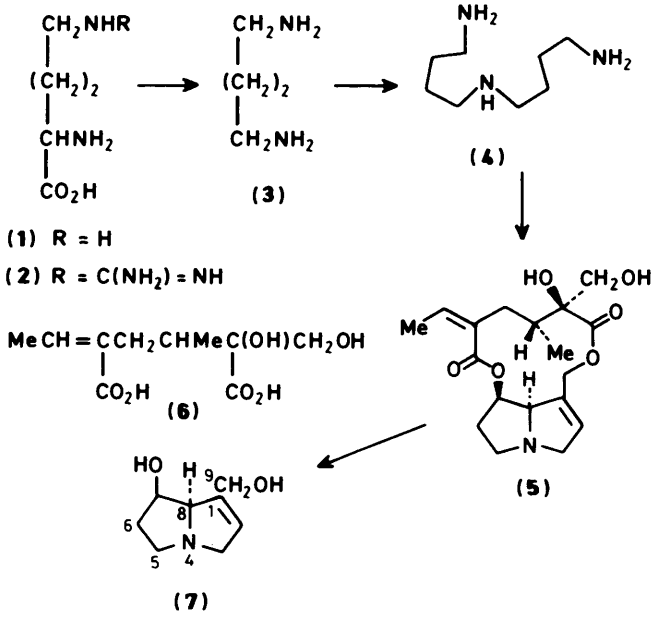
Application of ^2H N.M.R. Spectroscopy to Study the Incorporation of ^2H-Labelled Putrescines into the Pyrrolizidine Alkaloid Retrorsine[1]

Jatinder Rana and David J. Robins*
Department of Chemistry, University of Glasgow, Glasgow G12 8QQ

The biosynthesis of the retronecine (**7**) portion of the pyrrolizidine alkaloid retrorsine (**5**) has been studied in *Senecio isatideus* plants using ^2H-labelled putrescines. The labelling pattern in retrorsine (**9**) derived biosynthetically from [2,2,3,3-$^2H_4$]putrescine (**8**), established by ^2H n.m.r. spectroscopy, showed ^2H to be present at C-2, C-6α, C-6β, and C-7α. The retention of ^2H at C-7α indicates that the introduction of the hydroxy group at the 7-position of retronecine does not involve keto or enol intermediates. The use of [1,1,4,4-$^2H_4$]-putrescine (**10**) produced a sample of retrorsine (**12**) which gave ^2H n.m.r. spectroscopic signals chiefly for C-3α, C-3β, and the C-9 *pro-S* position. The operation of deuterium isotope effects is used to explain this labelling pattern. The formation of (9*S*)-[9-$^2H_1$]retrorsine (**12**) is consistent with stereospecific attack of a hydride donor on the *re*-face of an aldehyde precursor. (*R*)-[1-$^2H_1$]Putrescine (**19**) afforded retrorsine (**21**) equally labelled with ^2H at C-3β, C-5α, C-8α, and C-9 *pro-S*, while (*S*)-[1-$^2H_1$]putrescine (**22**) yielded retrorsine (**24**) with only the C-3α and C-5β positions labelled with ^2H. The stereochemistry of a number of the enzymic processes involved in retronecine biosynthesis has been established.

There is much interest in pyrrolizidine alkaloids because of their toxicity and wide distribution in a number of plant families.[2] Many of these alkaloids including retrorsine (**5**) contain retronecine (**7**) as the base portion of a macrocyclic dilactone system. Retrorsine is the major alkaloidal constituent of *Senecio isatideus* plants. Experiments with radioactive precursors have shown that two molecules of ornithine (**1**),[3–7] arginine (**2**),[6,7] and putrescine (**3**)[5,7] are required to form retronecine (Scheme 1), although incomplete labelling patterns were obtained for the distribution of radioactivity in the labelled alkaloids. This problem was overcome, and complete labelling patterns were obtained for retronecine from ^{13}C n.m.r. spectra after feeding experiments employing ^{13}C-labelled putrescines.[8,9] Furthermore, the use of [^{13}C-^{15}N]-labelled putrescines demonstrated that a later symmetrical intermediate formed from two molecules of putrescine is involved in retronecine biosynthesis.[9–11] Evidence that this later intermediate is homospermidine (**4**) has been provided by use of homospermidines labelled with ^{14}C[10,12] and ^{13}C,[13] in feeding experiments with *S. isatideus* plants. Retronecine is also known to be derived from L-ornithine and L-arginine rather than the D-enantiomers.[14] An attempt has been made to establish further stereochemical details of the biosynthetic pathway to retronecine by using ornithines specifically labelled with tritium.[15] However, only partial labelling patterns could be obtained by degradation of the samples of retronecine formed biosynthetically from [4-$^3H_1$]- and [5-$^3H_1$]-ornithine. The incompleteness of the degradation pathways for retronecine together with the lack of purity of one of the ^3H-precursors severely limited the usefulness of this approach. We believed more convincing evidence for the fate of hydrogen atoms in retronecine biosynthesis could be provided by the use of ^2H-labelled precursors in conjunction with ^2H n.m.r. spectroscopy on the retrorsine samples produced.



