Pyrrolizidine Alkaloid Biosynthesis. Synthesis of ^{3}H-Labelled Trachelanthamidine and Isoretronecanol and their Incorporation into Three Pyrrolizidine Bases (Necines) [1]

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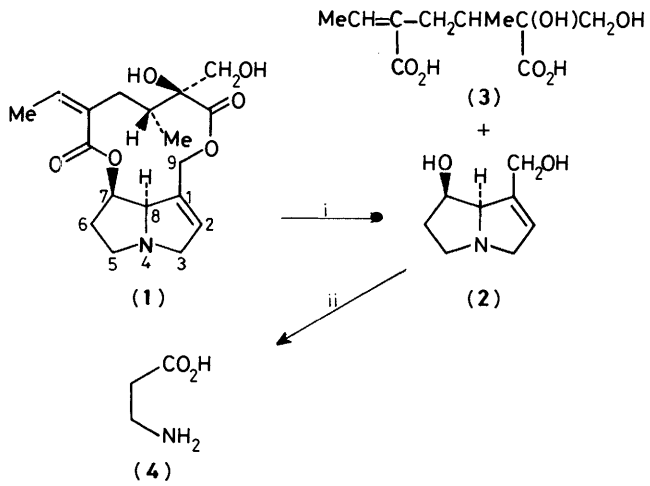
(±)-[5-^{3}H]Isoretronecanol (**22**) and (±)-[5-^{3}H]trachelanthamidine (**24**) were prepared by 1,3-dipolar cycloaddition of *N*-formyl[5-^{3}H]proline with ethyl propiolate followed by reduction steps. These ^{3}H-labelled 1-hydroxymethylpyrrolizidines together with [1,4-^{14}C]putrescine were fed to *Senecio isatideus* which produces retrorsine (**1**); *S. pleistocephalus* which yields rosmarinine (**8**); and *Cynoglossum officinale* which affords echinatine (**5**). The double labelling experiments demonstrated that isoretronecanol is incorporated much more efficiently into rosmarinine than into retrorsine or echinatine, whereas trachelanthamidine is a much more efficient precursor for retrorsine and echinatine. Base hydrolysis of retrorsine and echinatine labelled with [5-^{3}H]trachelanthamidine and of rosmarinine labelled with [5-^{3}H]isoretronecanol established that most of the ^{3}H-label was in the base portions, retronecine (**2**), heliotridine (**6**), and rosmarinecine (**9**), respectively. Further degradation of retronecine and rosmarinecine showed that most of the radioactivity was confined to the β-alanine (**4**) portion. The biosynthetic pathways to isoretronecanol and trachelanthamidine apparently diverge prior to the formation of these 1-hydroxymethylpyrrolizidines, probably during the cyclisation of an immonium ion (**14**) to form the 1-formylpyrrolizidines (**15**) and (**17**).

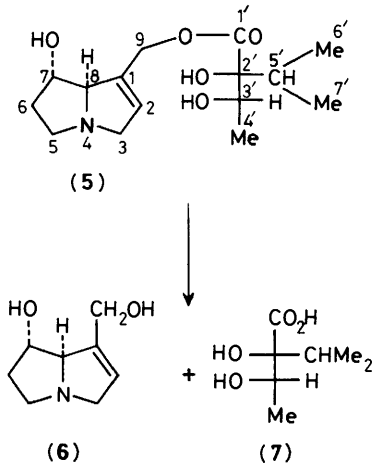
Pyrrolizidine alkaloids have a widespread distribution in plants and many of them are hepatotoxic.[2] The most toxic of these alkaloids are macrocyclic diesters of (+)-retronecine (**2**) such as retrorsine (**1**), which is the main constituent of *Senecio isatideus* plants (Scheme 1). The toxic action is believed to involve



