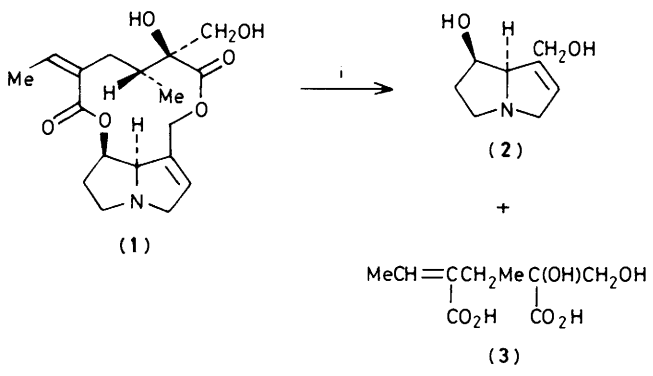
Pyrrolizidine Alkaloid Biosynthesis. Synthesis of ^{14}C-Labelled Homospermidines and their Incorporation into Retronecine[1]

Hassan A. Khan and David J. Robins*
Department of Chemistry, University of Glasgow, Glasgow G12 8QQ

[1,9-^{14}C]Homospermidine (**12**) was synthesized and fed to *Senecio isatideus* plants. The ^{14}C radioactivity was located predominantly in the base [retronecine (**2**)] portion of the alkaloid. Degradation of the retronecine showed that 43—44% of the radioactivity was at C-9 and 2—4% was at C-(5 + 6 + 7). In a complementary experiment, [4,6-^{14}C]homospermidine (**15**) was prepared and fed to *S. isatideus* plants. The ^{14}C radioactivity was again located mainly in retronecine (**2**). Degradation of retronecine demonstrated that 1—4% of the radioactivity was at C-9 and 45—47% was at C-(5 + 6 + 7). Homospermidine is therefore incorporated without significant breakdown into retronecine. Homospermidine was also shown to be present in *S. isatideus* plants by an intermediate trapping experiment.

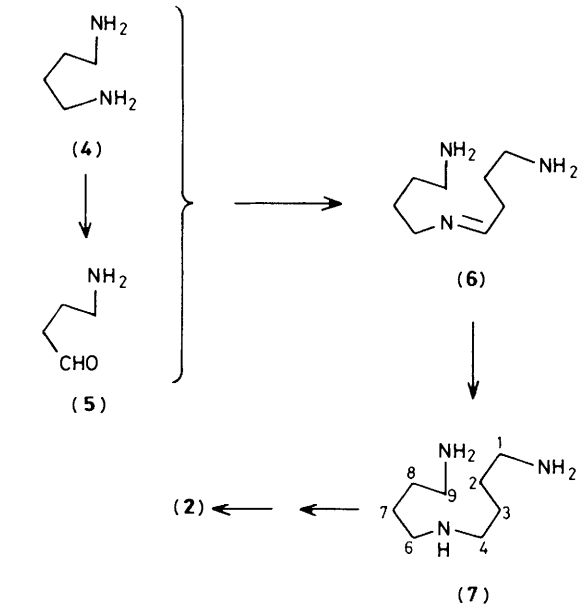
Pyrrolizidine alkaloids are of increasing economic importance, because of their widespread occurrence (*e.g.* ragworts, *Senecio* species), and the fact that many of them are hepatotoxic.[2] Retrorsine (**1**) is the major pyrrolizidine alkaloid in *Senecio isatideus* plants and yields retronecine (**2**) and isatinecic acid (**3**) on alkaline hydrolysis (Scheme 1).[3] Retronecine is known to be



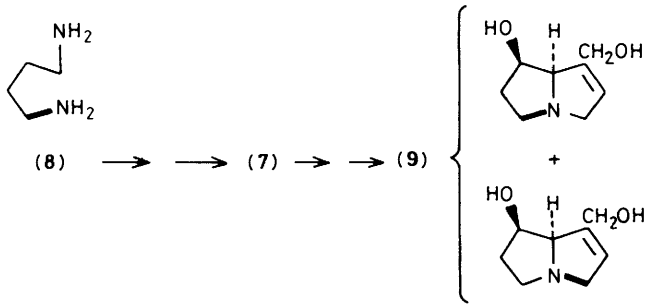
Scheme 1. *Reagent*: i, $Ba(OH)_2$

derived from two molecules of L-ornithine or L-arginine[4] *via* putrescine (**4**) (Scheme 2). Retronecine biosynthesis has been studied using ^{14}C-labelled precursors,[5,6] and ^{13}C-labelled putrescines.[7] In order to gain more information about the way in which the two putrescine molecules combine and then form retronecine, (1-*amino*-^{15}N,1-^{13}C) putrescine (**8**) was prepared and fed to *S. isatideus*[1] and *S. vulgaris*[7] plants. The ^{13}C-{^{1}H} n.m.r. spectrum of the biosynthetically derived retronecine (**9**) showed satellites of approximately equal intensity around the signals for C-3 and C-5 of retronecine (Scheme 3). This finding is consistent with the theory that a symmetrical C_4–N–C_4 intermediate is involved in retronecine biosynthesis.