Scheme 1.

Results and Discussion

The dihydrochloride of [2,2,3,3-$^2H_4$]putrescine (**8**) was made by exchanging the hydrogens of succinonitrile for deuterium by heating in deuterium oxide, followed by catalytic hydrogenation of the [2,2,3,3-$^2H_4$]succinonitrile and acidification of the product with hydrochloric acid. The putrescine sample (**8**) was estimated to contain >99% $^2H_4$ species by analysis of the ^1H n.m.r. spectra of the dihydrochloride of (**8**) and of the di-(phenylaminothiocarbonyl) derivative of (**8**). A sterile aqueous solution of a mixture of the dihydrochloride of (**8**) and [1,4-$^{14}C_2$]putrescine dihydrochloride was introduced into the xylems of *Senecio isatideus* plants through stem punctures.[7] Two weeks after completion of the feeding, the plants were harvested, and retrorsine (**5**) was extracted and recrystallised to constant specific radioactivity and the specific ^{14}C incorporation was calculated (see Table, expt. 1).

All of the protons in the retronecine portion of unlabelled retrorsine (**5**) were assigned from the 360 MHz ^1H n.m.r.

Table. Incorporation of ^{2}H-labelled putrescines into retrorsine (**5**) in *Senecio isatideus* plants.

Expt.	Precursor	Quantity fed (mg)	No. of plants	Length of expt. (weeks)	% ^{14}C Specific incorporation[a] in (**5**)	% Radioactivity in Retronecine (**7**)	% Radioactivity in Isatinecic acid (**6**)
1	(**8**)	500	4	5	2.4	96	4
2	(**10**)	250	2	5	2.3	102	2
3	(**19**)	150	4	4	2.0	96	3
4	(**22**)	150	4	4	1.9	99	2

[a] Specific ^{14}C incorporation per C_4 unit is calculated from [(Molar activity of product × 0.5)/(Molar activity of precursor)] × 100%.

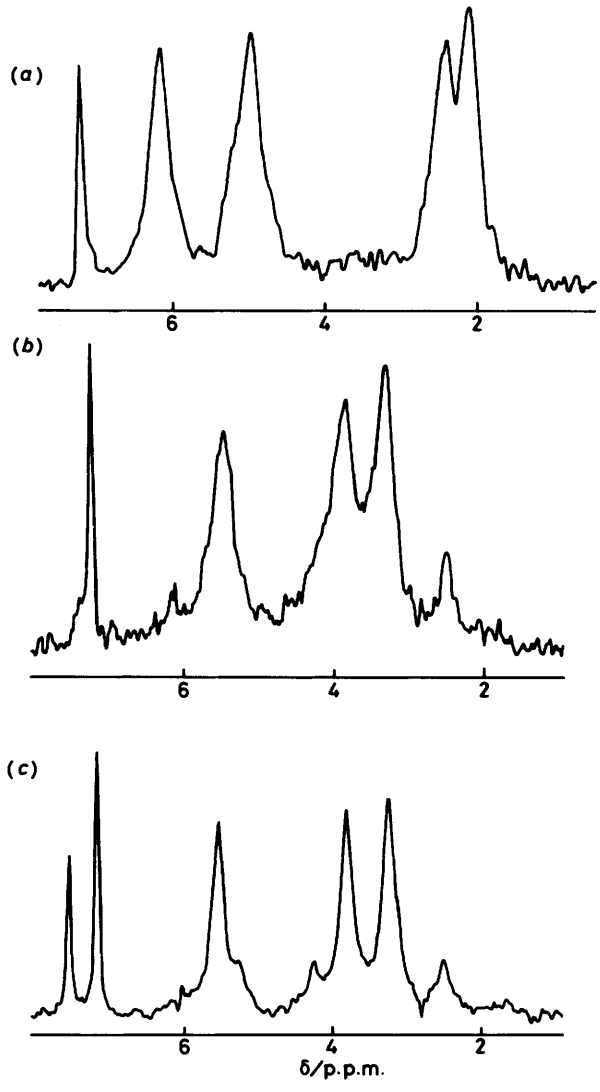
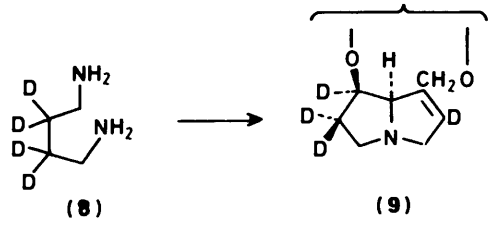