Scheme 1. *Reagents:* i, $Ba(OH)_2$; ii, CrO_3–H_2SO_4

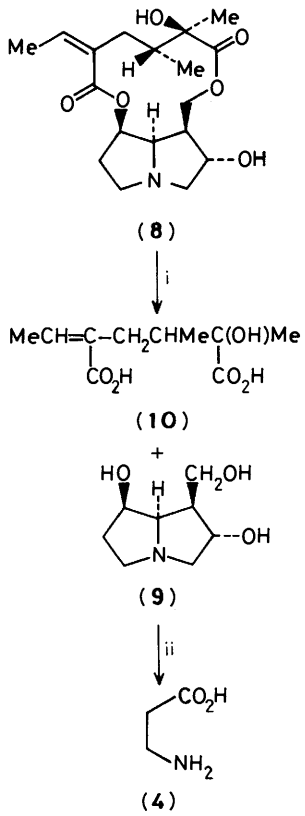
oxidation of the 1,2-unsaturated base portion (necine) by liver oxidase enzymes to the corresponding pyrrole derivatives which can act as bifunctional alkylating agents.[3] Monoesters of (+)-heliotridine (**6**), such as echinatine (**5**) from *Cynoglossum officinale* plants, are less toxic (Scheme 2). Alkaloids such as rosmarinine (**8**) which do not possess a 1,2-double bond are not hepatotoxic. Rosmarinine is the major alkaloidal constituent of *S. pleistocephalus* and yields (−)-rosmarinecine (**9**) on alkaline hydrolysis (Scheme 3).

Extensive feeding experiments on (+)-retronecine (**2**) with ^{14}C- and ^{13}C-labelled precursors have shown that it is formed

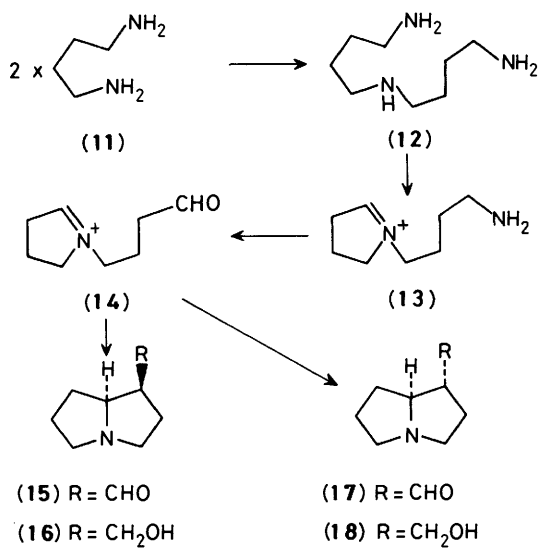


Scheme 2.

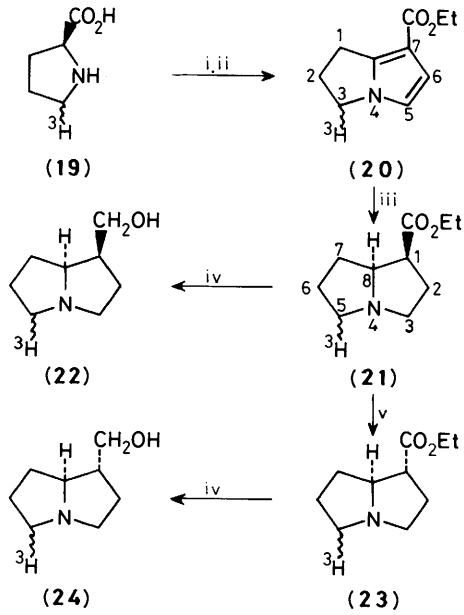
from two molecules of putrescine (**11**)[4] *via* homospermidine (**12**).[5] Similar results have recently been reported for rosmarinecine (**9**).[6] No biosynthetic experiments have so far been reported on (+)-heliotridine (**6**). An indication of how homospermidine is converted into necines was provided by the conversion of this triamine (**12**) into trachelanthamidine (**18**) using enzymes under physiological conditions (Scheme 4).[7] It is likely that oxidation of one terminal amino group of homospermidine produces an immonium ion (**13**)[8] which can then undergo oxidation of the remaining primary amino group, followed by a non-enzymic intramolecular cyclisation of the aldehyde (**14**) to give the more stable 1-formylpyrrolizidine (**17**). Reduction of this aldehyde (**17**) then affords trachelanthamidine (**18**). The formation of this base using enzymes suggested that the simple 1-hydroxymethylpyrrolizidines (**16**) and (**18**) should be tested as intermediates in the biosynthesis of more complex necines such as retronecine (**2**), rosmarinecine (**9**), and heliotridine (**6**).



Scheme 3. *Reagents:* i, $Ba(OH)_2$; ii, CrO_3–H_2SO_4



Scheme 4.



