Biosynthesis of Riddelliine: Incorporation of [3,5-^{14}C]Trachelanthamidine and [5-^{3}H]Isoretronecanol into the Retronecine Moiety of the Alkaloid

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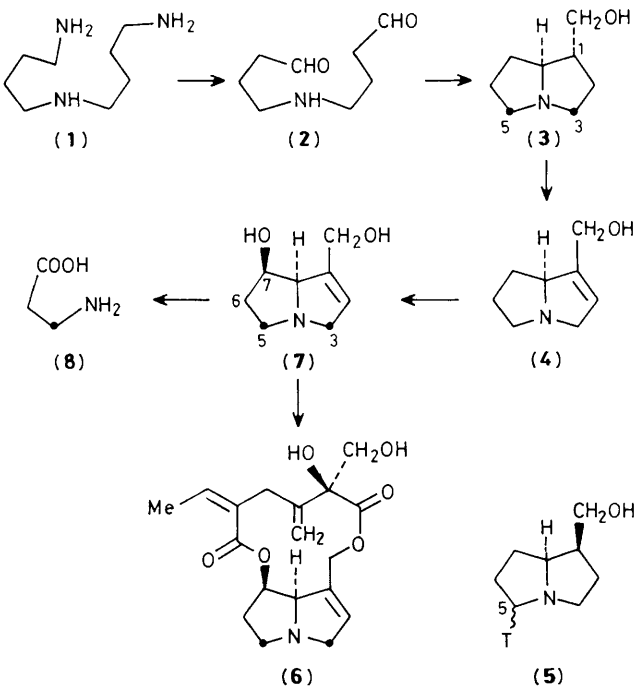
The two isomeric 1-hydroxymethylpyrrolizidines, trachelanthamidine and isoretronecanol labelled with ^{14}C and ^{3}H, respectively, were synthesized and fed to *Senecio riddellii* plants, resulting in the formation of riddelliine specifically labelled in its retronecine moiety, trachelanthamidine being the more efficient precursor (15.1% absolute incorporation).

Retronecine (**7**) is the basic moiety of many pyrrolizidine alkaloids[1] and its biosynthesis has been the subject of extensive tracer experiments which have been discussed in recent reviews.[2] The results obtained are consistent with the conversion of ornithine or arginine[3] into putrescine which is then transformed into homospermidine (**1**). It has been proposed that homospermidine, an established precursor of retronecine,[4] is oxidized to the dialdehydeamine (**2**). An intramolecular Mannich reaction followed by reduction affords trachelanthamidine (**3**). This 1-hydroxymethylpyrrolizidine has been obtained from (**2**) chemically[5,6] and from homospermidine by enzymic oxidation and reduction.[7] Short

Table 1. Incorporation of compounds into riddelliine.

Expt. no.	Precursor	Riddelliine incorporation (%)		Relative specific activities (riddelliine = 100)		
		Absolute	Specific	Riddellic acid	Retronecine	β-Alanine[b]
1	(±)-[3,5-^{14}C]-(**3**)[a] · HCl	15.1	3.6	1.8	99	46
2	(±)-[5-^{3}H]-(**5**)[a] · HCl	0.75	1.1	2.4	96	93
3	[1,4-^{14}C]Putrescine · 2HCl	0.91	0.6		Not determined	

[a] The compound fed was the depicted structure and its mirror image. [b] Isolated and assayed as *N*-2,4-dinitrophenyl-β-alanine.



Scheme 1. Proposed biosynthetic sequence from homospermidine to riddelliine.

term feeding of $^{14}CO_2$ to *Heliotropium spathulatum*[8] resulted in labelling of trachelanthamidine, supinidine (**4**), and retronecine. The specific activities of these bases were consistent with a biogenetic sequence: (**3**) → (**4**) → (**7**) as illustrated in Scheme 1.