Figure 1. 30.72 MHz ^{2}H {^{1}H} N.m.r. spectrum of retrorsine (**5**) (0.5 g): (*a*) sample of compound (**9**) derived from [2,2,3,3-$^2H_4$]putrescine (**8**) in $CHCl_3$ at 60 °C; (*b*) sample of compound (**12**) derived from [1,1,4,4-$^2H_4$]putrescine (**10**) in $CHCl_3$ at 60 °C; (*c*) sample of compound (**12**) in pyridine at 90 °C (natural abundance signals for pyridine are at δ 7.2 and 7.6)

spectrum taken in deuteriochloroform using selective ^{1}H decoupling and nuclear Overhauser enhancement (n.O.e.) experiments. For example, irradiation at δ 2.53 (5β-H) produced a much larger n.O.e. on the signal at δ 3.38 (*ca.* 25%), than that at δ 3.93 (*ca.* 4%), indicating that these should be assigned to 3β-H and 3α-H, respectively. It was also important to be certain of the assignment for the prochiral hydrogens at C-9 in retrorsine (**5**). There is a large chemical shift difference of 1.41 p.p.m. between these prochiral hydrogens. From previous ^{1}H n.m.r. spectroscopic studies,[16] supported by *X*-ray data on retrorsine,[17] the 9-H *pro-S* is believed to be deshielded by being in the plane of both the adjacent carbonyl and the double bond. This assignment was confirmed by observation of an n.O.e. at δ 5.50 (*ca.* 12%) when the hydrogen at δ 6.20 (2-H) was irradiated. A similar effect at δ 4.09 p.p.m. (*ca.* 11%) (9-H *pro-R*) was observed when the hydrogen at δ 4.26 (8-H) was irradiated.

The ^{2}H {^{1}H} n.m.r. spectra of the ^{2}H-labelled samples of retrorsine taken in chloroform showed very broad bands, but it was found that increased temperatures led to considerable narrowing of the signals.[18] Therefore, the ^{1}H and ^{2}H {^{1}H} n.m.r. spectra of retrorsine derived from feeding [2,2,3,3-$^2H_4$]putrescine (**8**) dihydrochloride to *S. isatideus* plants were obtained in chloroform at 60 °C and compared [Figure 1(*a*)]. Four signals were present at δ 2.15, 2.40, 5.0, and 6.20, corresponding to retrorsine (**9**) labelled with ^{2}H at C-6α, C-6β, C-7α, and C-2, respectively (Scheme 2). The four sites are



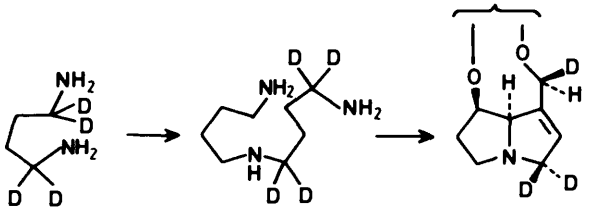
Scheme 2.

labelled to *ca.* the same extent with enrichment factors* for ^{2}H of 1.9 ± 0.1%. Thus the specific incorporation of ^{2}H into retrorsine per C_4 unit of putrescine is also *ca.* 1.9%. This is somewhat lower than the value for the ^{14}C specific incorporation of 2.4 ± 0.1%. This can be explained by the existence of intermolecular ^{2}H isotope effects on removal of one ^{2}H atom at the position which becomes C-7; two ^{2}H atoms must be removed from the carbon destined to become C-1; and one ^{2}H atom from the carbon which becomes C-2 of retrorsine. It should be noted that the presence of ^{2}H at C-7α in retrorsine (**9**) confirms that the introduction of the hydroxy group does not involve a keto or enol intermediate.[15]

The dihydrochloride of [1,1,4,4-$^2H_4$]putrescine (**10**) was prepared by catalytic reduction of succinonitrile under an atmosphere of deuterium gas, followed by acidification of the

* The enrichment factor for a labelled site in retrorsine is calculated from (integral of labelled site in retrorsine/concentration of retrorsine)/(natural abundance integral of ^{2}H in $CHCl_3$ at δ 7.25/concentration of $CHCl_3$) × 0.0156%.

product with hydrochloric acid. Analysis of the ^1H n.m.r. spectra of this material and of the di(phenylaminothiocarbonyl) derivative of the free base indicated that the sample of putrescine (**10**) contained *ca.* 96% of a $^2H_4$ species. A feeding experiment with the dihydrochloride of (**10**) on *Senecio isatideus* plants produced a sample of ^2H-labelled retrorsine. The ^2H $\{^1H\}$ n.m.r. spectrum of this material was obtained in chloroform at 60 °C. Three main signals were present at δ 3.35, 3.90, and 5.45 [Figure 1(*b*)] corresponding to retrorsine (**12**) labelled with ^2H at C-3β, C-3α, and C-9 *pro-S*, respectively (Scheme 3). The three sites are labelled to about the same extent