Scheme 5. *Reagents:* i, Ac_2O, HCO_2H; ii, Ac_2O, $HC{\equiv}CCO_2Et$; iii, H_2, Rd/C; iv, $LiAlH_4$; v, HCl

Results and Discussion

The synthesis of (±)-isoretronecanol (**16**) and (±)-trachelanthamidine (**18**) from L-proline has been reported by Pizzorno and Albonico.[9] This route was modified to produce ^{3}H-labelled samples of each necine as outlined in Scheme 5. [5-^{3}H]-L-Proline (**19**) was converted into its *N*-formyl derivative, and this was subjected to 1,3-dipolar cycloaddition with ethyl propiolate to yield the dihydropyrrolizine ester (**20**) after elimination of carbon dioxide. Hydrogenation of the pyrrole ring of (**20**) under the reported conditions[9,10] proved difficult. The best procedure was found to be use of a rhodium-on-carbon catalyst which gave a 62% yield of the saturated ester (**21**), and permitted recovery of starting material (**20**) (28%) which could be recycled. Reduction of the saturated ester (**21**) with lithium aluminium hydride gave (±)-[5-^{3}H]-isoretronecanol (**22**). Epimerisation of the 1β-ester (**21**) to the thermodynamically more stable 1α-isomer (**23**) has been reported using alkoxides.[11] We found that this procedure led to extensive degradation of the ester (**21**). An alternative method using acidic conditions succeeded in producing the 1α-ester (**23**). Reduction of this ester generated (±)-[5-^{3}H]trachelanthamidine (**24**). Careful examination of the ^{13}C n.m.r. spectra of both ^{3}H-labelled necines (**22**) and (**24**) indicated that each racemate contained less than 3% of the other racemate.

A preliminary feeding experiment was carried out with *Cynoglossum officinale* plants. [1,4-^{14}C]Putrescine dihydrochloride was fed to four *C. officinale* plants by direct absorption of a sterile aqueous solution through stem punctures.[12] After one week, the major alkaloidal constituent, echinatine (**5**), was extracted and purified by column chromatography on basic alumina. A reasonable specific ^{14}C incorporation of 0.6% (0.3% per C_4 unit) was observed in echinatine (92 mg). Alkaline hydrolysis of the ^{14}C-labelled echinatine gave heliotridine (**6**) and (−)-viridifloric acid (**7**) (Scheme 2). The distribution of radioactivity was 95% in the base and 2% in the acid, indicating that heliotridine is biosynthesized from putrescine.

For the feeding experiments with the necines (**22**) and (**24**) a double labelling technique was employed.[12] Each sample of the hydrochloride of the ^{3}H-labelled necines (**22**) and (**24**) was fed together with [1,4-^{14}C]putrescine dihydrochloride as a reference with an initial ^{3}H/^{14}C ratio of 10.0. The mixtures of isotopically labelled species were fed to *Senecio isatideus* and *Cynoglossum officinale* by direct absorption into the xylems through stem punctures,[12] and to *S. pleistocephalus* by the wick method.[6] All experiments were completed in one day and after one week, retrorsine (**1**) was isolated from *S. isatideus*, rosmarinine (**8**) from *S. pleistocephalus*, and echinatine (**5**) from *C. officinale*. Retrorsine and rosmarinine were recrystallised to constant specific activity, whereas echinatine was purified by chromatography and obtained as a gum. The ^{3}H/^{14}C ratios for

each feeding experiment (Table) provide a good measure of the relative efficiency of each ^{3}H-labelled necine as a precursor for the alkaloid relative to putrescine. (±)-[5-^{3}H]Isoretronecanol is incorporated well into rosmarinine (**8**) with an increase in the $^3H/^{14}C$ ratio from 10.0 to 17.0, but is a poor precursor of retrorsine (**1**) and echinatine (**5**) with the $^3H/^{14}C$ ratios decreasing from 10.0 to 0.7 and 1.0, respectively. By contrast, (±)-[5-^{3}H]trachelanthamidine is a good precursor of retrorsine (**1**) and echinatine (**5**) with the $^3H/^{14}C$ ratios increasing to 14.3 and 17.0 respectively, whereas no ^{3}H label could be detected in rosmarinine (**8**) after feeding of this compound.

