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

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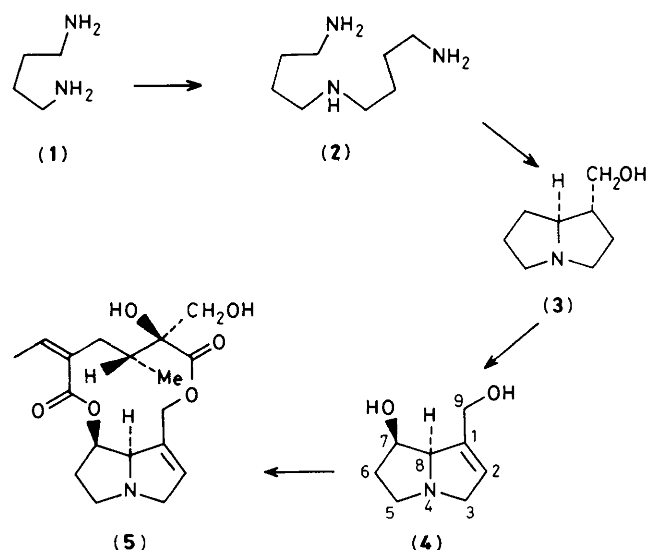
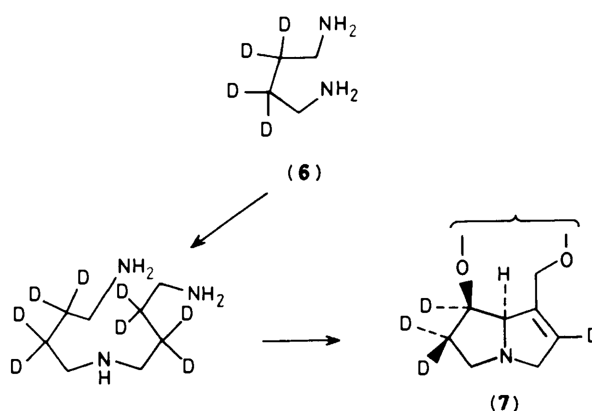
A sample of (2*R*)-[2-^{2}H]putrescine (**13**) dihydrochloride was prepared from (2*S*)-aspartic acid (**8**), and (2*S*)-[2-^{2}H]putrescine (**15**) dihydrochloride was synthesized from (2*R*)-aspartic acid. Feeding experiments carried out with these precursors on *Senecio isatideus* plants gave retrorsine (**5**) containing ^{2}H, and the distribution of ^{2}H from each experiment in retrorsine was determined by ^{2}H n.m.r. spectroscopy. All of the ^{2}H was confined to the base component of the alkaloid, retronecine (**4**). Retrorsine (**14**), derived biosynthetically from (2*R*)-[2-^{2}H]putrescine (**13**) dihydrochloride was labelled with ^{2}H at C-2 and C-6α, while retrorsine (**16**), produced from (2*S*)-[2-^{2}H]putrescine (**15**) dihydrochloride contained ^{2}H labels at C-6β and C-7α. These labelling patterns demonstrate that hydroxylation at C-7 of retronecine (**4**) proceeds with retention of configuration. In addition, the formation of the 1,2-double bond of retronecine involves removal of the *pro-S* hydrogen and retention of the *pro-R* hydrogen at the carbon atom which becomes C-2 of retronecine.

The major alkaloidal constituent of *Senecio isatideus* plants is retrorsine (**5**), which contains retronecine (**4**) as the base component (necine). Retronecine is the most commonly encountered pyrrolizidine base.[2] Experiments with ^{14}C-labelled precursors have established that retronecine is derived biosynthetically from L-ornithine or L-arginine[3] *via* putrescine (**1**) (Scheme 1).[4-7] Two molecules of putrescine combine to form homospermidine (**2**).[6,8] The pyrrolizidine ring is believed to be formed by a Mannich type cyclisation after oxidation of the primary amino groups in homospermidine to the corresponding dialdehyde.[9] Reduction of the 1-formylpyrrolizidine leads to the next intermediate which has been established in the biosynthetic pathway, trachelanthamidine (**3**).[10,11] In order to gain more information about the processes involved in the conversion of putrescine (**1**) into retronecine (**4**), samples of [1,4-^{2}H$_4$]- and [2,3-^{2}H$_4$]-putrescine (**6**) dihydrochlorides were fed to *S. isatideus* plants and the labelling patterns in retrorsine (**5**) were established by ^{2}H n.m.r. spectroscopy.[12] The stereochemical results of the enzymic processes concerned with removal of hydrogen from the 1- and 4-positions of putrescine were established using the enantiomeric [1-^{2}H]putrescines, which were prepared enzymically.[13,14] We had observed that the feeding experiment with [2,3-^{2}H$_4$]putrescine (**6**) dihydrochloride had given a sample of retrosine (**7**) which contained ^{2}H at C-2, C-6α, C-6β, and C-7α (Scheme 2). In order to determine the stereochemical consequences of the enzymic processes involving removal of hydrogen from the 2- and 3-positions of putrescine as it is converted into retronecine, the enantiomeric [2-^{2}H]-putrescines have been prepared chemically from optically active starting materials.