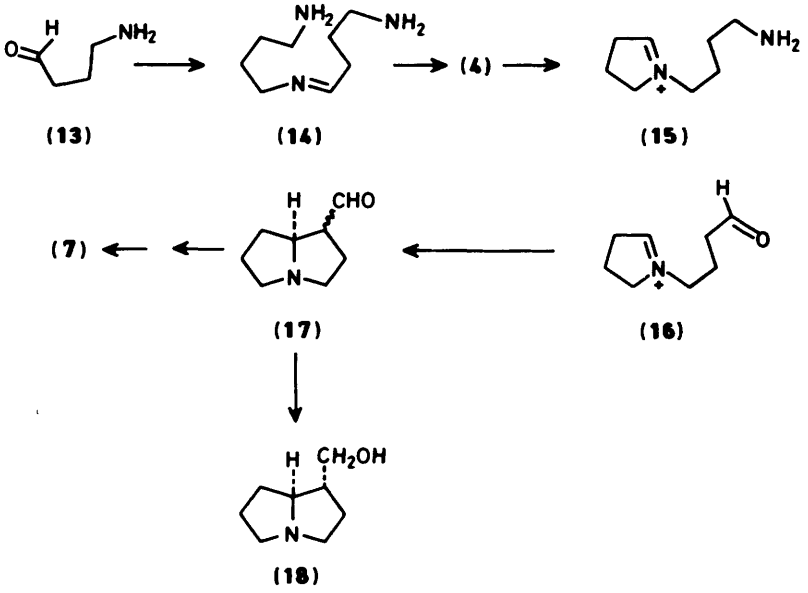
Scheme 3.

with enrichment factors of 1.5 ± 0.1% ^2H. Sharper signals were observed when the ^2H $\{^1H\}$ n.m.r. spectrum of compound (**12**) was taken in pyridine at 90 °C [Figure 1(*c*)] and smaller signals due to ^2H at C-5β and C-8 at δ 2.53 and 4.26, respectively, could be distinguished with enrichment factors of *ca.* 0.3% ^2H. The specific incorporation per C_4 unit is thus 0.5(1.5 + 0.3) × 100/96 = 0.9%. The likely presence of a signal for ^2H at C-5α at δ 3.25 could not be established because of the large resonance due to ^2H at C-3β (δ 3.35). It is clear however, that *ca.* 5/6 of the ^2H is located in the right hand half of the base portion [as in compound (**12**)].

A possible explanation for this result follows from consideration of the likely biosynthetic pathway to retronecine, outlined in Scheme 4, and the involvement of ^2H isotope effects during the biosynthesis. Homospermidine (**4**) could be produced after oxidation of putrescine by a diamine oxidase to 4-aminobutanal (**13**), then coupling of (**13**) with another molecule of putrescine, followed by reduction of the imine (**14**). Further oxidation of homospermidine (**4**) by diamine oxidase would afford the aldehyde (**16**) which could undergo a Mannich-type cyclisation to give the pyrrolizidine aldehyde (**17**). Reduction with a dehydrogenase would yield the saturated pyrrolizidine base trachelanthamidine (**18**), while further steps are required to form retronecine (**7**). Support for this proposed pathway comes from the recent synthesis of trachelanthamidine (**18**) from homospermidine using a diamine oxidase to give the aldehyde (**17**), followed by reduction with a coupled dehydrogenase system.[19] The initial oxidation of [1,1,4,4-$^2H_4$]putrescine (**10**) to [1,4,4-$^2H_3$]-4-aminobutanal catalysed by hog kidney diamine oxidase is known to be subject to an intermolecular ^2H isotope effect of 1.26.[20] If a similar effect operates with the diamine oxidase in *Senecio isatideus* plants, the most likely ^2H-labelled homospermidine species to be produced would be (**11**) formed by the reaction of [1,1,4,4-$^2H_4$]putrescine (**10**) with endogenous unlabelled 4-aminobutanal. This intermediate (**11**) is probably subject to an appreciable intramolecular ^2H isotope effect in the oxidation step (hog kidney diamine oxidase has a four times greater preference for oxidation of the unlabelled end of [1,1-$^2H_2$]putrescine[20]). The unlabelled end of the homospermidine (**11**) will then be preferentially converted into an aldehyde, leading to the intermediate (**15**), containing ^2H in the side chain. Retronecine will then be formed with most of the ^2H in the right hand portion [as in compound (**12**)].

Furthermore, the formation of (9*S*)-[9-$^2H_1$]retronecine (**12**) establishes the stereochemistry of the reduction of the aldehyde precursor. The hydride donor must add the hydrogen to the *re*-face of the carbonyl (-C^{2}HO) group, if it is assumed that subsequent ester formation occurs with retention of configuration at C-9 of retronecine. This stereospecificity is the same as that observed for normal coupled dehydrogenase enzyme systems.[21]

In order to establish the stereochemistry of more of the enzymic processes involved in retronecine (**7**) biosynthesis, it was necessary to prepare chirally deuteriated putrescines. (*R*)-[1-$^2H_1$]Putrescine (**19**) was therefore made by decarboxylation



Scheme 4.