These results (Table) show that isoretronecanol is incorporated more than 30 times more efficiently into rosmarinine (**8**) than is trachelanthamidine. On the other hand, trachelanthamidine is a better precursor for retrorsine (**1**) (20 times) and echinatine (**5**) (17 times) than isoretronecanol. Furthermore, the increase in $^3H/^{14}C$ ratios in Experiments 3—5 (Table) demonstrates that isoretronecanol is incorporated into rosmarinine, and trachelanthamidine is incorporated into retrorsine and echinatine, more efficiently than is putrescine. The small apparent incorporation of isoretronecanol into retrorsine and echinatine (Table) could arise after epimerisation of the base (**16**) in the biosynthetic pathway, but is more likely to be due to a small amount of trachelanthamidine in the isoretronecanol sample.

(±)-[5-^{3}H]Trachelanthamidine (**24**) was fed to *Senecio isatideus* and *Cynoglossum officinale*, and (±)-[5-^{3}H]isoretronecanol was administered to *S. pleistocephalus* to obtain ^{3}H-labelled samples of the alkaloids. Specific incorporations of ^{3}H were similar to those obtained in the double labelling experiments. The ^{3}H-labelled alkaloids were hydrolysed to give acidic and basic moieties and the ^{3}H distribution was measured. Retrorsine (**1**) gave retronecine (**2**) (95%) and isatinecic acid (**3**) (3%) (Scheme 1). Echinatine (**5**) yielded heliotridine (**6**) (99%) and (−)-viridifloric acid (**7**) (4%) (Scheme 2). Rosmarinine (**8**) afforded rosmarinecine (**9**) (97%) and senecic acid (**10**) (4%) (Scheme 3). Thus trachelanthamidine provides the necine portion of retrorsine and echinatine specifically, whereas isoretronecanol is a specific precursor for the base portion of rosmarinine. Further degradation of retronecine with chromic acid gave β-alanine (Scheme 1) corresponding to the C(5)–C(6)–C(7) unit. This was isolated as its *N*-2,4-dinitrophenyl derivative as described previously[12] and contained 95% of the specific radioactivity of retronecine. Similar degradation of rosmarinecine gave the derivative of β-alanine with 92% of the specific radioactivity of rosmarinecine. There was insufficient material for degradation of the heliotridine. The results of these degradations are consistent with the intact incorporation of trachelanthamidine into retronecine and isoretronecanol into rosmarinecine.

Leete and Rana have published similar evidence for the incorporation of ^{3}H-labelled trachelanthamidine rather than isoretronecanol into riddelliine in *Senecio riddellii*.[13] Riddelliine contains retronecine as the base portion.

Our results indicate that epimerisation of trachelanthamidine (**18**) and isoretronecanol (**16**) does not take place to any appreciable extent during the biosynthesis of retronecine (**2**), heliotridine (**6**), and rosmarinecine (**9**). The pathways probably diverge prior to the formation of the alcohols during the cyclisation of the immonium ion (**14**). It is unlikely that the aldehyde (**17**) is epimerised to compound (**15**) *en route* to isoretronecanol (**16**) because ^{2}H is retained at the 1α-position in rosmarinecine after feeding (*S*)-[2-^{2}H]putrescine to obtain ^{2}H-labelled rosmarinine.[14] Epimerisation of aldehyde (**15**) to the more stable (**17**) in the biosynthetic pathway to trachelanthamidine is a possibility.

Birecka and Catalfamo have carried out pulse labelling experiments with $^{14}CO_2$ on *Heliotropium spathulatum* (Boraginaceae).[15] They found that the specific activities of the labelled bases trachelanthamidine (**18**), supinidine (1,2-didehydrotrachelanthamidine), and retronecine (**2**) were consistent with the biosynthetic sequence trachelanthamidine→supinidine→retronecine. It has been suggested[1] that formation of retronecine from trachelanthamidine occurs by two hydroxylations at C-2α and C-7 with retention of configuration followed by *trans*-elimination of the elements of water from the 1,2-position. Labelling studies with (*R*)- and (*S*)-[2-^{2}H]putrescines on retrorsine are in accord with this theory.[16] Rosmarinecine (**9**) is formed from isoretronecanol (**16**) by two hydroxylations at C-2α and C-7 which both occur with retention of configuration.[14] The details of these late stages in the biosynthetic pathways to necines are under investigation.

Experimental

General.—M.p.s were measured with a Kofler hot-stage apparatus and are uncorrected. Organic solutions were dried with anhydrous $MgSO_4$, and solvents were removed under reduced pressure below 40 °C. N.m.r. spectra were obtained for solutions in deuteriochloroform on a Bruker WP200-SY spectrometer operating at 200 MHz for ^{1}H and 50 MHz for ^{13}C. 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 (except echinatine) were recrystallised to constant specific activity and they were counted in duplicate. Thin layer chromatography was carried out on silica gel G plates of 0.25 mm thickness developed with chloroform–methanol–conc. ammonia (85:14:1), and the bases were located by oxidation with *o*-chloranil, followed by treatment with Ehrlich's reagent.[17] A Panax thin-layer scanner RTLS-1A was used for the radioscanning of t.l.c. plates.