Scheme 1.

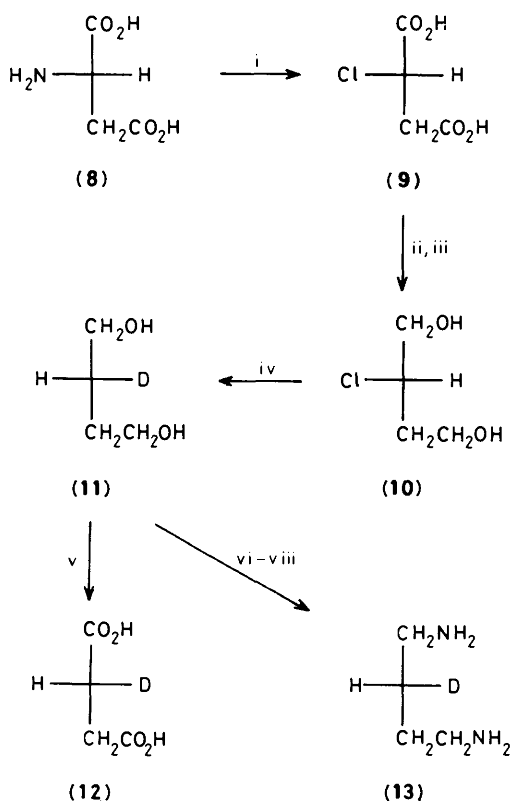


Scheme 2.

Results and Discussion

In order to prepare samples of the enantiomeric [2-^{2}H]-putrescines, it was decided to modify and extend one of the existing routes to the enantiomeric [2-^{2}H]butanedioic (succinic) acids. A number of routes to specifically deuteriated and tritiated succinic acids are available; most of these employ an enzymatic step.[15] A simple chemical route to (2*R*)- and (2*S*)-[2-

^{2}H]succinic acid starting from readily available (2*S*)- and (2*R*)-aspartic acid has been summarised by Arigoni and Eliel.[16] Treatment of (2*S*)-aspartic acid (**8**) with a mixture of hydrochloric and nitric acids in the presence of urea leads to replacement of the amino group by chlorine with retention of configuration (Scheme 3).[17] In the original route, this material (**9**) was reduced with lithium aluminium deuteride to give (2*R*)-[1,1,2,4,4-$^2H_5$]butane-1,4-diol, which was oxidised to (2*R*)-[2-^{2}H]succinic acid (**12**) with Jones' reagent. In order to avoid the insertion of undesired deuterium atoms at the 1- and 4-positions of putrescine, the (2*S*)-chlorosuccinic acid (**9**) was converted into the diester, and the ester groups were selectively reduced with di-isobutylaluminium hydride. Then treatment of the (2*S*)-2-chlorobutane-1,4-diol (**10**) with lithium aluminium deuteride introduced one deuterium atom with inversion of configuration