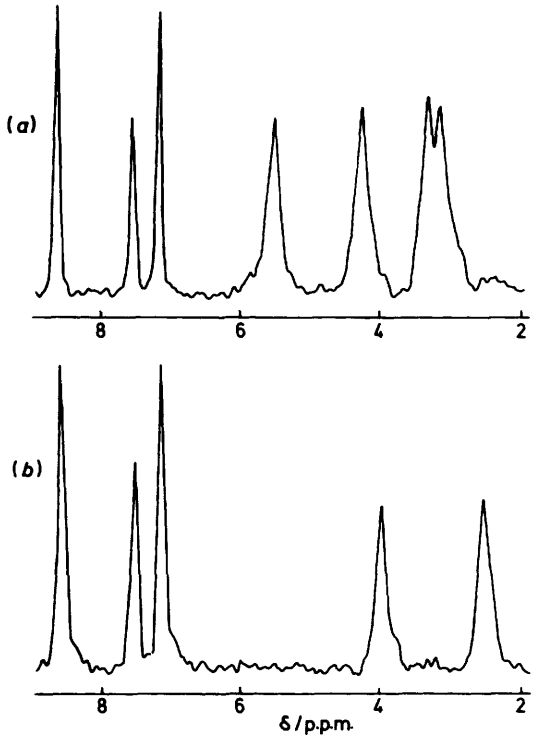
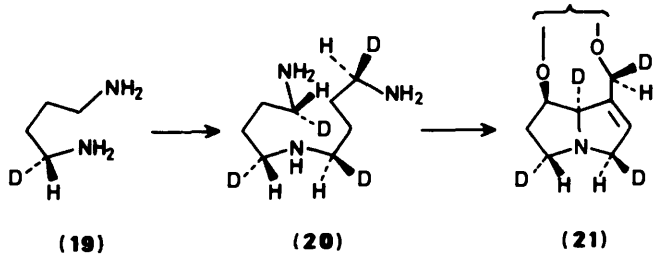


Figure 2. 30.72 MHz ^2H $\{^1H\}$ N.m.r. spectra of retrorsine (**5**) in pyridine at 90 °C: (*a*) sample of compound (**21**) derived from (*R*)-[1-$^2H_1$]putrescine (**19**); (*b*) sample of compound (**24**) derived from (*S*)-[1-$^2H_1$]putrescine (**22**) (natural abundance ^2H signals for pyridine are at δ 7.2, 7.6, and 8.7)

of L-ornithine in ^2H_2O using L-ornithine decarboxylase (E.C. 4.1.1.17).[22] This is an enzymic reaction which is known to proceed with retention of configuration.[22,23] Similar decarboxylation of DL-[2-$^2H_1$] ornithine in ^1H_2O yielded (*S*)-[1-$^2H_1$]-putrescine (**22**) and unchanged D-[2-$^2H_1$] ornithine, which were separated.[22] The ^2H content of each putrescine sample was estimated to be 96 and 91% $^2H_1$ species, respectively, by analysis of the ^1H n.m.r. spectra of the dihydrochlorides and the di(phenylaminothiocarbonyl) derivatives. Each precursor dihydrochloride together with [1,4-$^{14}C_2$]putrescine dihydrochloride was pulse fed to *Senecio isatideus* plants (Table 1, expts. 3 and 4).

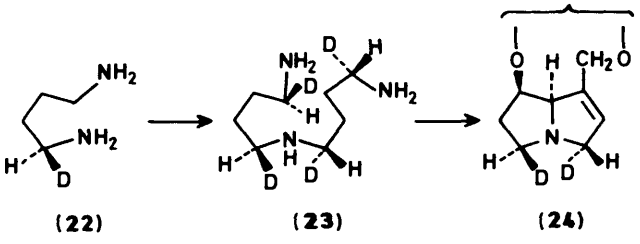
The ^2H $\{^1H\}$ n.m.r. spectrum of the retrorsine produced biosynthetically from (*R*)-[1-$^2H_1$]putrescine (**19**) was taken in pyridine at 90 °C [Figure 2(*a*)]. Four signals were visible at δ 3.15, 3.30, 4.25, and 5.52, corresponding to retrorsine (**21**) labelled with ^2H at C-5α, C-3β, C-8α, and C-9 *pro*-S, respectively (Scheme 5). The first two signals were not resolved when the ^2H



Scheme 5*

n.m.r. spectrum was taken in chloroform at 65 °C. Nearly equal enrichment factors of 1.05 ± 0.05% ^2H were observed for the four labelled sites. The specific incorporation of ^2H per C_4 unit is therefore 2.1% (1.05 × 2/96 × 100%) which agrees well with the ^{14}C specific incorporation of 2.0% per C_4 unit (Table 1, expt. 3). This agreement indicates that no ^2H is lost from the precursor (**19**) on its conversion into retrorsine (**21**). The labelling pattern can be explained by reference to Schemes 4 and 5.* It is known that the *pro-S* hydrogen is removed stereospecifically from the methylene group of primary amines by diamine oxidases.[24] The oxidation of putrescine (**3**) to 4-aminobutanal (**13**) therefore occurs with retention of all the deuterium, and coupling of compounds (**3**) and (**13**) produces the imine (**14**). Stereospecific reduction of this imine with attack of a hydride donor on the *si*-face of (**14**) is then necessary to form homospermidine labelled as shown in structure (**20**). The opposite stereospecificity of this reduction step would lead through homospermidine to retrorsine equally labelled at C-3α, C-3β, C-5α, and C-5β, because of the C_{2v} symmetry of homospermidine. Two further enzymic oxidations on homospermidine (**20**) each take place with removal of the *pro-S* hydrogen, again with retention of all the deuterium. Cyclisation of the iminium ion (**16**) occurs by attack on the *re*-face of the iminium ion to generate an 8α-pyrrolizidine aldehyde (**17**). Finally, reduction of the aldehyde function takes place by hydride attack on the *re*-face of the carbonyl group leading to retrorsine labelled as indicated in structure (**21**).