We believed that the most probable candidate for this symmetrical intermediate was homospermidine (4,4′-iminobisbutylamine) (**7**). Homospermidine was first isolated from the sandalwood tree, *Santalum album* L., by Kuttan *et al.*[8] where it comprises *ca.* 1% of the weight of the dried leaves. Because arginine and ornithine were found to be efficient precursors of homospermidine in sandalwood leaves, Kuttan and Radhakrishnan suggested[9] that the biosynthesis of homospermidine may involve formation of a Schiff base (**6**) between putrescine (**4**) and 4-aminobutanal (**5**) with subsequent reduction (Scheme 2). More recently, Srivenugopal and Adiga[10]



Scheme 2.



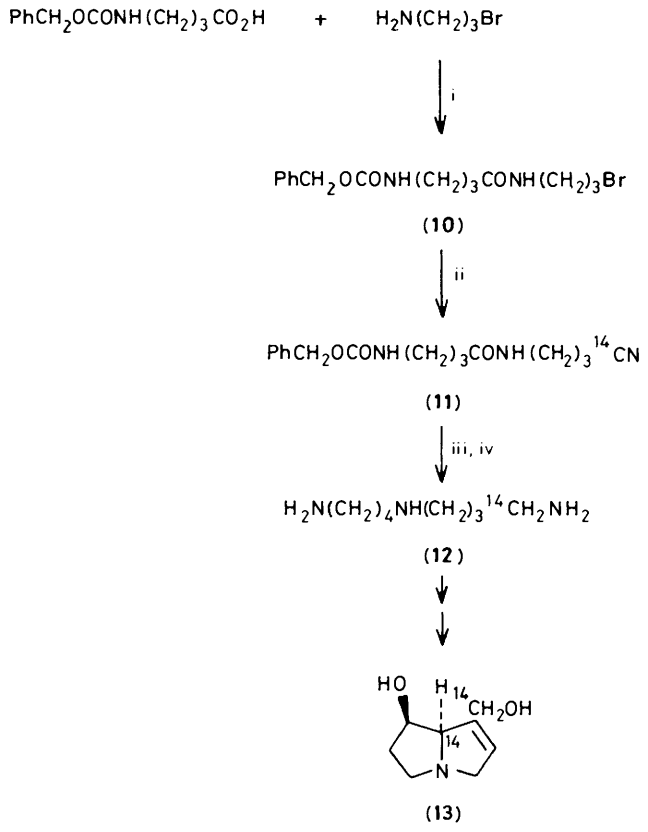
Scheme 3.

have isolated and partially purified an enzyme from *Lathyrus sativus* (grass pea) seedlings and from sandalwood leaves that catalyses the formation of homospermidine from putrescine and NAD^+ with concomitant liberation of ammonia. Tait has also partially purified an enzyme with similar properties from

the bacterium *Rhodopseudomonas viridis*.[11] We now present evidence using ^{14}C-labelled compounds that homospermidine is a key intermediate in retronecine biosynthesis.

Results and Discussion

A synthesis of [1,9-^{14}C]homospermidine (**12**) was carried out (Scheme 4). The *N*-benzyloxycarbonyl derivative of 4-amino-