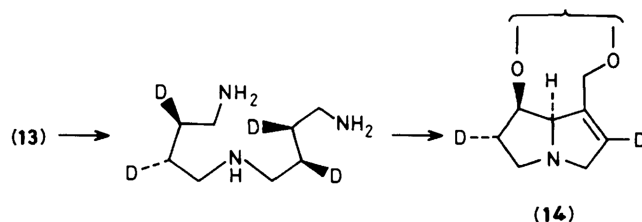


Scheme 3. *Reagents:* i, HCl, HNO_3, urea; ii, $SOCl_2$, MeOH; iii, DIBAH; iv, $LiAlD_4$; v, chromic acid; vi, HBr; vii, NaN_3; viii, $LiAlH_4$

to yield (2*R*)-[2-^{2}H]butane-1,4-diol (**11**) (Scheme 3). A sample of this diol was oxidised with chromic acid to give (2*R*)-[2-^{2}H]succinic acid (**12**). Optical rotatory dispersion (o.r.d.) data for this acid (**12**) were in accord with literature values,[18] and a circular dichroism (c.d.) curve was also recorded. Mass spectral data on (2*R*)-[2-^{2}H]succinic anhydride indicated the presence of *ca.* 98% $^2H_1$ species. After completion of these checks on the optical purity of the (2*R*)-[2-^{2}H]succinic acid, the preceding intermediate (2*R*)-[2-^{2}H]butane-1,4-diol (**11**) was converted into (2*R*)-[2-^{2}H]putrescine (**13**) by a sequence of reactions—formation of the dibromide, conversion into the diazide, and reduction of the diazide—which were not expected to affect the chiral centre. The dihydrochloride of (2*R*)-[2-^{2}H]putrescine (**13**) gave a single peak in its $^2H\{^1H\}$ n.m.r. spectrum at δ 1.76 p.p.m. Analysis of the ^{1}H n.m.r. spectra of the dihydrochloride and the di(phenylaminothiocarbonyl) derivative of this material (**13**) indicated a $^2H_1$ content of *ca.* 98%. A similar figure was estimated by a comparison of the mass spectra of the latter derivative with unlabelled material.

Samples of (2*S*)-[2-^{2}H]succinic acid and (2*S*)-[2-^{2}H]putrescine (**15**) dihydrochloride were prepared from (2*R*)-aspartic acid in an analogous manner to that outlined in Scheme 3. O.r.d. data for the (2*S*)-[2-^{2}H]succinic acid were again in agreement with literature values,[18] and the c.d. curve was the mirror image of that obtained for the (2*R*)-isomer (**12**). Mass spectral analysis on the (2*S*)-[2-^{2}H]succinic anhydride indicated *ca.* 83% $^2H_1$ species. However, it was clear from the $^2H\{^1H\}$ n.m.r. spectrum of the (2*S*)-[2-^{2}H]putrescine (**15**) dihydrochloride that there was an impurity of *ca.* 6% of [1,1-$^2H_2$]putrescine dihydrochloride. This impurity probably arose as a consequence of incomplete reduction of the dimethyl (2*R*)-chlorosuccinate with di-isobutylaluminium hydride. Distillation did not completely remove this impurity, and its reduction in the next step with lithium aluminium deuteride would lead to the introduction of deuterium on the terminal carbons of the putrescine (**15**) dihydrochloride. Analysis of the ^{1}H n.m.r. and mass spectra of putrescine (**15**) dihydrochloride and the di(phenylaminothiocarbonyl) derivative of putrescine (**15**) indicated a $^2H_1$ content of *ca.* 83% and a $^2H_2$ content of *ca.* 6% (estimated from the M + 2 and M + 3 ions). The absence of $^2H_4$ species in the mass spectra suggests that the impurity is mainly [1,1-$^2H_2$]putrescine. It was decided to proceed with the use of this mixture of deuteriated species, because the presence of a small amount of deuterium at C-1 should not interfere with the stereochemical results.

The ^{14}C specific incorporation * in retrorsine after feeding the (2*R*)-[2-^{2}H]putrescine (**13**) dihydrochloride mixture to *Senecio isatideus* plants was 0.30% per C_4 unit. The ^{1}H n.m.r. spectrum of retrorsine (**5**) was assigned completely from our previous work on the incorporation of ^{2}H-labelled putrescines into retrorsine.[12,14] The ^{2}H n.m.r. spectra were again obtained at 60 °C in chloroform, so that narrowing of the signals was observed. The $^2H\{^1H\}$ n.m.r. spectrum of the sample of retrorsine biosynthetically derived from the (2*R*)-isomer (**13**) [Figure 1(a)] showed two main signals at δ 2.15 and 6.20, corresponding to retrorsine (**14**) labelled with ^{2}H at C-6α and C-2, respectively (Scheme 4). The enrichment factors † for the two



