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SYNTHESIS OF [3,5-^{14}C]TRACHELANTHAMIDINE AND [5-^{3}H]ISORETRONECANOL AND THEIR INCORPORATION INTO THE RETRONECINE MOIETY OF RIDDELLIINE IN *SENECIO RIDDELLII*

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ABSTRACT.—(±)-[3,5-^{14}C]Trachelanthamidine and (±)-[5-^{3}H]isoretronecanol, which are diastereomers, were prepared from potassium [^{14}C]cyanide and [5-^{3}H]proline, respectively. These compounds and [1,4-^{14}C]putrescine were administered to *Senecio riddellii* plants resulting in the formation of labeled riddelliine, in which almost all the radioactivity was located in its retronecine moiety. The activity of the β-alanine obtained by degradation of the retronecine was consistent with specific labeling of this pyrrolizidine base at the expected positions. The extremely high absolute incorporation (15.1, 22.1%) of trachelanthamidine into riddelliine strongly favors this 1-hydroxymethylpyrrolizidine as the one on the main biosynthetic pathway to retronecine. The lower incorporation (0.75%) of isoretronecanol may represent a minor or aberrant pathway to retronecine.

The biosynthesis of retronecine (**7**), the basic moiety of a large number of pyrrolizidine alkaloids (2-5), has been the subject of many feeding experiments carried out by Robins, Spenser, and their co-workers. Figure 1 illustrates the later steps in the proposed biosynthesis of retronecine (6-8). It has been shown by Robins that homospermidine (**1**) is incorporated directly, without degradation, into retronecine (9,10). Oxidative deamination of the primary amino groups of **1** or transamination of these groups, affords the dialdehyde (**2**) which undergoes an intramolecular Mannich reaction to yield pyrrolizidine-1-aldehyde. This Mannich reaction was achieved chemically (11,12); 1-hydroxymethylpyrrolizidine being obtained after reduction. Robins obtained trachelanthamidine (**3**) from homospermidine by carrying out an enzymic oxidation, followed by an enzymic reduction (13). Spenser (8) considers that the main

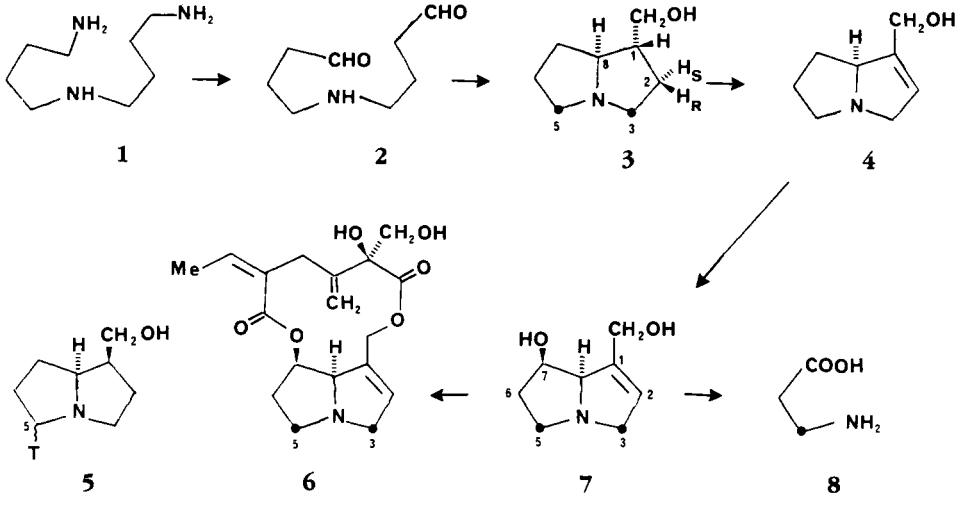

FIGURE 1. Biogenetic route from homospermidine to retronecine.