Additional support for this interpretation of the results came from the use of (*S*)-[1-$^2H_1$]putrescine (**22**) as a precursor of retronecine in *Senecio isatideus* plants. The ^2H $\{^1H\}$ n.m.r. spectrum of the retrorsine produced was taken in pyridine at 90 °C [Figure 2(*b*)]. Only two signals were observed at δ 2.55 and 3.95 corresponding to retrorsine (**24**) labelled with ^2H at C-5β and C-3α, respectively (Scheme 6).* Similar enrichment



Scheme 6*

factors for both sites were estimated at *ca.* 0.6 ± 0.05% ^2H. The specific incorporation of deuterium is thus 1.3% per C_4 unit (0.6 × 2/91 × 100%). Deuterium is evidently lost from this precursor due to the stereospecific removal of the *S*-^2H atoms during the three oxidation steps in the biosynthetic pathway involving oxidation of a primary amine to an aldehyde (Scheme 4). Deuterium is only retained when the C–N bond in putrescine remains intact in the formation of homospermidine (**23**). This leads to the appearance of deuterium at C-3α and C-5β in retrorsine (**24**) because of the C_{2v} symmetry of homospermidine. The deuterium incorporation is different from that for ^{14}C (see the Table, expt. 4), again probably due to the operation of an intramolecular deuterium isotope effect in the enzymic oxidation of the precursor (**22**) with diamine oxidase. This would favour the formation of (*S*)-[4-$^2H_1$]-4-aminobutanal over the unlabelled material produced by removal of the deuterium, leading to a sample of retrorsine (**24**) with a

* None of the molecules of putrescine can contain more than one ^2H atom. The structures (**20**), (**21**), (**23**), and (**24**) are therefore representations of all the ^2H-labelled species that are present.

retention of ^2H to ^{14}C of 1.3/1.9 = 68 ± 5%. This is higher than the figure of 50%, which would be expected from the operation of a biosynthetic pathway in the absence of any deuterium isotopic effects. The figure of 68% is in good agreement with that obtained by Grue-Sorensen and Spenser (69%). These workers observed similar patterns of incorporation for (*R*)- and (*S*)-[1-$^2H_1$]putrescines into a mixture of pyrrolizidine alkaloids in *Senecio vulgaris* plants.[25]

Samples of each ^{14}C-labelled retrorsine were hydrolysed with base to give isatinecic acid (**6**) and retronecine (**7**). Measurement of the distribution of the radioactivity showed that 96—102% of the total was in the base portion, and less than 4% resided in the acidic moiety, confirming that putrescine is a specific precursor for retronecine.

The stereochemical course of a number of steps involving removal or addition of hydrogen at the carbon atoms derived from C-1 and C-4 of putrescine have been determined by ^2H n.m.r. spectroscopy, after using ^2H-labelled putrescines to obtain samples of ^2H-labelled retrorsine. Further information about the stereochemical steps in pyrrolizidine alkaloid biosynthesis will be obtained by using other chirally deuteriated putrescines.

Experimental

M.p.s were measured with a Kofler hot-stage apparatus. Organic solutions were dried with anhydrous Na_2SO_4, and solvents were evaporated off under reduced pressure below 40 °C. ^1H N.m.r. spectra were obtained on a Bruker WH-360 spectrometer at 360 MHz and ^2H n.m.r. spectra were obtained on a Bruker WP-200SY spectrometer operating at 30.72 MHz. Mass spectra were obtained with A.E.I. MS 12 or MS 902 spectrometers. Radioactivity was measured with a Philips liquid scintillation analyser using toluene–methanol solution. Sufficient counts were accumulated to give a standard error of less than 1% for each determination. Radioactive samples were recrystallised to constant specific activity and were counted in duplicate. A Panax thin-layer scanner RTLS-1A was used for the radioscanning of t.l.c. plates.