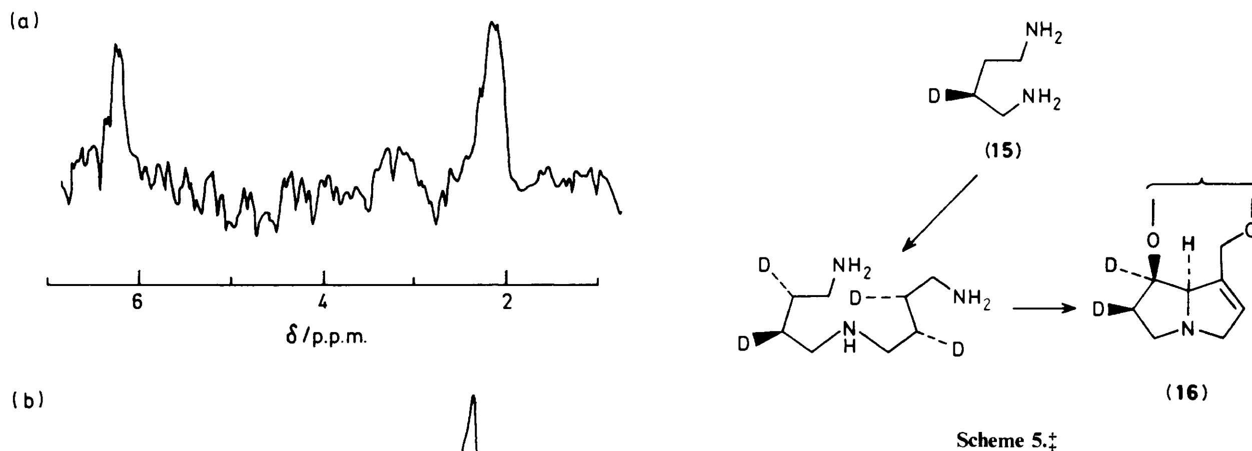
Scheme 4.‡

labelled sites were 0.22 ± 0.02% for C-6α and 0.14 ± 0.02% for C-2. The specific incorporation of ^{2}H was therefore 0.22 × 2/98 × 100 = 0.22% for the C_4 unit containing C-6α and

* Specific ^{14}C incorporation per C_4 unit is calculated from (molar activity of retrorsine (**5**) × 1/2)/(molar activity of putrescine) × 100%.

† 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 p.p.m./concentration of $CHCl_3$) × 0.0156%.

‡ No molecules of putrescine precursors (**13**) and (**15**) can contain more than one ^{2}H atom at the 2-position. The structures in Schemes 4 and 5 are therefore composite representations of all the ^{2}H-labelled species that are present.



(b)

6 4 2
δ/p.p.m.

Figure 1. 30.72 MHz $^2H\{^1H\}$ N.m.r. spectra of retrorsine in chloroform at 60 °C: (a) sample of (**14**) derived from (2*R*)-[2-^2H]putrescine (**13**); (b) sample of (**16**) derived from (2*S*)-[2-^2H]putrescine (**15**)

$0.14 \times 2/98 \times 100 = 0.14\%$ for the C_4 unit containing C-2 [98/2 atom % ^2H was the average enrichment of ^2H at each labelled site of the putrescine precursor (**13**)]. It is evident that two ^2H atoms are removed from the two molecules of putrescine (**13**) as they form retronecine. They are lost from the carbon atoms which become C-1 and C-7 in retronecine. A factor which may contribute to the difference in ^2H and ^{14}C specific incorporations is the operation of ^2H isotope effects in the biosynthetic pathway from putrescine to retronecine.