[1]Contribution No. 201 from this laboratory. Presented in part at the 191st National Meeting of the American Chemical Society, April 13-18, 1986, in New York, New York. A preliminary account of this work has appeared (1).

biosynthetic pathway from putrescine to the pyrrolizidine nucleus proceeds via 9-amino-5-azanonanal; the mono-aldehyde formed by the transamination of one of the primary amino groups of homospermidine. He regards homospermidine as a side product reversibly connected to the main biosynthetic sequence.

The present work was carried out to clarify later steps in the formation of retronecine. In particular, we wished to compare the ability of the two diastereomers of 1-hydroxypyrrolizidine, namely trachelanthamidine (**3**) and isoretronecanol (**5**), to serve as precursors of retronecine. Birecka (14) found that exposure of *Heliotropium spathulatum* plants to [^{14}C]CO_2 resulted in labeling of trachelanthamidine, supinidine (**4**), and retronecine, having specific activities consistent with the biosynthetic sequence illustrated in Figure 1. For this investigation we used the species *Senecio riddellii* Torr and Gray, which contains up to 18% (based on the dry weight) of riddelliine (**6**) and its *N*-oxide (15).

The synthetic routes to the labeled 1-hydroxymethylpyrrolizidines are illustrated in Figure 2. For convenience, only one enantiomer of chiral compounds is depicted. The synthesis of trachelanthamidine (**3**) is based on one previously described (16) with modifications for introduction of the label. 3-Chloropropanal diethyl acetal (**9**) was reacted with potassium [^{14}C]cyanide in aqueous EtOH of afford [1-^{14}C]-4,4-diethoxybutanonitrile (**10**) which was reduced with $LiAlH_4$ to yield [4-^{14}C]-4-aminobutanal diethyl acetal (**11**). This amine was converted to the secondary amine (**13**) by refluxing in C_6H_6 with Raney nickel. This compound was refluxed in aqueous EtOH con-

