrorsine) −(natural abundance integral/concentration of retrorsine)]/(natural abundance integral of ^{2}H in chloroform at δ 7.25 p.p.m./concentration of chloroform) × 0.0156%.

‡ Differences between the ^{2}H and ^{14}C specific incorporations, after taking into account the loss of ^{2}H from some carbon atoms in retronecine, may be due to the operation of ^{2}H isotope effects in the biosynthetic pathway.



Scheme 3§



Scheme 4§

^2H is lost from (2*S*)-[2-^2H]putrescine (**10**) as it is transformed into retrorsine (**11**). Removal of ^2H from the carbon atoms which become C-1 and C-2 of retronecine leads to the appearance of most of the ^2H in one C_4 unit derived from putrescine.

The hydroxylation at C-7 of retronecine thus occurs with retention of configuration, in agreement with the stereospecificity generally observed for direct hydroxylation at sp^3 carbon atoms.[14] Formation of the 1,2-double bond in retronecine takes place with retention of the *pro-R* hydrogen and loss of the *pro-S* hydrogen at the carbon atom which becomes C-2 of retronecine.

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§ No molecules of putrescine can contain more than one ^2H atom. The structures in Schemes 3 and 4 are therefore representations of all the ^2H-labelled species present.

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