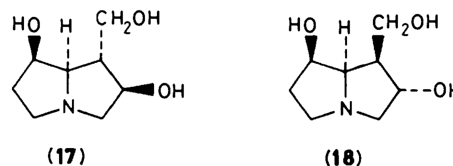
When (2*S*)-[2-^2H]putrescine (**15**) dihydrochloride was fed to *Senecio isatideus* plants, a sample of retrorsine was obtained with a ^{14}C specific incorporation* of 0.73% per C_4 unit. The $^2H\{^1H\}$ n.m.r. spectrum of this sample [Figure 1(b)] contained two major signals at δ 2.38 and 5.00. This indicates that retrorsine (**16**) is labelled with ^2H at C-6β and C-7α, respectively (Scheme 5). The enrichment factors† for the two labelled sites were estimated to be 0.34 ± 0.02% for C-6β and 0.20 ± 0.02% for C-7α. The ^2H specific incorporation for the C_4 unit in retrorsine derived from putrescine containing C-5 to C-8 was therefore $(0.34 + 0.20)/2 \times 2/83 \times 100 = 0.65\%$. Most of the ^2H appears in this C_4 unit, because ^2H is lost from the (2*S*)-[2-^2H]putrescine (**15**) as it is converted into retrorsine (**16**) by removal of ^2H from the carbon atoms which become C-1 and C-2 of retronecine. In the $^2H\{^1H\}$ n.m.r. spectrum of retrorsine (**16**) [Figure 1 (b)] small amounts of ^2H are discernible at δ 3.2—3.4 (5α-H and 3β-H), 3.9 (3α-H), 4.2 (8α-H), and 5.5 (9-H *pro-S*). These signals arise from the incorporation of the [1,1-$^2H_2$]-putrescine dihydrochloride impurity. The probable presence of a signal for 5β-H at δ 2.5 was masked by the larger signal for 6β-H, but allowance for the presence of this species was made in calculating the enrichment factor for 6β-H.

*·†·‡ see footnotes on p. 1090.

(16)

Scheme 5.‡

The labelling patterns established for samples of retrorsine [(**14**) and (**16**)] after feeding the (2*R*)- and (2*S*)-[2-^2H]putrescines (Schemes 4 and 5) allow certain stereochemical features in retronecine biosynthesis to be deduced. The hydroxylation process at C-7 of retronecine must occur with retention of configuration. This is the stereospecificity usually observed for direct hydroxylation at sp^3 carbon atoms.[19] Also, the formation of the double bond in retronecine takes place with retention of the *pro-R* hydrogen, and with loss of the *pro-S* hydrogen at the carbon atom which becomes C-2 of retronecine. It is known that trachelanthamidine (**3**) is on the biosynthetic pathway from putrescine (**1**) to retronecine (**4**),[10,11] and that a number of necines, such as croalbinecine (**17**) and rosmarinecine (**18**) possess hydroxy groups at C-2.[2] If it is assumed, on the basis



of this circumstantial evidence, that formation of the 1,2-double bond in retronecine occurs by hydroxylation of trachelanthamidine at C-2 followed by a dehydration process, the observed labelling patterns are consistent with hydroxylation at C-2α of trachelanthamidine (**3**) with retention of configuration, followed by *trans*-elimination of the elements of water. The alternative mode, hydroxylation with inversion of configuration followed by *cis*-elimination of water, seems much less probable. Further information about the stereochemistry of the enzymic processes involved in necine biosynthesis will be obtained by carrying out feeding experiments with the enantiomeric [2-^2H]putrescines to produce necines such as (**17**) and (**18**) lacking the 1,2-double bond.

Experimental

General.—M.p.s were measured with a Kofler hot-stage apparatus. Organic solutions were dried with anhydrous $MgSO_4$, and solvents were removed under reduced pressure below 40 °C. N.m.r. spectra were obtained on a Bruker WP 200-SY spectrometer operating at 200 MHz for ^1H and 30.72 MHz for ^2H. Mass spectra were obtained with A.E.I. MS 12 or 902

spectrometers. Optical rotations were measured with an Optical Activity Ltd. AA 10 Polarimeter. Radiochemicals were purchased from Amersham International. Radioactivity was measured with a Philips PW 4700 Liquid Scintillation Counter using toluene–methanol solutions. Sufficient counts were accumulated to give a standard error of less than 1% for each determination. Radioactive samples were recrystallised to constant specific radioactivity and they were counted in duplicate. A Panax thin-layer scanner RTLS-1A was used for the radioscanning of t.l.c. plates.

