Pyrrolizidine Alkaloids: Evidence for the Involvement of Spermidine and Spermine in the Biosynthesis of Retronecine

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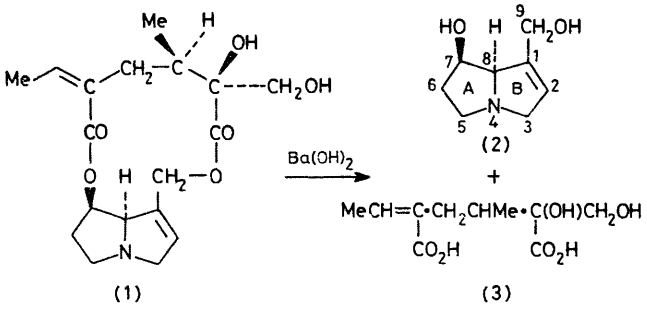
Summary Putrescine, spermidine, and spermine are efficient precursors of retronecine in *Senecio isatideus* plants; degradations of labelled alkaloids indicate that a symmetrical intermediate of the type C_4–N–C_4 is involved in the biosynthesis.

ORNITHINE,[1–3] putrescine,[2] and arginine[4] have been shown to be specific precursors of retronecine (**2**), the most common base portion (necine) of pyrrolizidine alkaloids. Degradations of retronecine, derived biosynthetically from [1,4-$^{14}C_2$]putrescine (**5**), [2-^{14}C]ornithine, or [5-^{14}C]ornithine (**4**) demonstrated that in each case one quarter of the total necine radioactivity was located at C-9, suggesting that C-2 and C-5 of the molecule of ornithine used to form ring B of retronecine (**2**) become equivalent. Further degradation of the retronecine is necessary to establish whether the second molecule of ornithine involved in the biosynthesis also passes through a symmetrical intermediate. We now report the first degradation to produce a fragment from ring A of a necine base. Furthermore, improved feeding techniques have led to much higher incorporations, and two new polyamine precursors have been identified.



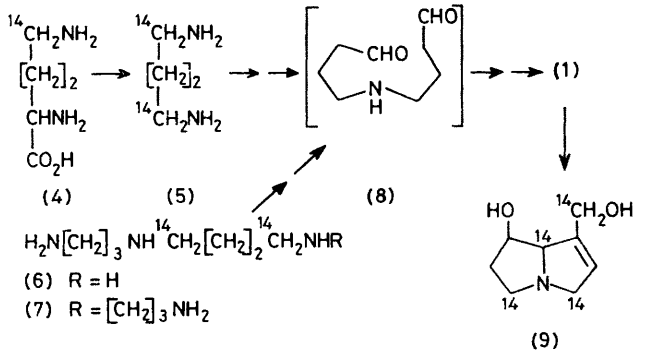
Retrorsine (**1**), the principal pyrrolizidine alkaloid in *Senecio isatideus* plants,[5] yields retronecine (**2**) and isatinecic acid (**3**) on alkaline hydrolysis. Good incorporations of precursors were achieved by direct absorption of a sterile aqueous solution of the precursor (as its hydrochloride) into the xylem of the growing plant through stem punctures. This technique gave incorporations which were consistently higher than those obtained by more usual methods (hydroponics, cut stem, wick method). ^{14}C-Labelled precursors

TABLE. Incorporation of precursors into retrorsine (1) in *Senecio isatideus* plants.

Expt.	Precursor	% Incorporation[a]	$^3H/^{14}C$ Ratio[b]	%^{14}C Radioactivity in (2)	%^{14}C Radioactivity in (3)
1	L-[*U*-^{14}C]Proline	0·04	77	68	30
2	[*U*-^{14}C]4-Aminobutanoic acid	0·04	37	52	48
3	L-[*U*-^{14}C]Glutamic acid	0·12	10	48	58
4	L-[*U*-^{14}C]Arginine	0·46	5·5	99	6
5	DL-[5-^{14}C]Ornithine (4)	0·25	6·2	97	5
6	[1,4-$^{14}C_2$]Putrescine (5)	1·6	0·60	94	1
7	[1,4-$^{14}C_2$-tetramethylene] Spermine (7)	5·2	0·73	103	1
8	[1,4-$^{14}C_2$-tetramethylene] Spermidine (6)	2·0	0·80	95	1

[a] Samples were recrystallised to constant specific activity. [b] L-[5-^{3}H]arginine was added to each precursor to give an initial $^3H/^{14}C$ ratio of 5·0.

were fed together with L-[5-^{3}H]arginine as a reference compound. Greater confidence can be placed in the $^3H/^{14}C$ ratios in the isolated retrorsine (1) (Table), than in incorporation figures which are known to vary markedly, even in duplicate experiments. The ratios indicate that the best precursors for retronecine biosynthesis are putrescine (5) and the previously untested polyamines, spermidine (6) and spermine (7). Putrescine is a much more efficient



precursor than ornithine, and this supports the theory[6] that putrescine follows ornithine in the biosynthetic pathway. Determination[2] of the amounts of radioactivity in the necine and acid portions of retrorsine (Table) showed that the poorer precursors, glutamate, 4-aminobutanoate, and proline are incorporated indiscriminately into both acid and base portions, while ornithine, arginine, putrescine, spermidine, and spermine specifically provide the necine portion of the alkaloid.

Degradations of retronecine (2) labelled with these specific precursors were carried out.[2] In experiments 5—8, 25, 24, 23, and 22%, respectively, of the total ^{14}C base activity was located at C-9 in agreement with previous results.[2] Modified Kuhn–Roth oxidation[7] of retronecine (2) gave β-alanine, isolated as its 2,4-dinitrophenyl derivative. This corresponds to C-(5+6+7) of retronecine. Again, in experiments 5—8, 22, 22, 23, and 24%, respectively, of the total ^{14}C base activity was found in this fragment. Experiment 5 shows that C-2 and C-5 of ornithine become equivalent in the formation of ring A of retronecine. These results suggest that a later, symmetrical intermediate, such as (8), derived from two molecules of putrescine, is involved in retronecine, (9), biosynthesis.[6]

The discovery of spermidine and spermine as efficient precursors for necine biosynthesis is interesting since both polyamines are known to produce pyrrolines on oxidation with the polyamine oxidases present in higher plants.[8]

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