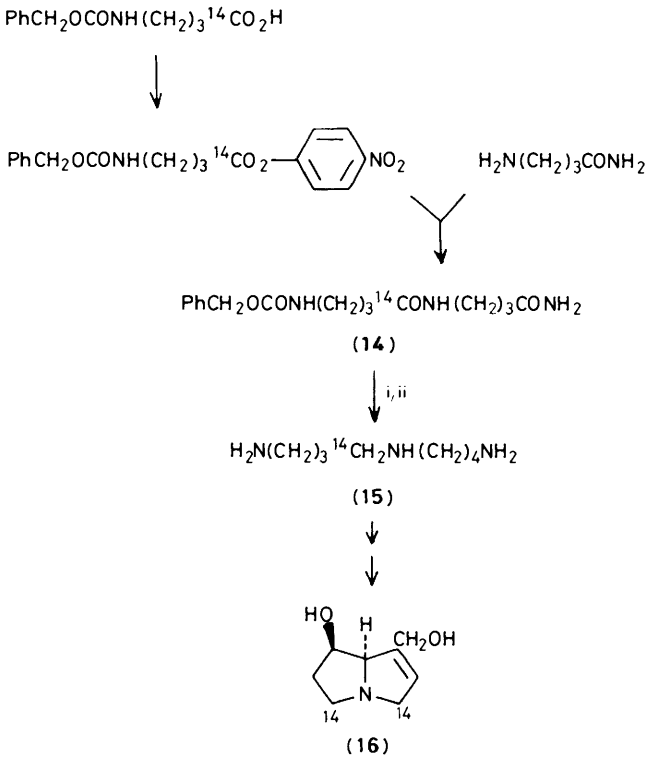


Scheme 4. *Reagents*: i, PhCOCl; ii, K^{14}CN; iii, H_2, Pd–C; vi, BH_3·THF

butanoic acid was coupled with 3-bromopropylamine by the mixed anhydride method. The radiolabel was introduced by treatment of the protected bromoamide (**10**) with [^{14}C]potassium cyanide. Reduction of the nitrile and amide functions and removal of the protecting group of the intermediate (**11**) yielded [1,9-^{14}C]homospermidine (**12**), which was isolated and purified as the trihydrochloride. This material was fed to *Senecio isatideus* plants as detailed in Table 1 (experiments 1—3). Retrorsine (**1**) was isolated and recrystallised to constant specific radioactivity. Total incorporations of 0.48—0.97% were observed in the isolated retrorsine, whereas the specific incorporations* varied from 0.04—0.3%. The lowest value for the specific incorporation was obtained with the oldest plants, where appreciable dilution due to endogenous unlabelled retrorsine (**1**) had taken place. The radioactive retrorsine from these three experiments was diluted with inactive retrorsine, and the mixture was hydrolysed to give retronecine (**2**) and isatinecic acid (**3**). It was found that the specific radioactivity of the retronecine was 95—100% of the value of the diluted retrorsine, whereas the specific radioactivity of the isatinecic acid was less than 3% of the value for the diluted retrorsine (Table 2, experiments 1—3). Thus homospermidine is a specific precursor for the base portion of retrorsine.

The partial distribution of radioactivity in retronecine obtained in experiments 1 and 2 was determined by two separate degradations.[6] Treatment of retronecine (**1**) with osmium tetraoxide and sodium periodate gave formaldehyde, isolated as its dimedone (5,5-dimethylcyclohexane-1,3-dione) derivative, corresponding to C-9 of retronecine (**2**). The specific activity of this derivative was 43 and 44 ± 4% of the value of retronecine. Modified Kuhn–Roth oxidation of retronecine gave β-alanine isolated as its *N*-2,4-dinitrophenyl derivative, corresponding to C-(5 + 6 + 7) of retronecine (**2**). Less than 4% of the specific radioactivity of retronecine was present in this derivative in the two experiments (Table 2). The rest of the radioactivity is assumed to be present at C-8 of retronecine to give the labelling pattern as shown in structure (**13**). These results are consistent with incorporation of homospermidine (**12**) into labelled retronecine (**13**) without significant breakdown of the precursor.

In order to provide additional evidence for homospermidine as an intermediate in retronecine biosynthesis, a different ^{14}C-labelled precursor was prepared, based on the synthesis of Kuttan *et al.* (Scheme 5).[8] The *N*-benzyloxycarbonyl derivative



Scheme 5. *Reagents*: i, H_2, Pd–C; ii, BH_3·THF

of [1-^{14}C]-4-aminobutanoic acid was converted into its *p*-nitrophenyl ester, and this was coupled with 4-aminobutanamide to give the ^{14}C-labelled *N*-protected diamide (**14**). Reduction of the amide function and removal of the protecting group yielded [4,6-^{14}C]homospermidine (**15**), isolated and purified as its trihydrochloride.

Two feeding experiments with this labelled homospermidine (**15**) were carried out on *Senecio isatideus* plants as specified in

* Specific ^{14}C incorporation is calculated from {(molar activity of retrorsine)/(molar activity of [^{14}C]homospermidine)} × 100%.

Table 1. Incorporation of ^{14}C-homospermidines into retrorsine in *Senecio isatideus* plants

Experiment	Precursor	Quantity fed/mg	No. of plants	Age of plants/months	Length of expt./days	%Total incorporation	% Specific* in (1)
1	(12)	18	2	6	14	0.48	0.043
2	(12)	18	2	2	10	0.50	0.30
3	(12)	2.7	1	1	7	0.97	0.41
4	(15)	24	4	3	14	0.61	0.22
5	(15)	24	2	2	10	0.82	0.37

* See footnote on p. 820.