[2,2,3,3-$^2H_4$]*Butane-1,4-diamine* (**8**) (*Putrescine*) *Dihydrochloride*.—A solution of succinonitrile (2.0 g, 25 mmol) in ^2H_2O (20 ml; Fluorochem Ltd., 99.8 atom % ^2H) was heated at reflux for 24 h. The mixture was evaporated to dryness under reduced pressure, and this procedure was repeated with fresh batches of ^2H_2O (3 × 20 ml). [2,2,3,3-$^2H_4$]Succinonitrile solidified to a clear waxy solid and was recrystallised from benzene (1.7 g, 85%), m.p. 55—56 °C (lit.,[26] 57 °C), $\nu_{max.}(CHCl_3)$ 2 250 cm^{-1}; δ_H ($CDCl_3$) no signals detected; $\delta_D(CHCl_3)$ 2.78 (s); *m/z* 84 and 56 (Found: M^+ 84.0620. Calc. for $C_4{}^2H_4N_2$: 84.0622). [2,2,3,3-$^2H_4$]Succinonitrile (1.5 g, 17.85 mmol) was added to a suspension of platinum oxide (225 mg) in glacial acetic acid (60 ml), and the mixture was stirred under a hydrogen atmosphere for 3 days. The mixture was filtered, and concentrated to a syrup. This syrup was dissolved in 2M-HCl and the solution was evaporated to dryness. The dihydrochloride of [2,2,3,3-$^2H_4$]-butane-1,4-diamine (**8**) was crystallised from aqueous ethanol (1.8 g, 60%); $\delta_H(D_2O)$ 3.08 (s); $\delta_D(H_2O)$ 1.76 (s). The di(phenylaminothiocarbonyl) derivative had m.p. 178—179 °C (lit.,[27] 177—179 °C); δ_H[$(CD_3)_2SO$; 80 °C] 3.48 (s, 4 H), 7.25 (m, 10 H), 7.75 (s, 2 × NH), and 9.35 (s, 2 × NH); *m/z* 362 (unlabelled material *m/z* 358).

[1,1,4,4-$^2H_4$]*Butane-1,4-diamine* (**10**) *Dihydrochloride*.—Succinonitrile (1.5 g, 18.75 mmol) was added to a suspension of platinum oxide (225 mg) in perdeuterioacetic acid (Aldrich; 98 atom % ^2H; 50 ml). The mixture was stirred under a deuterium atmosphere for 3 days. The mixture was filtered and concentrated to a syrup, which was dissolved in 2M-HCl and evaporated to dryness. The dihydrochloride of [1,1,4,4-$^2H_4$]butane-1,4-diamine (**10**) was crystallised from aqueous ethanol (1.4 g, 64%); $\delta_H(D_2O)$ 1.77 (4 H, s) and 3.06 (*ca.* 0.15 H, s); $\delta_D(H_2O)$ 3.07 (s). The di(phenylaminothiocarbonyl) derivative had m.p. 178—179 °C; δ_H [$(CD_3)_2SO$; 90 °C] 1.56 (s, 4 H), 7.25 (m, 10 H), 7.75 (s, 2 × NH), and 9.35 (s, 2 × NH); *m/z* 362 (unlabelled material *m/z* 358). (R)-[1-^2H]*putrescine* (**19**) (96% $^2H_1$ species) and (S)-[1-^2H]-*putrescine* (**22**) (91% $^2H_1$ species) were prepared as described earlier.[22]

Feeding Methods.—*Senecio isatideus* plants were propagated from stem cuttings and grown on in a standard compost. The number of plants used in each experiment is shown in Table 1. Feeding experiments were commenced at the onset of flowering. [1,4-$^{14}C_2$]Putrescine dihydrochloride (10 μCi, expts. 1 and 2; 25 μCi, expts. 3 and 4; Amersham International) was added to each ^2H-labelled precursor. Aqueous solutions of the precursor were introduced into the xylems of the plants through stem punctures made with a sterile needle. Each precursor was fed on alternate days. Two weeks after administration of the precursor had been completed, the plants were harvested and retrorsine (**5**) was isolated as described previously. Retrorsine was recrystallised to constant specific activity from acetone, m.p. 214—215 °C (lit.,[7] 216—217 °C). Radioscans of silica gel G t.l.c. plates of 0.25 mm thickness developed with chloroform–methanol–conc. ammonia (85:14:1) showed one radioactive band, coincident with authentic unlabelled retrorsine at R_F 0.35. Retrorsine was visualised by oxidation with *o*-chloranil,* followed by treatment with Ehrlich's reagent.[28] δ_H (360 MHz; $CDCl_3$) (Retronecine portion only) 2.15 (1 H, m, 6α-H), 2.38 (1 H, dd, 6β-H), 2.53 (1 H, m, 5β-H), 3.25 (1 H, t, 5α-H), 3.38 (1 H, m, 3β-H), 3.93 (1 H, dd, 3α-H), 4.09 (1 H, d, 9-H *pro-R*), 4.26 (1 H, m, 8α-H), 5.00 (1 H, t, 7α-H), 5.50 (1 H, d, 9-H *pro-S*), and 6.20 (1 H, d, 2-H). The coupling constants (Hz) are: $J_{2.3\alpha}$ 1.8, $J_{2.3\beta}$ 1.7, $J_{2.8\alpha}$ 1, $J_{3\alpha.3\beta}$ 15.8, $J_{3\alpha.8\alpha}$ 1.8, $J_{3\beta.8\alpha}$ 6.2, $J_{5\alpha.5\beta}$ 9, $J_{5\alpha.6\alpha}$ 8.3, $J_{5\beta.6\alpha}$ 12, $J_{5\beta.6\beta}$ 5.8, $J_{6\alpha.6\beta}$ 13.8, $J_{6\alpha.7\alpha}$ 4, $J_{7\alpha.8\alpha}$ 4, and *J* 9 *pro-R*, 9 *pro-S* 11.8.

