Stereochemistry of Pyrrolizidine Alkaloid Biosynthesis: Incorporation of Chiral [1-^{2}H]Putrescines into Retrorsine

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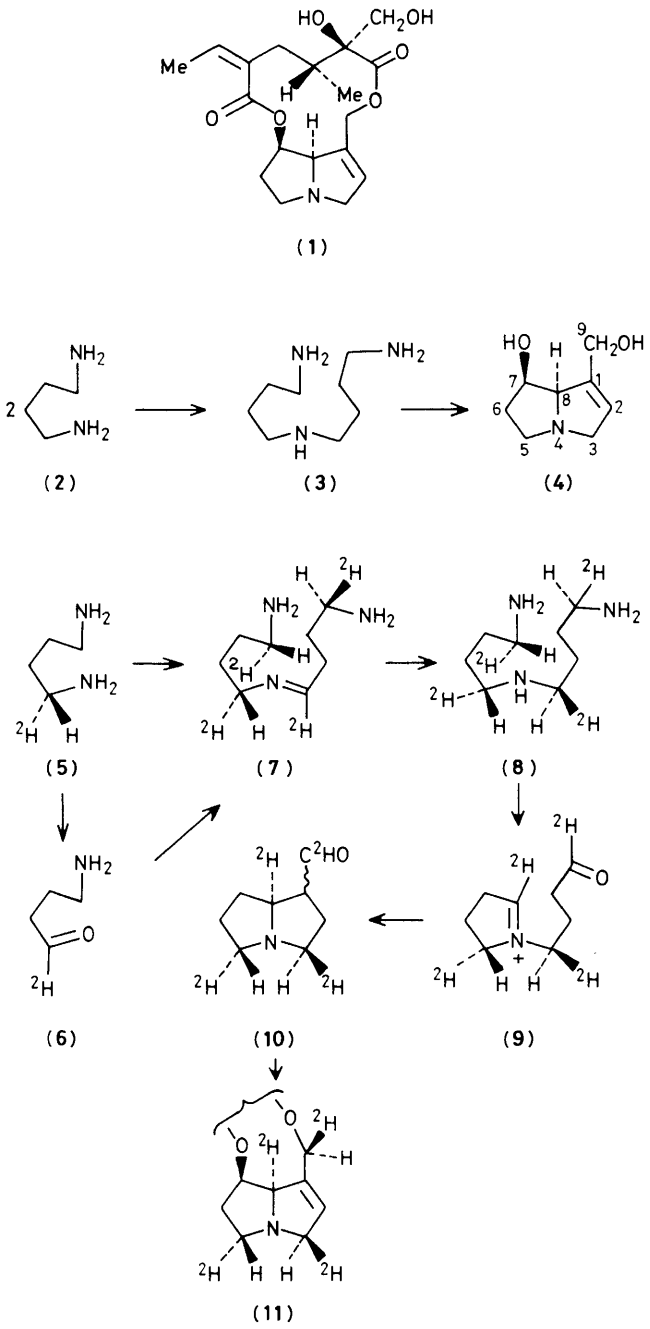
^{2}H N.m.r. spectroscopy has been used to establish the labelling patterns in retrorsine (**1**) derived biosynthetically from (*R*)-[1-^{2}H]- and (*S*)-[1-^{2}H]-putrescine; in the former case retrorsine (**11**) is equally labelled with ^{2}H at the 3β, 5α, 8α, and 9-*pro-S* positions, while with the latter precursor only the 3α and the 5β positions in retrorsine (**15**) are labelled with ^{2}H.

Many pyrrolizidine alkaloids, including retrorsine (**1**), contain retronecine (**4**) as the base portion.[1,2] Experiments with isotopically labelled compounds have shown that retronecine is derived biosynthetically from two molecules of L-ornithine or L-arginine[3] *via* putrescine (**2**),[4,5] and a symmetrical C_4–N–C_4 intermediate.[6,7] This latter symmetrical intermediate has been identified as homospermidine (**3**).[6,8] We have recently used ^{2}H n.m.r. spectroscopy to establish complete labelling patterns in retrorsine derived biosynthetically from [1,4-$^2H_4$]- and [2,3-$^2H_4$]-putrescine.[9] We have now fed chiral deuteriated putrescines to *Senecio isatideus* plants to produce samples of ^{2}H-labelled retrorsine. ^{2}H N.m.r. spectroscopy has been used to establish the stereochemical course of a number of the enzymic processes involved in retronecine biosynthesis.