Table 2. Distribution of radioactivity in retrorsine (1) and retronecine (2)

Experiment	% Specific radioactivity in Isatinecic acid (3)	% Specific radioactivity in Retronecine (2)	% of Retronecine radioactivity at C-9	% of Retronecine radioactivity at C-(5 + 6 + 7)
1	3	96	43 ±4	4 ±4
2	2	100	44 ±4	2 ±2
3	0	95		
4	3	98	4 ±4	45 ±4
5	1	98	1 ±1	47 ±4

Table 1 (experiments 4 and 5). The total incorporations were similar to those obtained with the previous precursor (**12**), and the use of fairly young plants gave reasonable specific incorporations into retrorsine (**1**). Measurement of the distribution of the radioactivity in the retrorsine samples was carried out by hydrolysis to give retronecine (**2**) which contained 98% of the specific radioactivity, and isatinecic acid (**3**) which retained only 1—3% of the specific radioactivity of the retrorsine (Table 2, experiments 4 and 5). Further degradation of retronecine yielded samples of formaldehyde (as dimedone derivative) which contained very little radioactivity, and samples of β-alanine with specific activities of 45 and 47 ± 4% compared to the retronecine. Assuming that most of the radioactivity in these two experiments is at C-3 and C-5 of retronecine [as in (**16**)] the results are again consistent with the intact conversion of homospermidine into retronecine. The breakdown to a small extent of homospermidine and recombination to form retronecine cannot, however, be ruled out.

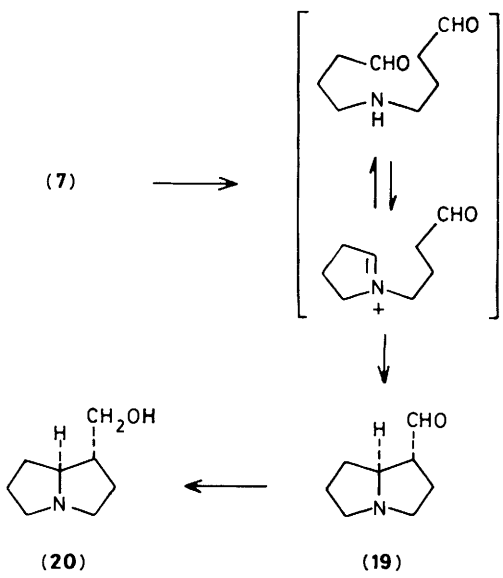
The presence of homospermidine in *Senecio isatideus* plants was demonstrated by an intermediate trapping experiment. DL-[5-^{14}C]Ornithine was fed to one *S. isatideus* plant. After one day, the plant was harvested and inactive homospermidine trihydrochloride was added to the acid extract. The *N*-phenylamino(thiocarbonyl) derivative of homospermidine (**17**) (and of the other polyamines present) was formed as described by Golding and Nassereddin[12] from isothiocyanatobenzene. T.l.c. showed that most of the radioactivity (*ca.* 80%) in the mixture was due to the derivative (**18**) of putrescine. The

PhNHCSNH(CH$_2$)$_4$N(CSNHPh)R

(**17**) R = (CH$_2$)$_4$NHCSNHPh
(**18**) R = H

remainder of the radioactivity was associated with the derivative (**17**) of homospermidine (*ca.* 10%) and baseline material (*ca.* 10%). Traces of radioactivity were also observed for the *N*-phenylamino(thiocarbonyl) derivatives of spermine and spermidine. The radioactive bands were separated by p.l.c. and the derivatives of homospermidine and putrescine (after dilution with inactive material) were recrystallised to constant specific radioactivity. The fact that the homospermidine derivative (**17**) contained *ca.* 0.5% of the original radioactivity fed as DL-[5-^{14}C]ornithine shows that homospermidine is produced from ornithine in the *S. isatideus* plant, and it suggests that homospermidine is a normal intermediate in retronecine biosynthesis.