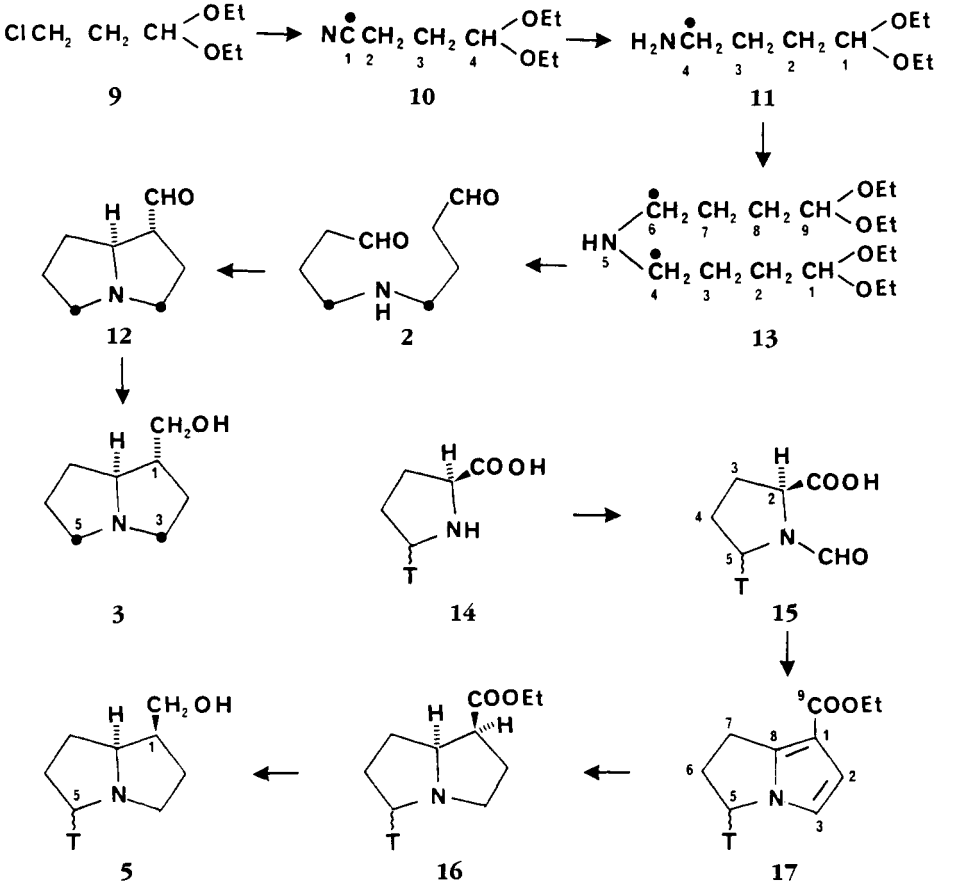

FIGURE 2. Synthesis of labeled trachelanthamidine and isoretronecanol.

taining HCl to yield the dialdehyde amine (**2**) which cyclized to yield the pyrrolizidine-1-aldehyde (**12**). Reduction of this aldehyde with $NaBH_4$ in MeOH afforded (±)-trachelanthamidine. This material was 98.1% pure by gc. The overall yield from potassium [^{14}C]cyanide was 8.4%. The route to [5-^{3}H]isoretronecanol (**5**) is based on a method briefly described in a communication (17). Commercially available (2*S*)-[5-^{3}H]proline (**14**) { the chirality of the tritium was not indicated, and it is assumed that the starting material was a mixture of the (5*S*)- and (5*R*)-[5-^{3}H]proline } was converted to *N*-formylproline (**15**) by stirring with a mixture of HCOOH and Ac_2O. A 1,3 dipolar addition of ethyl propiolate to this proline derivative in boiling Ac_2O yielded the dihydropyrrolizine (**17**). Hydrogenation in EtOH in the presence of palladium on carbon afforded the 1-carboethoxypyrrolizidine (**16**) with the indicated relative stereochemistry. Reduction of this ester with $LiAlH_4$ yielded (±)-[5-^{3}H]isoretronecanol, 98.8% pure by gc. The overall yield from [5-^{3}H]proline was 29%.

These 1-hydroxymethylpyrrolizidines and [1,4-^{14}C]putrescine were administered as their hydrochloride salts, dissolved in H_2O, to *S. riddellii* plants growing in a greenhouse. A drop of the aqueous solution was placed on the stem (held in a horizontal position) where a tiny hole had been made with a sewing needle. This feeding technique was introduced by Robins (18). When putrescine was fed by this method (Exp. No. 4) and via a cotton wick inserted in the stems (Exp. No. 5), a significantly higher incorporation of activity into riddelliine was obtained by the former method. A few days prior to feeding, watering of the plants was stopped since it has been reported that plants subjected to stress of this type produce more riddelliine (15). After 2 to 3 weeks the aerial parts of the plants were cut off and extracted with a reductive work up to convert riddelliine *N*-oxide to the tertiary amine. Details of the amounts of precursor fed, the yield and activity of the resultant riddelline, and the absolute and specific incorporations, are recorded in Table 1. All the experiments were not carried out under exactly the same environmental conditions. However, the very high absolute incorporations of (±)-trachelanthamidine (15.1 and 21.2%) are remarkable. Incorporations of this magnitude are seldom obtained in biosynthetic studies with higher plants. The incorporation of (±)-isoretronecanol was lower (0.75%) but still significant.

TABLE 1. Compounds Fed to *Senecio riddellii* and Activity of the Resultant Riddelliine