Preparation of (2R)-[2-^{2}H]*Butane*-**1**,4-*diamine* (**13**) (*Putrescine*) *Dihydrochloride*.—(2S)-*Chlorobutanedioic acid* (**9**). (2*S*)-Aspartic acid (**8**) (100 g, 0.752 mol) and urea (10 g) were dissolved in a mixture of 10M hydrochloric acid (160 ml) and concentrated nitric acid (160 ml). The solution was heated at 70 °C for 5 h, and vigorous evolution of gas was observed. The solution was allowed to cool to room temperature and left for 18 h. Crystals of (2*S*)-chlorobutanedioic acid (**9**) were filtered off, washed with cold water, and dried *in vacuo* (80 g, 70%), m.p. 180—182 °C (lit.,[17] m.p. 176 °C); $[\alpha]_D^{19}$ $-21.5°$ (*c* 1, H_2O) {lit.,[17] $[\alpha]_D$ $-20.1°$ (H_2O)}; $\nu_{max.}$(KBr) 3 000 and 1 710 cm^{-1}; δ_H(D_2O) 3.0 (2 H, ABX system, J_{AB} 17 Hz, $J_{AX,BX}$ 8 Hz) and 4.2 (1 H, t, *J* 8 Hz) (Found: C, 31.45; H, 3.0; Cl, 23.05. Calc. for $C_4H_5ClO_4$: C, 31.47; H, 3.28; Cl, 23.28%).

Dimethyl (2S)-*chlorobutanedioate*. (2*S*)-Chlorobutanedioic acid (**9**) (70 g, 0.46 mol) was esterified with methanol (500 ml) and thionyl chloride (110 g, 0.92 mol). Excess of reagents was removed under reduced pressure, and the residue was distilled to afford dimethyl (2*S*)-chlorobutanedioate (77.0 g, 93%), b.p. 106—110 °C at 15 mmHg; $[\alpha]_D^{19}$ $-42.8°$ (*c* 1, $CHCl_3$) (lit.,[20] $[\alpha]_D^{20}$ $-41.96°$); $\nu_{max.}$(CCl_4) 1 755 cm^{-1}; δ_H($CDCl_3$) 3.04 (2 H, ABX system, J_{AB} 17 Hz, $J_{AX,BX}$ 8 Hz), 3.6 (3 H, s), 3.76 (3 H, s), and 4.2 (1 H, t, *J* 8 Hz) (Found: C, 39.7; H, 5.0; Cl, 19.9. Calc. for $C_6H_9ClO_4$: C, 39.9; H, 5.0; Cl, 19.6%).

(2S)-2-*Chlorobutane*-1,4-*diol* (**10**). A solution of dimethyl (2*S*)-chlorobutanedioate (36 g, 0.2 mol) in toluene (500 ml) was cooled to 0 °C and di-isobutylaluminium hydride (1.5M in toluene; 600 ml) was added over 30 m with cooling. After a further 30 min, ethyl acetate (30 ml) was added, and the mixture was added to a suspension of Celite (250 g) in acetone (600 ml). Methanol was added in portions over 15 min and the mixture was shaken vigorously. Heat was evolved and evolution of gas was observed. Shaking was continued until gelling had occurred, and then the mixture was left for 30 min. Water (500 ml) was added and the mixture was filtered. The filtrate was concentrated under reduced pressure, and final traces of water were removed by azeotropic distillation with benzene. Distillation of the residue yielded (2*S*)-2-chlorobutane-1,4-diol (**10**) as a pale yellow oil (11 g, 45%), b.p. 95—104 °C at 0.5 mmHg; $[\alpha]_D^{19}$ $-45.0°$ (*c*. 1.2, MeOH); $\nu_{max.}$(film) 3 000br cm^{-1}; δ_H[$CDCl_3$/($CD_3)_2SO$] 1.4—1.8 (2 H, m), 3.35—3.60 (4 H, m), 3.4—3.6 (2 H, br s, D_2O exch.), and 3.65—3.90 (1 H, m) (Found: C, 38.35; H, 7.15; Cl, 28.45. $C_4H_9ClO_2$ requires C, 38.55; H, 7.23; Cl, 28.51%).

(2R)-[2-^{2}H]*Butane*-1,4-*diol* (**11**). A solution of (2*S*)-2-chlorobutane-1,4-diol (10) (8 g, 64 mmol) in tetrahydrofuran (THF) (50 ml) was added to a suspension of lithium aluminium deuteride (98 atom % ^{2}H, 3.2 g) in THF (50 ml) with cooling in ice. The mixture was stirred for 18 h at room temperature, and then saturated aqueous sodium sulphate solution (1 ml) was added. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was distilled to afford (2*R*)-[2-^{2}H]butane-1,4-diol (**11**) (4.92 g, 84%), b.p. 94—97 °C at 1 mmHg; $\nu_{max.}$(film) 3 000br cm^{-1}; δ_H[($CD_3)_2CO$)] 1.55—1.85 (3 H, m), 3.45—3.9 (4 H, m), and 3.7 (2 H, s); δ_D(acetone) 1.5 p.p.m. (s).