Further evidence for homospermidine (**7**) as an intermediate in retronecine (**2**) biosynthesis was reported recently by us. [1,9-^{13}C$_2$]Homospermidine was prepared and used to produce ^{13}C-labelled retrorsine in *Senecio isatideus* plants.[13] The ^{13}C-{^{1}H} n.m.r. spectrum of the retronecine, obtained by alkaline hydrolysis, showed a geminal coupling of 6 Hz between C-8 and C-9, demonstrating that homospermidine is incorporated intact into retronecine. Moreover, homospermidine has been converted into the saturated pyrrolizidine base, 1-hydroxymethylpyrrolizidine (**20**) using enzymes and physiological conditions (Scheme 6).[14] Enzymic oxidation of the primary



Scheme 6.

amino groups in homospermidine by diamine oxidase, followed by Mannich cyclisation, presumably generated 1-formylpyrrolizidine (**19**), from which the saturated pyrrolizidine base (**20**) was obtained by either chemical or enzymic reduction. The ease with which these transformations occur does add support to the theory that homospermidine is a key intermediate in retronecine biosynthesis.

Experimental

General.—M.p.s were measured with a Kofler hot-stage apparatus. Organic solutions were dried with anhydrous $MgSO_4$, and solvents were evaporated off under reduced pressure below 40 °C. N.m.r. spectra were run for solutions in deuteriochloroform with tetramethylsilane as internal standard unless otherwise stated. Mass spectra were obtained with A.E.I. MS 12 or MS 902 spectrometers. T.l.c. was carried out on silica gel G plates of 0.25-mm thickness developed with chloroform–methanol–conc. ammonia (85:14:1), and alkaloids were detected by oxidation with *o*-chloranil, followed by treatment with Ehrlich's reagent.[15]

Radiochemical Methods.—All radiochemicals were purchased from the Radiochemical Centre, Amersham (Amersham International), or the New England Nuclear Corporation. Radioactivity was measured with a Philips liquid scintillation analyser 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 activity and were counted in duplicate. A Panax thin-layer scanner RTLS-IA was used for the radioscanning of t.l.c. plates.

4-*Benzyloxycarbonylamino*-*N*-(3-*bromopropyl*)*butanamide* (**10**).—A mixture of 4-benzyloxycarbonylaminobutanoic acid (9.48 g, 40 mmol) and triethylamine (4.08 g, 40 mmol) in dry dichloromethane (100 ml) was cooled to −5 °C, and freshly distilled benzoyl chloride (5.62 g, 40 mmol) was added slowly with stirring during 1 h. A cooled solution of 3-bromopropylamine hydrobromide (9.76 g, 40 mmol) and triethylamine (4.08 g, 40 mmol) in dry dichloromethane (100 ml) was added, and the reaction mixture was allowed to warm to 5 °C and left at this temperature for 18 h. The organic solution was washed with 1M-HCl (2 × 100 ml), water (100 ml), 1M-$NaHCO_3$ (2 × 100 ml), and water (100 ml), dried, filtered and concentrated to give a white solid (13 g, 91%). Recrystallisation from ethyl acetate gave 4-*benzyloxycarbonylamino*-N-(3-*bromopropyl*)*butanamide* (**10**) (6.8 g, 48%); m.p. 87—88 °C; $\nu_{max.}$ (KBr) 3 380, 3 290, 1 710, 1 660, and 700 cm^{-1}; δ_H 1.85 (4 H, m), 2.15 (2 H, m), 3.3 (6 H, m), 5.07 (2 H, s), 5.4 and 6.6 (2 H, each br s, NH), and 7.32 (5 H, s); *m/z* 358, 356, and 277 (Found: C, 50.7; H, 5.5; Br, 22.8; N, 8.0. $C_{15}H_{21}BrN_2O_3$ requires C, 50.42; H, 5.88; Br, 22.4; H, 7.84%).

4-*Benzyloxycarbonylamino*-N-(3-[^{14}C]*cyanopropyl*)*butanamide* (**11**).—Finely ground potassium [^{14}C]cyanide (98 mg, 0.75 mmol; 1 mCi) was added to a solution of the bromoamide (**10**) (357 mg, 1.00 mmol) and 18-crown-6 (140 mg) in dry hexamethylphosphoric triamide (2 ml). The mixture was heated for 1.5 h at 50—60 °C and was then poured into water (10 ml). The aqueous solution was extracted with ethyl acetate (3 × 10 ml). The combined organic extracts were washed with water (8 × 2 ml), dried, filtered and concentrated to a thick oil of 4-*benzyloxycarbonylamino*-N-(3-[^{14}C]*cyanopropyl*)*butanamide* (**11**) (268 mg, 88%; 341 μCi $mmol^{-1}$); $\nu_{max.}$ ($CHCl_3$) 2 260 cm^{-1} (CN); δ_H 1.76 (4 H, m), 2.16 (4 H, m), 3.23 (4 H, m), 5.1 (2 H, s), 5.25 and 6.65 (2 H, each br s, NH), and 7.36 (5 H, s); *m/z* 303 and 276 (Found for unlabelled sample: M^+ 303.1580. $C_{16}H_{21}N_3O_3$ requires *M* 303.1578).