Exp. No.	Compound fed (wt. activity)	Month of initial feeding	Duration of feeding (days)	Fresh weight of plants (g)	Riddellii			
					Wt (mg)	Riddelliine dpm/mmol	Absolute inc. (%)	Specific inc.(%)
1	(±)-[3,5-^{14}C]-**3**·HCl (91.2 mg, 1.64×10^8 dpm)	August	21	230	794	1.08×10^7	15.1	3.6
2	(±)-[3,5-^{14}C]-**3**·HCl (100 mg, 2.46×10^9 dpm)	October	20	390	1260	1.45×10^8	21.2	3.3
3	(±)-[5-^{3}H]-**5**·HCl (674 mg, 4.84×10^8 dpm)	August	21	256	911	1.40×10^6	0.75	1.1
4	[1,4-^{14}C]Putrescine·2HCl (50 mg, 5.50×10^7 dpm)	November	15	140	168	1.04×10^6	0.91	0.6
5	[1,4-^{14}C]Putrescine·2HCl[a] (50 mg, 5.50×10^7 dpm)	November	15	135	170	6.30×10^5	0.56	0.53

[a]Fed by means of cotton wicks inserted into the stems. All other feedings by absorption through a stem puncture.

Degradations were carried out on the alkaloid isolated from Exps. No. 1 and No. 3 to determine whether the labeled trachelanthamidine and isoretronecanol had been incorporated specifically into the retronecine moiety of riddelliine. Hydrolysis of the alkaloid with $Ba(OH)_2$ yielded riddellic acid and retronecine which contained almost all the radioactivity of the original alkaloid. Oxidation of retronecine with chromium

trioxide yields β-alanine (**8**) along with other amino acids. This compound is derived from C-5, C-6, and C-7 of retronecine, and it was isolated as its *N*-2,4-dinitrophenyl derivative as previously described (18,19). The activity of these degradation products is recorded in Table 2, and it is evident that the results are consistent with the riddelliine being labeled at C-3 and C-5 from [3,5-^{14}C]trachelanthamidine and at C-5 with ^{3}H from [5-^{3}H]isoretronecanol.

TABLE 2. Activity of Riddelliine and Its Degradation Products

Source of labeled riddelline	Activity (dpm/mmol)			
	Experiment 1 (RSA)[a]		Experiment 3 (RSA)[a]	
Riddelliine	1.08×10^{7}	100	1.40×10^{6}	100
Riddellic Acid	1.94×10^{5}	1.8	3.3×10^{4}	2.4
Retronecine·HCl	1.07×10^{7}	99	1.34×10^{6}	96
N-2,4-Dinitrophenyl-β-alanine	4.95×10^{6}	46	1.30×10^{6}	93

[a]RSA=Relative specific activity.

The high level of incorporation of trachelanthamidine into the retronecine moiety of riddelliine strongly suggests that this hydroxymethylpyrrolizidine is on the main pathway from homospermidine to retronecine. Recently, Robins has investigated the stereochemistry of the final steps in retronecine biosynthesis in *Senecio isatideus* (20). It was discovered that the pro-*S* hydrogen is lost from the position which ultimately becomes C-2 of retronecine. This result, considered with our observations on the incorporation of trachelanthamidine, indicates that the introduction of the double bond into retronecine proceeds with a *trans*-elimination of the hydrogens at C-1 and C-2 of trachelanthamidine, if it is assumed that it is the (1*R*)-isomer (depicted as (**3**) in Figure 1) which is the precursor of retronecine. This assumption seems reasonable in view of the stereochemistry at C-8 but will be checked in the future by feeding the enantiomers of trachelanthamidine. Independently, Robins (21) has found that (±)-[5-^{3}H]trachelanthamidine is a precursor of retronecine in *S. isatideus*.

The incorporation of isoretronecanol into riddelliine may represent a minor, perhaps aberrant, pathway to retronecine. It seems unlikely that a change in the stereochemistry of its hydroxymethyl group will occur in the absence of any activating group. The lower level of incorporation of putrescine into riddelliine is consistent with this compound being a more remote precursor of the alkaloid.