We have now examined the ability of trachelanthamidine and its stereoisomer isoretronecanol (**5**) to serve as precursors of the retronecine moiety of the alkaloid riddelliine (**6**) and its *N*-oxide. These alkaloids are very abundant (up to 18% of the dry weight of the plant) in *Senecio riddellii*, a species which has been responsible for fatalities in cattle grazing in the Great Plains of the U.S.A.[9] (±)-[3,5-^{14}C]Trachelanthamidine was synthesized from [4-^{14}C]-4-aminobutanal diethyl acetal.[10] This labelled compound was obtained by the reduction ($LiAlH_4$) of [1-^{14}C]-4,4-diethoxybutanonitrile formed by reaction of 3-chloropropanal diethyl acetal with potassium [^{14}C]cyanide. (±)-[5-^{3}H]Isoretronecanol was prepared from [5-^{3}H]proline.[11] These compounds, and [1,4-^{14}C]putrescine, were administered to *S. riddellii* plants (2 years old) by absorption of aqueous solutions of their hydrochloride salts through stem punctures.[12] The plants were harvested 3 weeks after the initial feeding and riddelliine isolated from the aerial parts as previously described,[9] with a reductive work-up to convert the alkaloid *N*-oxide into the tertiary amine. In experiment 1 (±)-[3,5-^{14}C]trachelanthamidine hydrochloride (91.2 mg, 1.64×10^8 d.p.m.) afforded riddelliine (794 mg, 2.48×10^7 d.p.m.) from 230 g of the fresh plant. The labelled riddelliine obtained from the 1-hydroxymethylpyrrolizidines was hydrolysed to yield riddellic acid and retronecine which was then oxidized with CrO_3 in sulphuric acid to yield β-alanine (**8**)[12] which is derived from carbons 5, 6, and 7 of retronecine. The results are recorded in Table 1 and the relative specific activities of these degradation products of riddelliine indicate that the labelled (±)-trachelanthamidine and (±)-isoretronecanol were incorporated specifically into the retronecine moiety of the alkaloid without significant prior breakdown. The results indicate that both these 1-hydroxymethylpyrrolizidines can serve as precursors of retronecine. However the superior incorporation (both absolute and specific) of trachelanthamidine, indicates that this isomer is the most likely intermediate between homospermidine and retronecine. We do not know as yet which enantiomer of trachelanthamidine is involved. The incorporation of putrescine (expt. 3) into riddelliine was less than that of trachelanthamidine, which is consistent with this compound being a more remote precursor of the alkaloid.

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References

1 D. J. Robins, *Fortschr. Chem. Org. Naturst.*, 1982, **41**, 115.
2 I. D. Spenser, *Pure Appl. Chem.*, 1985, **57**, 453; R. B. Herbert, *Nat. Prod. Rep.*, 1985, **2**, 163; E. Leete, in 'Biosynthesis,' 1983, vol. 7, eds. R. B. Herbert and T. J. Simpson (Specialist Periodical Report, The Royal Society of Chemistry), p. 102.
3 D. J. Robins and J. R. Sweeney, *Phytochemistry*, 1983, **22**, 457.
4 H. A. Khan and D. J. Robins, *J. Chem. Soc., Chem. Commun.*, 1981, 554; *J. Chem. Soc., Perkin Trans. 1*, 1985, 819.
5 K. Babor, I. Ježo, V. Kaláč, and M. Karvaš, *Chem. Zvesti*, 1959, **13**, 163.
6 N. J. Leonard and S. W. Blum, *J. Am. Chem. Soc.*, 1960, **82**, 503.
7 D. J. Robins, *J. Chem. Soc., Chem. Commun.*, 1982, 1289.
8 H. Birecka and J. L. Catalfamo, *Phytochemistry*, 1982, **21**, 2645.
9 R. J. Molyneux and A. E. Johnson, *J. Nat. Prod.*, 1984, **47**, 1030.
10 S. Takano, N. Ogawa, and K. Ogasawara, *Heterocycles*, 1981, **16**, 915.
11 M. T. Pizzorno and S. M. Albonico, *J. Org. Chem.*, 1974, **39**, 731.
12 D. J. Robins and J. R. Sweeney, *J. Chem. Soc., Perkin Trans. 1*, 1981, 3083.