[1,9-^{14}C]*Homospermidine* (**12**).—The [^{14}C]nitrile (**11**) (265 mg, 0.87 mmol) in methanol (20 ml) was hydrogenolysed for 1.5 h at room temperature in the presence of 10% Pd–C (50 mg). The ^{1}H n.m.r. spectrum of the crude product (120 mg) obtained by filtration and removal of solvent indicated complete removal of the protecting group. This material was used directly in the next step. A solution of 1M-borane in tetrahydrofuran (THF) (8 ml) was added to this material in dry THF (20 ml), and the solution was heated at reflux for 20 h. Dry ethanol (5 ml) was added to the cooled solution, and, after 1 h, dry HCl gas was passed through the solution. The solid trihydrochloride of [1,9-^{14}C]homospermidine (**12**) (54 mg; 348 μCi $mmol^{-1}$) was filtered off and recrystallised to constant specific activity from aqueous ethanol; m.p. 288—289 °C (decomp.) (lit.,[8] m.p. 290—294 °C; undepressed mixed m.p. with authentic material). Radioscans of a t.l.c. cellulose-coated plate developed in propan-2-ol–conc. ammonia (7:3) indicated one radioactive band at R_F 0.64 (ninhydrin) coincident with authentic unlabelled homospermidine trihydrochloride.

N-*Aminocarbonylpropyl*-4-*benzyloxycarbonylamino*[1-^{14}C]*butanamide* (**14**).—This procedure is based on the method of Kuttan *et al.*[8] A solution of dicyclohexylcarbodi-imide (0.4 g, 1.94 mmol) in ethyl acetate (1 ml) was added to a solution of *N*-benzyloxycarbonyl-4-amino[1-^{14}C]butanoic acid (230 mg, 0.97 mmol; 108 μCi $mmol^{-1}$) and *p*-nitrophenol (160 mg, 1.15 mmol) in ethyl acetate (4 ml) at 0 °C. The reaction was kept at 0 °C for 4 h and then at room temperature for 18 h. Dicyclohexylurea was filtered off and the filtrate was washed with water, dil. $NaHCO_3$ (2 ×), water, dil. H_2SO_4 (4 ×), and water (each 5 ml). The organic layer was dried, filtered, and concentrated to an oil (0.4 g) which gave crystals of 4-nitrophenyl 4-benzyloxycarbonylamino[1-^{14}C]butanoate from ethanol, (270 mg, 77%); m.p. 83—84 °C (lit.,[8] 84—85 °C); 111.6 μCi $mmol^{-1}$; $\nu_{max.}$ (KBr) 3 360, 1 760, 1 720, 1 690, 1 600, and 1 525 cm^{-1}; δ_H 2.05 (2 H, m), 2.65 (2 H, m), 3.40 (2 H, m), 5.15 (2 H, s), 7.23 and 8.23 (4 H, A_2B_2), and 7.33 (5 H, s); *m/z* 358 (M^+), 220 (Found for unlabelled material: C, 60.15; H, 5.4; N, 7.8. Calc. for $C_{18}H_{18}N_2O_6$: C, 60.32; H, 5.06; N, 7.82%).

Ammonia gas was passed through a solution of unlabelled 4-nitrophenyl 4-benzyloxycarbonylaminobutanoate (1 g, 2.8 mmol) in dry ethanol (15 ml) at 0 °C for 15 min. The flask was sealed and left at room temperature for 3 h. Ethanol was removed under reduced pressure and the residue was poured into ice–water (20 ml). The aqueous mixture was extracted with chloroform (3 × 20 ml). The organic extracts were combined and washed with water (2 × 50 ml), dried, filtered and concentrated to give a solid which gave crystals from aqueous ethanol of 4-benzyloxycarbonylaminobutanamide (0.50 g, 75%), m.p. 130—132 °C; $\nu_{max.}$ (KBr) 3 395, 3 355, 1 694, 1 650, and 1 540 cm^{-1}; δ ([$^2H_4$]MeOH), 1.8 (2 H, m), 2.16 (2 H, m), 3.13 (2 H, m), 5.03 (2 H, s), and 7.26 (5 H, s); *m/z*, 236 (M^+) and 219 (Found: C, 60.8; H, 6.6; N, 11.6. $C_{12}H_{16}N_2O_3$ requires C, 61.02; H, 6.78; N, 11.86%). A solution of 4-benzyloxycarbonylaminobutanamide (0.50 g) in dry ethanol (25 ml) was hydrogenated at atmospheric pressure and at room temperature for 2 h in the presence of 10% Pd–C (0.2 g). The mixture was filtered through Celite, and the filtrate was concentrated to yield 4-aminobutanamide (0.18 g, 85%) as an unstable oil; δ ([$^2H_4$]MeOH) 1.8 (2 H, m), 2.35 (2 H, m), and 2.75 (2 H, m). This was used immediately in the next step.