Our results have established that it is possible to produce radioactive riddelliine labeled at specific positions by feeding to *S. riddellii* specifically labeled compounds which are advanced intermediates in the biosynthetic pathway to the ultimate alkaloid. A mixture of labeled pyrrolizidine alkaloids (retrorsine, seneciphylline, and senecionine) was obtained by growing *Senecio vulgaris* plants in an atmosphere containing [^{14}C]CO_2 (22), but the absolute incorporation of activity was only 0.05%, and the radioactivity was presumably present in both the retronecine and necic acid portions of these alkaloids. The labeled riddelliine will be used to study its metabolism in animals to elucidate the mechanism whereby it and other pyrrolizidine alkaloids are hepatotoxic. A recent report (23) that the retronecine moiety of senecionine is metabolized to 4-hydroxy-2(*E*)-hexenal, which is hepatotoxic, will be evaluated.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are corrected. Radioactivity measurements were carried out in a Tracor Analytic Mark III liquid scintillation counter, with dioxane-EtOH as

the solvent with the usual scintillators (24). Mass spectra were determined by Dr. E. Larka and his associates at the University of Minnesota on an AE1-30 spectrometer. Nmr measurements were carried out on a Nicolet 300 spectrometer, operating at 300 MHz (^{1}H) and 75.5 (MHz) (^{13}C), with the assistance of Dr. S.B. Philson. Gc analyses were carried out on a glass capillary column in a Hewlett-Packard model 5890A gas chromatogram.

[1-^{14}C]-4,4-DIETHOXYBUTANONITRILE (**10**).—Potassium [^{14}C]cyanide (586 mg, 2.00×10^9 dpm, 9.0 mmol) dissolved in H_2O (3 ml) was added dropwise to a solution of 3-chloropropanal diethyl acetal (1.0 g, 6 mmol) and KI (0.5 g, 3 mmol) in EtOH (15 ml) at 20°. After refluxing for 30 h, the reaction mixture was cooled and filtered, the residual salts being washed with $CHCl_3$. The filtrate was evaporated to small bulk, diluted with H_2O (10 ml) and extracted with Et_2O (7×5 ml). The residue obtained on evaporation of the dried (Na_2SO_4) extract was distilled (104°, 10 mm) to afforded the nitrile **10** as a colorless oil (700 mg, 1.36×10^9 dpm, radiochemical yield 68%). The following spectral data were obtained on unlabeled material: ir (neat) 2250 cm^{-1} (CN); ^{1}H nmr ($CDCl_3$) δ 1.23 (6H, t, CH_3), 1.91 (2H, m, C-3), 2.45 (2H, m, C-2), 3.52 (4H, m, CH_2 of Et), 4.58 (1H, t, C-4); ^{13}C nmr $CDCl_3$) δ 119.5 (CN), 100.8 (C-4), 62.3 (-OCH_2CH_3), 29.6 (C-3, 15.3 (-OCH_2CH_3), 12.5 (C-2); ms *m/z* (rel. int.) 156 (M^+H) (0.5), 128 (0.7), 112 (46), 103 (41), 85 (6), 84 (100), 75 (27), 54 (16), 47 (55), 41 (32), 29 (28).

[4-^{14}C]-4-AMINOBUTANAL DIETHYL ACETAL (**11**).—[1-^{14}C]-4,4-Diethoxybutanonitrile (700 mg) dissolved in dry Et_2O (10 ml) was added dropwise to a stirred suspension of $LiAlH_4$ (500 mg) in Et_2O (10 ml) at 20 °. The mixture was then refluxed for 24 h. Excess $LiAlH_4$ was destroyed by the addition of a few drops of aqueous potassium sodium tartrate to the cooled reaction mixture, which was then filtered, the residue being washed well with Et_2O. Evaporation of the dried (Na_2SO_4) filtrates afforded the amine **11** as a colorless oil (611 mg, 1.21×10^9 dpm) 97.8% pure by gc. Spectral data were obtained on unlabeled material: ir (neat) 3398 cm^{-1} (NH_2); ^{1}H nmr ($CDCl_3$) δ 1.2-1.36 (6H, t, CH_3, 1.45-1.6 (2H, m), 2.63-2.79 (2H, t), 3.40-3.66 (4H, q, -OCH_2CH_3), 4.49 (1H, t); ^{13}C nmr ($CDCl_3$) δ 102.8 (C-1), 61.0 (-OCH_2CH_3), 42.1 (C-4), 31.0 (C-2), 29.1 (C-3), 15.4 (-OCH_2CH_3); ms (CI/CH_4) *m/z* (rel. int.) 162 (M^+H) (5.2), 116 (30), 98 (9), 70 (70), 47 (27), 29 (100).