(2R)-[2-^{2}H]*Butanedioic acid* (**12**). (2*R*)-[2-^{2}H]Butane-1,4-diol (**11**) (250 mg, 2.78 mmol) was added to a solution of sodium dichromate dihydrate (2 g) in dilute sulphuric acid (1M; 12 ml) at 0 °C. The mixture was warmed at 50 °C for 1 h, cooled, and extracted with diethyl ether (3 × 25 ml). The ether extracts were dried, filtered through Celite, and concentrated under reduced pressure. The residue was recrystallised (acetone) to yield (2*R*)-[2-^{2}H]butanedioic acid (300 mg, 91%), m.p. 186—188 °C; $[\alpha]_{250}^{25}$ $-20.1°$ (*c*. 1.7, MeOH) (lit.,[18] $[\alpha]_{250}$ $-20°$); c.d. $\lambda_{max.}$ 210 nm, $\Delta\varepsilon$ -0.056.

A portion of (2*R*)-[2-^{2}H]butanedioic acid (**12**) was heated with acetyl chloride for 3 h to give the anhydride. The solution was concentrated and the anhydride was recrystallised from chloroform, m.p. 118—120 °C. Comparison of the mass spectrum of the parent ion of this anhydride at *m/z* 101 with that of unlabelled anhydride at *m/z* 100 indicated a $^2H_1$ content of 98%.

(2R)-[2-^{2}H]*Putrescine* (**13**) *dihydrochloride*. (2*R*)-[2-^{2}H]Butane-1,4-diol (**11**) (4.0 g, 44 mmol) was added to a mixture of hydrobromic acid (48% w/w; 100 ml) and sulphuric acid (10M; 75 ml) at 0 °C. The mixture was stirred at room temperature for 18 h and then heated at reflux for 3 h. The mixture was cooled and extracted with diethyl ether (2 × 50 ml). The ether extracts were washed with water (100 ml) and saturated aqueous sodium hydrogen carbonate (50 ml), dried, filtered, and concentrated under reduced pressure. The residue was subjected to Kugelrohr distillation to yield (2*R*)-[2-^{2}H]-1,4-dibromobutane (5.8 g, 63%), b.p. 75 °C (oven temp.) at 0.5 mmHg; R_F 0.8 [light petroleum (b.p. 60—80 °C)]; δ_H($CDCl_3$) 2.05 (3 H, m) and 3.5 (4 H, m); δ_D($CHCl_3$) 2.02 (s). The dibromide (5 g, 23 mmol) was dissolved in dimethyl sulphoxide (DMSO) (25 ml) and added to a solution of sodium azide (1.7 g, 26 mmol) in DMSO (50 ml); the mixture was then stirred for 18 h at room temperature. It was then poured into water (500 ml) and extracted with diethyl ether (2 × 200 ml). The combined ether extracts were washed with water (2 × 100 ml), dried, filtered, and concentrated to give a yellow oil (2.6 g), $\nu_{max.}$ 2 150 cm^{-1}. This oil was redissolved in diethyl ether (50 ml), lithium aluminium hydride (300 mg) was added, and the mixture was stirred at room temperature for 18 h. Saturated aqueous sodium sulphate (1 ml) was added to the mixture, which was then filtered. The filtrate was dried and filtered again. Dry hydrogen chloride gas was passed through the filtrate and (2*R*)-[2-^{2}H]putrescine (**13**) dihydrochloride was filtered off and dried (2.7 g, 72% from the dibromide), m.p. 300 °C (decomp.); δ_H(D_2O) 1.6 (3 H, m) and 2.7 (4 H, m); δ_D(H_2O) 1.76 (s) (Found: C, 29.5; H, 9.0; Cl, 44.0; N, 16.98. $C_4H_{13}{}^2H_1Cl_2N_2$ requires C, 29.62; H, 9.25; Cl, 43.82; N, 17.29%). The bis(phenylaminothiocarbonyl) derivative had m.p. 177—178 °C (lit.,[21] 177—179 °C); δ_H[($CD_3)_2SO$; 80 °C)] 1.56 (s, 3 H), 3.48 (s, 4 H), 7.25 (m, 10 H), 7.75 (s, 2 × NH), and 9.35 (s, 2 × NH); *m/z* 359 (unlabelled material *m/z* 358).