Hydrolysis of Retrorsine (**5**).—This was carried out as described previously[7] to yield isatinecic acid (**6**) and retronecine (**7**) hydrochloride. Both products were recrystallised to constant specific radioactivity, m.p. 144—146 °C (lit.,[29] 148 °C) and 163—164 °C (lit.,[30] 164 °C), respectively. The results obtained from feeding the ^2H-labelled putrescine precursors (**8**), (**10**), (**19**), and (**22**) are summarised in Table 1.

* *o*-Chloranil = 3,4,5,6-tetrachloro-*o*-benzoquinone

Acknowledgements

We are grateful to Dr. D. S. Rycroft (Glasgow) and Dr. I. Sadler (Edinburgh) for running the n.m.r. spectra. We thank the S.E.R.C. for a Research Assistantship (to J. R.) and for the use of the high-field n.m.r. service.

References

1 Preliminary accounts; J. Rana and D. J. Robins, *J. Chem. Soc., Chem. Commun.*, 1983, 1222; 1984, 517.
2 D. J. Robins, *Fortschr. Chem. Org. Naturst.*, 1982, **41**, 115; D. J. Robins, 'The Alkaloids,' Specialist Periodical Reports, The Royal Society of Chemistry, London, 1978—1983, vols. 8—13; D. J. Robins, *Nat. Prod. Rep.*, 1984, **1**, 235; 1985, **2**, 213.
3 E. Nowacki and R. U. Byerrum, *Life Sci.*, 1962, **1**, 157.
4 C. A. Hughes, R. Letcher, and F. L. Warren, *J. Chem. Soc.*, 1964, 4974.
5 W. Bottomley and T. A. Geissman, *Phytochemistry*, 1964, **3**, 357.

6 N. M. Bale and D. H. G. Crout, *Phytochemistry*, 1975, **14**, 2617.
7 D. J. Robins and J. R. Sweeney, *J. Chem. Soc., Chem. Commun.*, 1979, 120; *J. Chem. Soc., Perkin Trans. 1*, 1981, 3083.
8 H. A. Khan and D. J. Robins, *J. Chem. Soc., Chem. Commun.*, 1981, 146.
9 H. A. Khan and D. J. Robins, *J. Chem. Soc., Perkin Trans. 1*, 1985, 101.
10 H. A. Khan and D. J. Robins, *J. Chem. Soc., Chem. Commun.*, 1981, 554.
11 G. Grue-Sorensen and I. D. Spenser, *J. Am. Chem. Soc.*, 1981, **103**, 3208.
12 H. A. Khan and D. J. Robins, *J. Chem. Soc., Perkin Trans. 1*, 1985, 819.
13 J. Rana and D. J. Robins, *J. Chem. Res. (S)*, 1983, 146.
14 D. J. Robins and J. R. Sweeney, *Phytochemistry*, 1983, **22**, 457.
15 G. Grue-Sorensen and I. D. Spenser, *Can. J. Chem.*, 1982, **60**, 643.
16 L. B. Bull, C. C. J. Culvenor, and A. T. Dick, 'The Pyrrolizidine Alkaloids,' North-Holland, Amsterdam, 1968.
17 H. Stoeckli-Evans, *Acta Crystallogr., Sect. B*, 1979, **35**, 2798.
18 H. H. Mantsch, H. Saito, and I. C. P. Smith, in 'Progress in N.M.R. Spectroscopy,' eds. J. Emsley, J. Feeney, and L. Sutcliffe, Pergamon Press, 1976, vol. 11.
19 D. J. Robins, *J. Chem. Soc., Chem. Commun.*, 1982, 1289.
20 P. S. Callery, M. S. B. Nayar, E. M. Jakubowski, and M. Stogniew, *Experientia*, 1982, **38**, 431.
21 R. Bentley, 'Molecular Asymmetry in Biology,' Academic Press, New York, 1970, vol. 2, p. 18.
22 D. J. Robins, *Phytochemistry*, 1983, **22**, 1133; J. C. Richards and I. D. Spenser, *Can. J. Chem.*, 1982, **60**, 2810.
23 G. R. Orr and S. J. Gould, *Tetrahedron Lett.*, 1982, **23**, 3139.
24 A. R. Battersby, J. Staunton, and M. C. Summers, *J. Chem. Soc., Perkin Trans. 1*, 1976, 1052.
25 G. Grue-Sorensen and I. D. Spenser, *J. Am. Chem. Soc.*, 1983, **105**, 7401.
26 C. R. Witschonke, *Anal. Chem.*, 1954, **26**, 562.
27 T. Tarantelli, *J. Chem. Soc., Dalton Trans.*, 1974, 837.
28 H. J. Huizing, F. De Boer, and T. M. Malingré, *J. Chromatogr.*, 1980, **195**, 407; R. J. Molyneux and J. N. Roitman, *ibid.*, 1980, **195**, 412.
29 S. M. H. Christie, M. Kropman, E. C. Leisegang, and F. L. Warren, *J. Chem. Soc.*, 1949, 1700.
30 D. H. G. Crout, N. M. Davies, E. H. Smith, and D. Whitehouse, *J. Chem. Soc., Perkin Trans. 1*, 1972, 671.

Received 30*th July* 1985; *Paper* 5/1312