[4,6-^{14}C]-1,1,9,9-TETRAETHOXY-5-AZANONANE (**13**).—A solution of [4-^{14}C]-4-aminobutanal diethyl acetal (611 mg) in dry C_6H_6 (5 ml) was added to a suspension of freshly prepared Raney Ni (W-2) (ca. 800 mg) in dry C_6H_6 (20 ml), and the mixture was refluxed in a N_2 atmosphere for 30 h. The cooled mixture was then filtered through Celite, dried (Na_2SO_4), evaporated, and the residue distilled (110-115°, 0.2 mm) to yield **13** as a colorless oil (446 mg, 1.07×10^9 dpm) >99% pure by gc. Spectral data on unlabeled material: ir (neat) 3300 (NH), 1130, 1053 cm^{-1}; ^{1}H ($CDCl_3$) δ 1.11-1.29 (12H, t, CH_3), 1.52-1.65 (8H, m), 1.95 (1H, br, NH, exchangeable with D_2O), 2.63 (4H, m), 3.41-3.66 (8H, q), 4.51 (2H, m); ^{13}C nmr ($CDCl_3$) δ 102.8 (C-1,9), 60.9 (-OCH_2CH_3), 49.8 (C-4,6), 31.5 (C-3,7), 25.5 (C-2,8), 15.4 (O-CH_2CH_3); ms *m/z* (rel. int.) 304 (M^+-H) (1.3), 288 (1.2, 274 (0.5), 202 (5), 188 (11), 172 (14), 156 (7), 135 (8), 103 (32), 99 (20), 86 (100), 75 (19), 71 (18), 58 (16), 47 (19).

(±)-[3,5-^{14}C]TRACHELANTHAMIDINE (**3**).—The amine **13** (446 mg) was dissolved in a mixture of EtOH (10 ml) and concentrated HCl (2.5 ml), and the solution refluxed in a N_2 atmosphere for 4 h. The residue obtained on evaporation of this solution (containing the aldehyde **12**) was dissolved in MeOH (10 ml) at 0° and $NaBH_4$ (200 mg) was added to the stirred solution. After 3 h at 20°, solid K_2CO_3 was added, and the solution was concentrated to a small bulk. The mixture was extracted with $CHCl_3$ (7×10 ml). Evaporation of the dried (K_2CO_3) extract afforded a pale brown oil that was distilled (115-120°, 0.08 mm) to yield **3** as a colorless oil, 98.1% pure by gc. The base was converted to its HCl salt (91.4 mg, 1.66×10^8 dpm). Non-labeled material afforded a picrate, mp 173-174°, lit (16) 177-178°. Spectral data on unlabeled free base: ir (neat) 3300 cm^{-1} (OH); ^{1}H nmr ($CDCl_3$) δ 1.42-3.57 (12H, m), 3.61 (2H, d, C-9), 4.18 (1H, s, OH); ms *m/z* (rel. int.) 141 (M^+) (14), 83 (100), 82 (48), 55 (49), 41 (30).