(2S)-[2-^{2}H]*Putrescine* (**15**) *Dihydrochloride*.—This compound was prepared in an analogous manner from (2*R*)-aspartic acid. (2*R*)-Chlorobutanedioic acid had $[\alpha]_D^{20}$ $+20.8°$ (*c* 1.2, H_2O) (Found: C, 31.58; H, 3.30; Cl, 23.12. Calc. for $C_4H_5ClO_4$: C, 31.47; H, 3.28; Cl, 23.28%). Dimethyl (2*R*)-chlorobutanedioate had $[\alpha]_D^{21}$ $+45.0°$ (*c* 1, $CHCl_3$) (Found: C, 39.8; H, 4.9; Cl, 19.8. Calc. for $C_6H_9ClO_4$: C, 39.9; H, 5.0; Cl, 19.6%). (2*R*)-2-Chlorobutane-1,4-diol had $[\alpha]_D^{21}$ $+36.7°$ (*c* 1.2, MeOH). The ^{1}H n.m.r. spectrum of this material revealed the presence of some unchanged ester, which could not be completely removed by distillation. This mixture was used to prepare (2*S*)-[2-^{2}H]butanedioic acid, m.p. 186—188 °C, $[\alpha]_{250}^{25}$ $+19.5°$ (*c* 1.5, MeOH), c.d. $\lambda_{max.}$ 210 nm, $\Delta\varepsilon$ $+0.048$. Analysis of the mass spectra of this anhydride and unlabelled material indicated a $^2H_1$ content of 83%. (2*S*)-[2-^{2}H]Putrescine (**15**) dihydrochloride, m.p. 300 °C (decomp.); δ_H(D_2O) 1.6 (3 H, m) and 2.7 (4 H, m); δ_D(H_2O) 1.58 (s) and 3.08 (s). The latter signal indicated an impurity of *ca*. 6% [1,1-$^2H_2$]putrescine dihydro-

chloride. (Found: C, 29.5; H, 9.05; Cl, 44.1; N, 17.15. $C_4H_{13}{}^2H_1Cl_2N_2$ requires C, 29.62; H, 9.25; Cl, 43.82; N, 17.29%). The bis(phenylaminothiocarbonyl) derivative had m.p. 176—178 °C (lit.,[21] 177—179 °C); δ_H[$(CD_3)_2SO$; 80 °C] 1.56 (s, *ca.* 3 H), 3.48 (s, *ca.* 4 H), 7.25 (m, 10 H), 7.75 (s, 2 × NH), and 9.35 (s, 2 × NH); *m/z* 361, 360, and 359 (M^+) (unlabelled material *m/z* 358).

Feeding Methods.—Senecio isatideus plants were propagated from stem cuttings and grown in a standard compost. Four well-established plants were used for each experiment. [1,4-^{14}C]Putrescine dihydrochloride (5 μCi) was added to each ^{2}H-labelled precursor (200 mg). Sterile aqueous solutions of the precursor mixtures were introduced into the xylems of the plants through stem punctures made with a sterile needle. Feeding was carried out on alternate days over a period of 2 weeks. After a further 2 weeks, the plants were harvested and retrorsine (**5**) was isolated as described previously.[4] Retrorsine was recrystallised to constant specific radioactivity from acetone, m.p. 215—216 °C (lit.,[4] 216—217 °C). Radioscans of silica gel G t.l.c. plates of 0.25 mm thickness developed with chloroform–methanol–concentrated ammonia (85:14:1) showed one radioactive band, coincident with authentic retrorsine at R_F 0.35. Visualisation of retrorsine was carried out by oxidation with 3,4,5,6-tetrachloro-*o*-benzoquinone, followed by treatment with Ehrlich's reagent.[22] ^{1}H N.m.r. data[14] for retronecine portion of retrorsine (360 MHz, $CDCl_3$): δ 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).

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