(*R*)-[1-^{2}H]Putrescine (**5**) (96% $^2H_1$ species) was prepared by decarboxylation of L-ornithine in ^2H_2O with ornithine



Scheme 1.‡

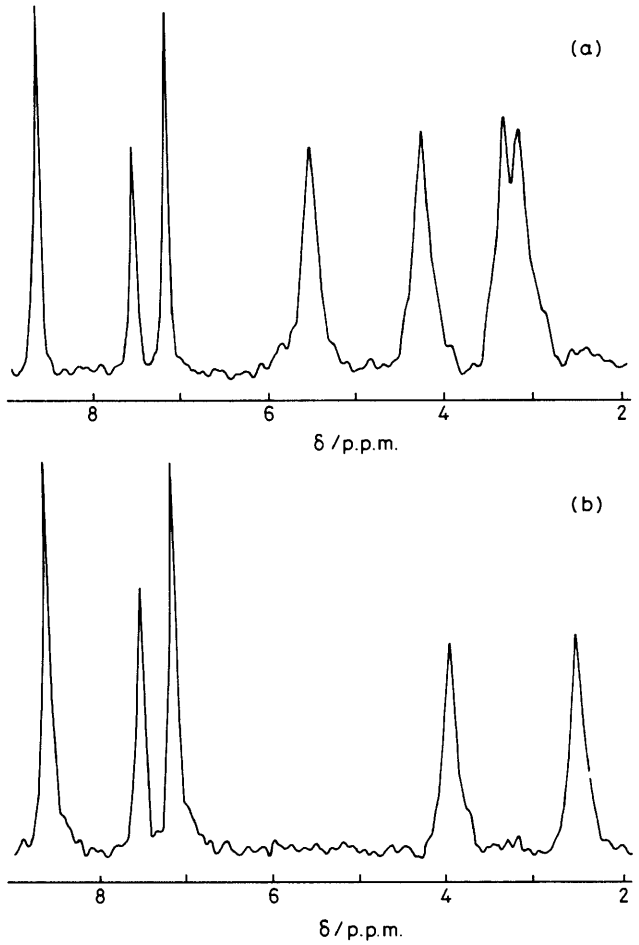


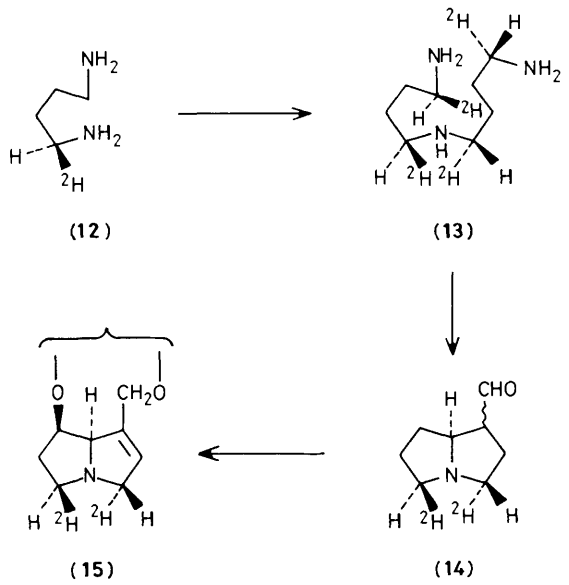
Figure 1. 30.72 MHz $^2H\{^1H\}$ N.m.r. spectra of retrorsine in pyridine at 90 °C: (a) sample of (**11**) derived from (*R*)-[1-^{2}H]putrescine (**5**); (b) sample of (**15**) derived from (*S*)-[1-^{2}H]putrescine (**12**). (Natural abundance ^2H signals for pyridine are at δ 7.2, 7.6, and 8.7 p.p.m.)

decarboxylase.[10] Similar decarboxylation of [2-^{2}H]-DL-ornithine in H_2O yielded (*S*)-[1-^{2}H]putrescine (**12**) (91% $^2H_1$ species).[10] Both samples were converted into their dihydrochlorides, $^2H\{^1H\}$ n.m.r. (H_2O) δ 3.08 p.p.m. (s). Each precursor (150 mg) together with [1,4-$^{14}C_2$]putrescine dihydrochloride (25 μCi) was pulse fed into the xylems of four *S. isatideus* plants over a two week period. After a further two weeks, retrorsine (**1**) was isolated and recrystallised to constant specific radioactivity.