(*S*)-[5-^{3}H]-*N*-FORMYLPROLINE (**15**).—Ac_2O (12.75 g) and HCOOH (11.5 g) were stirred at 20° for 90 min. (*S*)-[5-^{3}H]Proline (1.436 g, 1.66×10^9 dpm) dissolved in HCOOH (5 ml) was added and the mixture stirred at 20° for 12 h. The syrupy residue obtained on evaporation of the solvent on a rotary evaporator was dissolved in hot EtOH. On cooling overnight at 0°, (*S*)-[5-^{3}H]-*N*-formylproline separated as colorless prisms (1.25 g, 1.14×10^9 dpm) mp 94-95.5°, lit (17) mp 88-91°. Spectral data on unlabeled material: ir (nujol) 1720, 1630 cm^{-1}; ^{1}H nmr (D_2O) some signals were clearly observable as doublets due to the two isomers present arising from restricted rotation of the *N*-formyl group. δ 1.95 (2H, m, C-4), 2.3 (2H, m, C-3), 3.48, 3.73 (2H, m, C-4), 4.40, 4.68 (1H, m, C-2), 8.18, 8.24 (1H, s, CHO); ^{13}C nmr (D_2O) δ 178.4, 177.4 (COOH), 167.0, 165.8 (CHO, 62.0, 59.3 (C-2), 49.8, 46.7 (C-5), 31.91, 31.82 (C-3), 26.1, 24.9 (C-4); ms *m/z* (rel. int.) 143 (M^+) (14), 115 (2.2), 99 (40), 98 (100), 71 (23), 70 (66), 68 (10), 43 (23).

ETHYL [5-^{3}H]-6,7-DIHYDRO-5H-PYRROLIZINE-1-CARBOXYLATE (**17**).—Ethyl propiolate (4.9 g, 50 mmol) was added to a stirred solution of **15** (1.25 g, 8.74 mmol) in Ac_2O (15 ml), which was then heated at 145° (oil bath temp) in a N_2 atmosphere for 4 h. Evaporation of the cooled reaction mixture furnished a brown viscous oil that was subjected to column chromatography on SiO_2. Gradient elution with $CHCl_3$-MeOH (250:1 to 49:1) afforded the pyrrolizine **17** as a pale colored oil (1.35 g, 9.75×10^8 dpm). Spectral data on unlabeled material: ir (neat) 1690 (C=0), 1550 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 1.32 (3H, t, CH_3), 2.61 (2H, m, C-6), 3.05 (2H, t, C-7), 4.21 (2H, t, C-5), 4.30 (2H, q, -OCH_2CH_3), 6.56 (2H, dd, C-2+C-3); ^{13}C nmr ($CDCl_3$) δ 165.2 (C-9), 143.9 (C-8), 114.6 (C-3, 113.2 (C-2), 107.2 (107.2), 59.2 (-OCH_2CH_3), 46.9 (C-5), 27.1 (C-7), 25.5 (-6), 14.6 (-OCH_2CH_3); ms *m/z* (rel. int.) 179 (M^+) (45), 150 (63), 134 (100), 106 (37), 51 (17).

(±)-[5-^{3}H]ISORETRONECANOL (**5**).—A solution of the pyrrolizine **17** (1.35 g) in EtOH (25 ml) was hydrogenated in the presence of 10% Pd/C (1.5 g) at 20° and 3 atmospheres pressure for 3 days. Evaporation of filtered reaction mixture yielded a colorless oil (1.03 g) whose spectral characteristics were consistent with ethyl (±)-8α-pyrrolizidine-1β-carboxylate (**16**): ir (neat) 1720 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 1.26 (3H, t, CH_3), 1.73-1.96 (7H, m), 2.51-3.04 (4H, m), 3.5-3.85 (1H, m). The ester **16** (1.03 g) dissolved in Et_2O (20 ml) was added to a suspension of $LiAlH_4$ (425 mg) in Et_2O (15 ml) and the mixture stirred at 20° in a N_2 atmosphere for 2 h. Excess $LiAlH_4$ was destroyed by the addition of wet Et_2O and a drop of 10% NaOH. The filtered reaction mixture was dried (Na_2SO_4), and the oil obtained on evaporation was distilled (96-98°, 0.01 mm) to afford **5** as a colorless oil, which was 98.8% pure by gc. It was converted to its hydrochloride (674 mg, 4.84×10^8 dpm). Spectral data on the unlabeled free base: ir (neat) 3300 cm^{-1} (OH); ^{1}H nmr ($CDCl_3$) δ 1.50-3.35 (12H, m) 3.62 (2H, d, C-9), 5.45 (1H, s, OH); ms *m/z* (rel. int.) 141 (M^+) (3.2), 140 (15), 124 (17), 110 (15), 83 (100), 82 (42), 55 (20). It afforded a picrate, mp 190-191°, lit (25) mp 190-192°.