Ethyl (±)-[3-^{3}H]-2,3-*Dihydro*-1H-*pyrrolizine*-7-*carboxylate* (**20**).—L-Proline (500 mg, 4.3 mmol) was added to an aqueous solution of L-[5-^{3}H]proline (**19**) (1 ml, 1 Ci mmol^{-1}, 1 mCi), and the solution was evaporated to dryness. The residue was dissolved in formic acid (10 ml) and was added to a mixture of acetic anhydride (10 ml) and formic acid (12 ml) at 0 °C. The solution was allowed to warm to room temperature and was left for 18 h. The solution was concentrated and the residue of [5-^{3}H]-*N*-formylproline (700 mg) was dissolved in acetic anhydride (40 ml). Ethyl propiolate (1.7 g, 17.3 mmol) was added, and the mixture was heated at reflux under nitrogen for 3 h. The solution was cooled and concentrated to a brown oil which was subjected to column chromatography on silica. Elution with diethyl ether–light petroleum (b.p. 60—80 °C) (1:2) afforded the dihydropyrrolizine (**20**) as a colourless oil (548 mg, 72%), 130 μCi mmol^{-1}; $\nu_{max.}$(CCl_4) 1 680 and 1 560 cm^{-1}; δ_H 1.30 (3 H, t, *J* 7 Hz, Me), 2.51 (2 H, m, 2-H_2), 3.05 (2 H, t, *J* 8 Hz, 1-H_2), 3.92 (2 H, m, 3-H_2), 4.24 (2 H, q, *J* 7 Hz, CH_2Me), and 6.50 and 6.60 (2 H, AB system, *J* 3 Hz, 5-H and 6-H).

Ethyl (±)-[5-^{3}H]*Pyrrolizidine*-1β-*carboxylate* (**21**).—The dihydropyrrolizine ester (**20**) (500 mg, 2.8 mmol) in acetic acid (40 ml) was hydrogenated using 5% rhodium-on-carbon (500 mg) at 7 atm for 18 h at room temperature. The catalyst was filtered off, and the filtrate was concentrated. The residual oil was partitioned between 1M HCl (25 ml) and ether (25 ml). The acid layer was washed with ether (4 × 25 ml), basified with conc. ammonia, and then extracted with ether (6 × 30 ml). The combined ether extracts were dried, filtered, and concentrated to give the ester (**21**) as a colourless oil (320 mg, 62%),

Table. Incorporation of precursors into retrorsine (**1**), echinatine (**5**), and rosmarinine (**8**)

Experiment	Precursor	Alkaloid	Quantity of alkaloid (mg)	^3H Specific Incorporation	$^3H/^{14}C$ ratio[a]
1	(±)-[5-^{3}H]Isoretronecanol (**22**)	(**1**)	165	0.3	0.7
2	(±)-[5-^{3}H]Isoretronecanol (**22**)	(**5**)	83	0.04	1.0
3	(±)-[5-^{3}H]Isoretronecanol (**22**)	(**8**)	198	2.4	17.0
4	(±)-[5-^{3}H]Trachelanthamidine (**24**)	(**1**)	152	2.8	14.3
5	(±)-[5-^{3}H]Trachelanthamidine (**24**)	(**5**)	71	0.35	17.0
6	(±)-[5-^{3}H]Trachelanthamidine (**24**)	(**8**)	227	<0.1	<0.5

[a] Initial $^3H/^{14}C$ ratio 10.0.

126 μCi mmol^{-1}; $\nu_{max.}$ 1 740 cm^{-1}; δ_H 1.29 (3 H, t, *J* 7 Hz), 2.1—3.35 (11 H, complex), 3.80 (1 H, m), and 4.2 (2 H, q, *J* 7 Hz). The picrate had m.p. 118—120 °C (from ethanol) (lit.,[9] 119—121 °C). The ether washings were dried, filtered, and concentrated to give unreacted starting material (**20**) (140 mg, 28%). The hydrogenation was repeated to yield a further 108 mg of the product (**21**).