A solution of 4-nitrophenyl 4-benzyloxycarbonylamino-[1-^{14}C]butanoate (265 mg, 0.74 mmol) and 4-aminobutanamide (80 mg, 0.78 mmol) in dry *N*,*N*-dimethylformamide (5 ml) was left to stand at room temperature for 3 h. Water (10 ml) was added and the white precipitate was filtered off and washed with dil. $NaHCO_3$, dil. HCl, water, and dried. This material (240 mg) gave crystals of *N*-aminocarbonylpropyl-4-benzyl-

oxycarbonylamino[1-^{14}C]butanamide (**14**), from methanol–chloroform (195 mg, 82%); m.p. 171—172 °C (lit.,[8] 171—172 °C); 110.9 μCi mmol^{-1}; $\nu_{max.}$ (KBr) 3 400, 3 360, 3 320, 3 200, 1 690, 1 650, 1 630, and 1 540 cm^{-1}; δ [$(CD_3)_2SO$] 1.65 (4 H, m), 2.05 (4 H, m), 3.05 (4 H, m), 5.0 (2 H, s), and 7.35 (5 H, s); *m/z* 321 (M^+) and 263 (Found: C, 60.1; H, 6.9; N, 12.8. Calc. for $C_{16}H_{23}N_3O_4$: C, 59.80; H, 7.21; N, 13.08%).

N-*Aminocarbonylpropyl*[1-^{14}C]*butanamide*.—The protected diamide (**14**) (189 mg, 0.589 mmol) was hydrogenated in methanol (20 ml) in the presence of 10% Pd–C (100 mg) for 1.5 h at room temperature. The catalyst was filtered off (Celite) and the filtrate was concentrated to an oil (106 mg) which gave crystals of N-*aminocarbonylpropyl*[1-^{14}C]*butanamide* from ethanol (81 mg, 73%); 108.7 μCi mmol^{-1}; m.p. 98—100 °C; $\nu_{max.}$ (KBr) 3 400, 3 320, 3 210, 1 650, 1 630, and 1 540 cm^{-1}; δ ([$^2H_4$]MeOH) 1.76 (4 H, m), 2.22 (4 H, m), 2.69 (2 H, m), and 3.19 (2 H, m); *m/z* 187 (M^+), 143, 129, 127, 112, 99, and 86 (Found: C, 51.2; H, 9.3; N, 22.2. $C_8H_{17}N_3O_2$ requires C, 51.31; H, 9.15; N, 22.45%).

[4,6-^{14}C]*Homospermidine* (**15**).—A 1M-solution of borane in THF (10 ml) was added to *N*-aminocarbonylpropyl[1-^{14}C]-butanamide (80 mg, 0.428 mmol) in dry THF (10 ml) and the mixture was heated at reflux for 4 h. Dry ethanol (8 ml) was added to the cooled solution, which was left to stand for 1 h. Dry HCl gas was then passed through the solution, and the white precipitate was filtered and dried. The trihydrochloride of [4,6-^{14}C]homospermidine (**15**) was recrystallised to constant specific activity from aqueous ethanol (48 mg, 42%); 109.1 μCi mmol^{-1}; m.p. 286—288 °C (lit.,[8] m.p. 290—294 °C, undepressed mixed m.p. with authentic sample). Radioscans of a t.l.c. cellulose-coated plate developed in propan-2-ol–conc. ammonia (7:3) showed one radioactive band at R_F 0.62 (ninhydrin) coincident with authentic unlabelled homospermidine trihydrochloride.

Feeding Method.—The growth of *Senecio isatideus* plants and administration of precursors by pulsed feeding were carried out as described previously.[6,7] Details of each experiment are given in Table 1. Retrorsine was isolated[6] and recrystallised to constant specific activity from acetone, m.p. 215—216 °C (lit.,[6] 216—217 °C). In each experiment radioscans of t.l.c. plates showed one radioactive band coincident with authentic unlabelled retrorsine at R_F 0.34. Incorporation figures for each experiment are provided in Table 1. Radioactive retrorsine was diluted with inactive material (100—200 mg), and hydrolysed[7] to isatinecic acid (**3**) and retronecine (**2**). Isatinecic acid was recrystallised to constant specific activity from ethyl acetate, m.p. 145—146 °C (lit.[16] 148 °C). Retronecine was recrystallised to constant specific activity from acetone, m.p. 119—120 °C (lit.,[5b] 119—120 °C). The distribution of radioactivity in retronecine (**2**) and isatinecic acid (**3**) is shown in Table 2.