ADMINISTRATION OF THE POTENTIAL PRECURSORS TO *S. RIDDELLII* AND ISOLATION OF THE RIDDELLIINE.—The plant *S. riddellii* (Riddell's groundsel) was cultivated in a greenhouse. The plants were between 1 and 2 years old at the time of feeding. At the time of feeding the plants were about 1 m in height and were producing flowers. A few days prior to feeding, the plants were subjected to stress by not watering. Details of the amounts and activities of the precursors fed are recorded in Table 1. The compounds were fed as aqueous solutions of their hydrochloride salts. No deleterious effects were noted at the sites of feeding. In experiments No. 1-4 the solutions were applied as a single drop to a puncture made in the stem with a sewing needle. This type of feeding was spread over several days. In experiments No. 5 the precursor was fed via a cotton wick inserted through the stem of the plant. The isolation of riddelline from experiment No. 1, which follows, is typical of the method of extraction.

The aerial parts of the plant were cut off near to ground level and the fresh material (230 g) chopped up in a Waring blender with MeOH (2 liters). The mixture was then filtered, the residue being washed with more MeOH. The combined filtrates were evaporated and the residue was dissolved in 2 N H_2SO_4 (40 ml). This aqueous solution was washed with $CHCl_3$ (7×20 ml) to remove lipids and pigments. Zn dust (2 g) was added to the acidic solution which was stirred for 1 h. The mixture was then filtered through Celite and the filtrate extracted with $CHCl_3$ (3×20 ml). The cooled aqueous solution was then made basic with concentrated NH_3 and extracted with $CHCl_3$ (6×25 ml). Evaporation of the dried (Na_2SO_4) extract yielded a cream colored solid which was crystallized from absolute EtOH to afford riddelliine (794 mg), mp 192-194°. Tlc [on SiO_2 PF-254, developing with $CHCl_3$-MeOH-NH_3 (85:14:1)] showed a single spot (detected by uv), Rf 0.40, coincident with authentic riddelliine. Its ^{13}C-nmr spectrum was identical with that previously reported (26).

DEGRADATION OF THE LABELED RIDDELLIINE.—These reactions were applied to the labeled riddelliine obtained from experiments No. 1 and No. 3. Activities of the degradation products are recorded in Table 2.

A mixture of riddelliine (94 mg) and $Ba(OH)_2\cdot8H_2O$ (200 mg) was dissolved in H_2O (5 ml) and the solution refluxed for 3 h. Then CO_2 was passed into the cold solution, and the resultant $BaCO_3$ filtered off. The filtrate was acidified with HCl and extracted continously with Et_2O for 48 h. Evaporation of the dried (Na_2SO_4) extract afforded riddellic acid (32 mg), having properties (tlc, [α]) identical with an authentic specimen. Evaporation of the aqueous solution which had been extracted with Et_2O afforded a residue which was crystallized from MeOH yielding retronecine hydrochloride (48.9 mg) mp 162-163°, identical with an authentic specimen.

A solution of retronecine hydrochloride (48 mg) in 6N H_2SO_4 (2 ml) was mixed with a solution of CrO_3 (250 mg) in H_2O (2 ml) and refluxed for 48 h. The reaction mixture was worked up as previously described (19), and reacted with 1-fluoro-2,4-dinitrobenzene. The resultant dinitrophenyl derivatives of amino acids were separated by tlc (18), yielding *N*-2,4-dinitrophenyl-β-alanine (4.8 mg) as the major component.

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