(±)-[5-^{3}H]*Isoretronecanol* (**22**).—Lithium aluminium hydride (10 mg) was added to a solution of the pyrrolizidine ester (**21**) (66 mg, 0.36 mmol) in dry diethyl ether (10 ml) at 0 °C under nitrogen. The mixture was stirred for 1 h at 0 °C, then wet ether was added, followed by 20% aqueous NaOH (0.3 ml). The mixture was filtered through anhydrous Na_2SO_4, and the filtrate was concentrated to yield (±)-[5-^{3}H]isoretronecanol (**22**) (50 mg, 98%), 115 μCi mmol^{-1}; $\nu_{max.}$(neat) 3 300 cm^{-1} (br); δ_H 1.6—3.2 (12 H, complex), 3.65 (2 H, d, *J* 7 Hz, 9-H_2), and 3.90 (1 H, br s, exch. with D_2O, OH); δ_C 26.0, 26.5, and 27.3 (C-2, -6, and -7), 44.5 (C-1), 54.1 and 55.7 (C-3 and C-5), 63.0 (C-9), and 66.3 (C-8). The picrate had m.p. 190—192 °C (from ethanol) (lit.,[9] 187—189 °C).

Ethyl (±)-[5-^{3}H]*Pyrrolizidine-1α-carboxylate* (**23**).—The *endo*-ester (**21**) (65 mg, 0.36 mmol) was dissolved in 10M HCl (2 ml) and left for 2 h at room temperature. The solution was then heated at 180 °C for 18 h in a sealed tube then cooled and evaporated to dryness. The residue was dissolved in ethanol (10 ml) at 0 °C and thionyl chloride (0.2 ml) was added. The mixture was left for 18 h at room temperature, then concentrated to an oil which was partitioned between 1M HCl (25 ml) and diethyl ether (25 ml). The aqueous layer was washed with ether (3 × 25 ml), then basified with conc. ammonia and extracted with diethyl ether (5 × 30 ml). The combined ether extracts were dried, filtered, and concentrated to yield the *exo*-ester (**23**) as a colourless oil (41 mg, 63%), 84.1 μCi mmol^{-1}; $\nu_{max.}$ 1 740 cm^{-1}; δ_H 1.25 (3 H, t, *J* 7 Hz), 1.60—3.35 (11 H, complex), 3.65 (1 H, m), and 4.15 (2 H, q, *J* 7 Hz). The picrate had m.p. 180—181 °C (from ethanol) (lit.,[18] 180—181 °C).

(±)-[5-^{3}H]*Trachelanthamidine* (**24**).—The 1α-ester (**23**) (39 mg, 0.27 mmol) was reduced as described for the 1β-ester (**21**) to yield (±)-[5-^{3}H]trachelanthamidine (**24**) as a colourless oil (19 mg, 62%), 84.9 μCi mmol^{-1}; $\nu_{max.}$ 3 300 cm^{-1} (br); δ_H 1.8—3.7 (14 H, complex) and 5.2 (1 H, br s, exch. with D_2O, OH); δ_C 25.7, 30.2, and 32.0 (C-2, -6, and -7), 48.6 (C-1), 55.1 and 55.6 (C-3 and -5), 68.0 (C-9), and 68.3 (C-8). The picrate had m.p. 171—173 °C (from ethanol) (lit.,[19] 177—178 °C).

Feeding Methods.—*Senecio isatideus* and *S. pleistocephalus* plants were propagated by stem cuttings, whereas *Cynoglossum officinale* was grown from seed (Suttons Seeds). All plants were grown in a standard compost in a greenhouse. A sample of [1,4-^{14}C]putrescine dihydrochloride was added to each ^{3}H-labelled precursor to give an initial $^3H/^{14}C$ ratio of 10.0. The precursor mixture was dissolved in sterile water and over one day fed by adsorption through stem punctures for *S. isatideus* and *C. officinale*, and by the wick method to *S. pleistocephalus*. One well established plant was used for each experiment with *S. pleistocephalus* and four with *S. isatideus* and *C. officinale*. One week later, the plants were harvested and the alkaloids were isolated as described.[12]

Retrorsine (**1**) was recrystallised to constant specific radioactivity from acetone, m.p. 215—216 °C (lit.,[12] 216—217 °C), R_F 0.35.

Rosmarinine (**8**) was recrystallised to constant specific radioactivity from dichloromethane–acetone, m.p. 202—203 °C (lit.,[20] 202—204 °C), R_F 0.30.