Degradation for C-9 *of Retronecine* (**2**).—This was carried out as described previously.[5b] Formaldehyde (as the dimedone derivative) was recrystallised to constant specific activity from aqueous ethanol, m.p. 189—190 °C (mixed m.p. undepressed with authentic material).

Degradation for C-(5 + 6 + 7) of *Retronecine* (**2**).—This was carried out as described previously.[6] *N*-2,4-Dinitrophenyl-β-alanine was recrystallised from aqueous ethanol, m.p. 144—145 °C (mixed m.p. undepressed with authentic material).

1,6,11-*Tris*[*phenylamino*(*thiocarbonyl*)]-1,6,11-*triazaundecane* (**17**).—This was prepared from homospermidine trihydrochloride by treatment with isothiocyanatobenzene in aqueous ethanol as described by Golding and Nassereddin.[12] The derivative had m.p. 150—151 °C (EtOH); R_F 0.24 (CH_2Cl_2–MeCN, 9:1); $\nu_{max.}$ (KBr) 3 400, 3 160, 1 600, 1 500, and 1 450 cm^{-1}; δ ([$(CD_3)_2SO$] 1.65—1.95 (8 H, complex), 3.5—3.8 (8 H, complex), 7.1—7.3 (15 H, m), 7.65 (2 H, m, NH), and 8.9—9.3 (3 H, br, NH) (Found: C, 61.6; H, 6.6; N, 14.75; S, 17.1. $C_{29}H_{36}N_6S_3$ requires C, 61.66; H, 6.42; N, 14.88; S, 17.03%).

The following derivatives were also prepared for reference purposes as described:[12] 1,4-bis[phenylamino(thiocarbonylamino)]butane (**18**), m.p. 177—178 °C (lit.[12] 178—180 °C); R_F 0.32 (CH_2Cl_2–MeCN, 9:1); 1,5,10,14-tetrakis[phenylamino(thiocarbonyl)]-1,5,10,14-tetra-azatetradecane had m.p. 173—175 °C (lit.,[12] 173—175 °C); R_F 0.20 (CH_2Cl_2–MeCN, 9:1); 1,5,10-tris[phenylamino(thiocarbonyl)]-1,5,10-triazadecane had m.p. 140—141 °C (lit.,[12] 141—143 °C); R_F 0.28 (CH_2Cl_2–MeCN, 9:1).

Intermediate Trapping Experiment.—A solution of DL-[5-^{14}C]ornithine (100 μCi) was fed to a six-month old *S. isatideus* plant as described above. After 24 h, the plant was blended in 0.4M-trichloroacetic acid (400 ml) and the mixture was sonicated (2 × 15 min). The aqueous solution was washed with ether (2 × 400 ml). A solution of unlabelled homospermidine trihydrochloride (35 mg) in water (1 ml) was added to the aqueous solution and it was basified to pH 9 with KOH. Isothiocyanatobenzene (0.5 ml) in ethanol (5 ml) was added to the solution and it was stirred for 3 days at room temperature. The aqueous solution was extracted with CH_2Cl_2 (3 × 200 ml). The organic extracts were dried, filtered, and concentrated to a white solid (260 mg). Total incorporation was 4.4%. Radioscans of a t.l.c. plate developed in CH_2Cl_2–MeCN (9:1) indicated two major bands at R_F 0.24 and 0.32. This mixture was subjected to p.l.c. The band at R_F 0.24 was removed and the material extracted was recrystallised to constant specific activity from ethanol, m.p. 150—151 °C (undepressed m.p. with the authentic derivative of homospermidine); specific activity 3.85 μCi mmol^{-1}. The band at R_F 0.32 was removed and the material extracted was diluted with the phenylamino(thiocarbonyl) derivative of putrescine (35 mg). This was recrystallised to a constant specific activity of 29.6 μCi mmol^{-1}. Some radioactivity was observed at R_F 0.0. Weak radioactive bands were observed at R_F 0.20 and 0.28 corresponding to the phenylaminothiocarbonyl derivatives of spermine and spermidine respectively.

Acknowledgements

We thank the S.E.R.C. for a Research Assistantship (to H. A. K.).

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Received 31*st July* 1984; *Paper* 4/1355