With (*R*)-[1-^{2}H]putrescine (**5**) as precursor, the $^2H\{^1H\}$ n.m.r. spectrum[9] of retrorsine taken in pyridine at 90 °C [Figure 1(a)] showed four signals at δ 3.15, 3.30, 4.25, and 5.52 p.p.m. corresponding to retrorsine (**11**) labelled with ^2H at C-3β, C-5α, C-8α, and C-9 *pro-S*. (The two signals at δ 3.15 and 3.30 p.p.m. could not be resolved when the ^2H n.m.r. spectrum was taken in chloroform at 65 °C). The enrichment factors† for the four labelled sites are nearly equal at *ca.* 1.05% ^2H. The specific incorporation of ^2H per C_4 unit is therefore 2.1% (1.05 × 2/96 × 100%), which is in good agreement with the ^{14}C specific incorporation of 2.0% per C_4 unit. These results suggest that no ^2H is lost from (*R*)-[1-^{2}H]-putrescine (**5**) on its conversion into retrorsine. An explanation for these observations is outlined in Scheme 1. It is known that diamine oxidase removes the *pro-S* hydrogen stereospecifically from the methylene group of primary amines.[11] The initial step in retronecine biosynthesis probably involves the oxidation of putrescine to 4-aminobutanal. The transformation of (**5**) → (**6**) would thus occur with retention of all the ^2H. Coupling between (**5**) and (**6**) is then envisaged to produce an imine (**7**). Stereospecific reduction of this imine with attack of a hydride donor on the *si*-face of the imine is

‡ No molecules of putrescine can contain more than one ^2H atom. The structures (**7**)—(**11**) and (**13**)—(**15**) are therefore representations of all the ^2H-labelled species present.

† The enrichment factor for a labelled site in retrorsine is calculated from {[(integral of labelled site in retrorsine) − (natural abundance contribution)]/(concentration of retrorsine)}/(natural abundance integral of ^2H in pyridine at δ 7.6 p.p.m./concentration of pyridine) × 0.0156%.



Scheme 2.‡

then required to form homospermidine (**8**). (The opposite mode of reduction would lead to homospermidine equally labelled at C-3α, C-3β, C-5α, and C-5β, because of the symmetry of the molecule). Two further enzymic oxidations on homospermidine (**8**) with removal of the *pro-S* hydrogens would then yield a dialdehyde or its cyclised form (**9**). Mannich cyclisation of (**9**) would produce 1-formylpyrrolizidine (**10**). Finally, stereospecific reduction of the aldehyde (**10**) with attack of a hydride donor on the *re*-face of the carbonyl group would lead to retrorsine labelled as shown in structure (**11**). The feasibility of part of the proposed pathway (Scheme 1) has been demonstrated by a synthesis of unlabelled (**10**) from (**3**) using diamine oxidase and physiological conditions.[12]

The feeding experiment with (*S*)-[1-^{2}H]putrescine (**12**) as precursor yielded a sample of retrorsine with a ^{14}C specific incorporation of 1.9% per C_4 unit. The ^{2}H{^{1}H} n.m.r. spectrum of this sample taken in pyridine at 90 °C [Figure 1(b)] showed only two signals at δ 2.55 and 3.95 p.p.m. corresponding to retrorsine labelled with ^{2}H at C-3α and C-5β. The enrichment factors† for these two labelled sites are both *ca.* 0.6% ^{2}H. The specific incorporation of ^{2}H is thus 1.3% per C_4 unit (0.6 × 2/91 × 100%). The loss of ^{2}H relative to ^{14}C from the precursor (**12**) is due to the removal of the (*S*)-[1-^{2}H] atoms during the three oxidation steps of primary amine to aldehyde (**12**) → (**14**) (Scheme 2). The ^{2}H is only retained when it is next to the nitrogen atom that is incorporated into the pyrrolizidine ring. This leads to the appearance of ^{2}H at C-3α and C-5β in retrorsine (**15**) because of the symmetry of the intermediate (**13**).

These results are consistent with the following stereochemical details in retronecine biosynthesis. Initial oxidation of putrescine to 4-aminobutanal takes place with loss of the *pro-S* hydrogen. Reduction of the imine formed by coupling of putrescine with 4-aminobutanal occurs by hydride attack on the *si*-face of the imine to yield homospermidine. Two further oxidation steps each result in the removal of *pro-S* hydrogens to give a dialdehyde. Cyclisation of the iminium ion (**9**) occurs by attack on the *re*-face of the iminium ion to yield the 8α-pyrrolizidine (**10**). Finally, reduction of the aldehyde takes place on the *re*-face of the carbonyl group.§

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§ *Note added in proof:* The mode of incorporation of (*R*)- and (*S*)-[1-^{2}H]putrescine into a mixture of pyrrolizidine alkaloids has recently been determined, G. Grue-Sorensen and I. D. Spenser, *J. Am. Chem. Soc.*, 1983, **105**, 7401.