Echinatine (**5**) was purified by column chromatography on basic alumina with dichloromethane–chloroform (3:1) as eluant to give the alkaloid as a gum, R_F 0.28, $[\alpha]_D^{20}$ +12.3° (*c* 0.94 in $CHCl_3$) (lit.,[21] $[\alpha]_D^{22}$+15.0°); $\nu_{max.}$(thin film) 3 400 and 1 728 cm^{-1}; δ_H 0.89 and 0.93 (both 3 H, d, *J* 6.8 Hz, 6′- and 7′-H_3), 1.27 (3 H, d, *J* 6.6 Hz, 4′-H_3), 1.86 (1 H, m, 6-H), 1.96 (1 H, m, 6-H), 2.18 (1 H, dq, *J* 6.8 Hz, 5′-H), 2.62 (1 H, ddd, *J* 10.7, 7.0, and 6.1 Hz, 5-H), 3.27 (1 H, dd, *J* 10.8 and 6.5 Hz, 5-H), 3.37 (1 H, dd, *J* 3.0 and 1.5 Hz, 3-H), 3.60 (1 H, br s, OH), 3.88 (1 H, dd, *J* 3.1 and 1.5 Hz, 3-H), 3.97 (1 H, m, 8-H), 3.99 (1 H, q, *J* 6.6 Hz, 3′-H), 4.01 (2 H, br s, 2 × OH), 4.15 (1 H, dt, *J* 6.0 Hz, 7-H), 4.79 and 4.96 (2 H, AB system, *J* 13.4 Hz, 9-H_2), and 5.70 (1 H, br s, 2-H); δ_C 15.7 and 17.8 (C-6′ and -7′), 17.2 (C-4′), 32.2 (C-5′), 33.5 (C-6), 54.2 (C-5), 61.7 (C-3), 62.0 (C-9), 71.6 (C-3′), 74.2 (C-7), 79.7 (C-8), 84.1 (C-2′), 125.6 (C-2), 136.1 (C-1), and 173.9 (C-1′); *m/z* 299 (M^+), 156, 139, 138, 137, 136, 120, and 95 (Found: M^+ 299.1735; C, 60.60; H, 8.21; N, 4.35%. $C_{15}H_{25}NO_5$ requires *M*, 299.1732; C, 60.18; H, 8.42; N, 4.68%). The picrolonate had m.p. 210—212 °C (lit.,[21] 214 °C) (Found: C, 53.4; H, 6.1; N, 12.6. $C_{25}H_{33}N_5O_{10}$ requires C, 53.3; H, 5.9; N, 12.4%).

After each feeding experiment, radioscans of t.l.c. plates of purified alkaloids showed only one radioactive band, coincident with authentic unlabelled alkaloid.

Hydrolysis of Alkaloids.—This was carried out for all three alkaloids as described for the hydrolysis of senecionine.[22] From the hydrolysis of retrorsine (**1**), isatinecic acid (**3**) was crystallised from ethyl acetate–light petroleum (b.p. 60—80 °C), m.p. 146—147 °C (lit.,[12] 147—149 °C), and retronecine (**2**) was crystallised from acetone–light petroleum (b.p. 60—80 °C), m.p. 119—120 °C (lit.,[12] 118—120 °C). From the hydrolysis of rosmarinine (**8**), senecic acid (**10**) was crystallised from ethyl acetate–light petroleum (b.p. 60—80 °C), m.p. 142—144 °C (lit.,[16] 142—144 °C), and rosmarinecine was crystallised from acetone–light petroleum (b.p. 60—80 °C), m.p. 170—171 °C (lit.,[20] 171—172 °C). Hydrolysis of echinatine (**5**) gave (−)-viridifloric acid (**7**), which was crystallised from ethyl acetate–light petroleum (b.p. 40—60 °C), m.p. 120—121 °C (lit.,[23] 119—120 °C), and (+)-heliotridine (**6**) which was crystallised from acetone, m.p. 116—117 °C (lit.,[22] 115—116 °C).

Degradation for C(5)–C(6)–C(7) *of Retronecine* (**2**) *and Rosmarinecine* (**9**).—This was carried out as described previously for retronecine.[12] Samples of *N*-2,4-dinitrophenyl-β-alanine were obtained from retronecine and rosmarinecine in *ca.* 10% yield, m.p. 145—146 °C (lit.,[12] 144—146 °C).

Acknowledgements

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