Evidence for Different 1-Hydroxymethylpyrrolizidines as Intermediates in the Biosynthesis of Retronecine and Rosmarinecine

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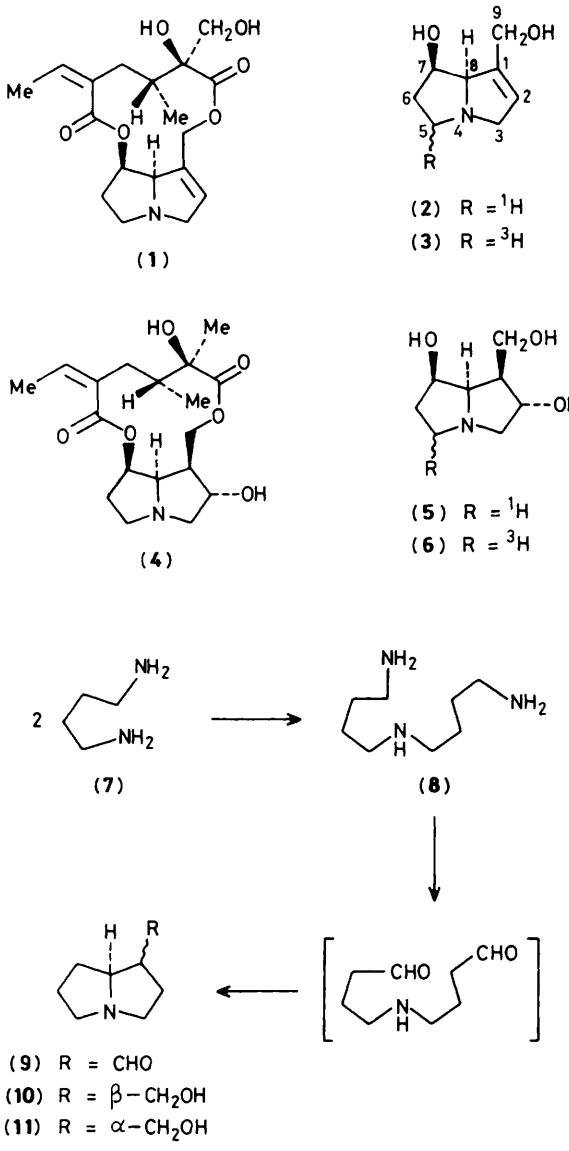
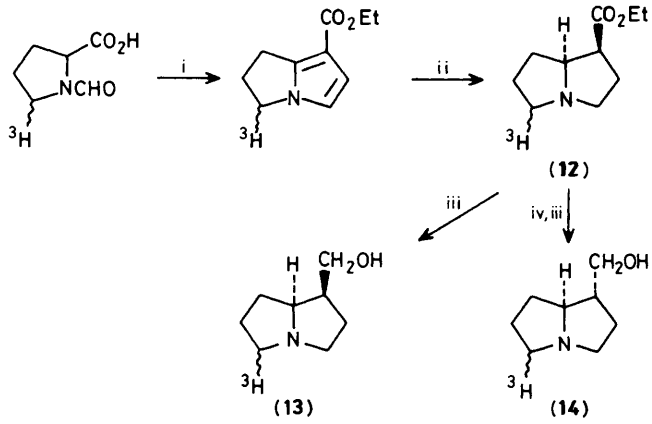
(±)-[5-^{3}H]Isoretronecanol (**13**) is incorporated specifically into rosmarinecine (**5**) in *Senecio pleistocephalus,* whereas (±)-[5-^{3}H]trachelanthamidine (**14**) is an efficient precursor for retronecine (**2**) in *Senecio isatideus.*

Plants containing pyrrolizidine alkaloids have a widespread occurrence.[1] Retrorsine (**1**) is the major alkaloidal constitutent of *Senecio isatideus* and gives retronecine (**2**), the most common necine, on alkaline hydrolysis. A different necine, rosmarinecine (**5**), is present in the alkaloid rosmarinine (**4**), which has recently been isolated from *S. pleistocephalus.*[2]

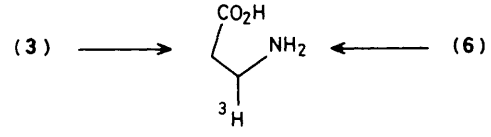
Experiments with ^{14}C- and ^{13}C-labelled precursors have established that retronecine (**2**)[3] and rosmarinecine (**5**)[2] are derived biosynthetically from two molecules of putrescine (**7**) *via* a symmetrical intermediate, homospermidine (**8**). Additional support for homospermidine as an intermediate in retronecine biosynthesis was provided by its conversion into



Scheme 1



Scheme 2. *Reagents:* i, Ac_2O, $HC{\equiv}CCO_2Et$; ii, H_2, Pd/C; iii, $LiAlH_4$; iv, HCl.



Scheme 3

the necine trachelanthamidine (**11**) using enzymes under physiological conditions.[4] Oxidation of homospermidine (**8**) with diamine oxidase is believed to yield a dialdehyde, which undergoes intramolecular cyclisation to give 1-formylpyrrolizidine (**9**). Reduction of this aldehyde then affords trachelanthamidine (**11**) (Scheme 1). The ease of formation of the simple necine (**11**) using enzymes suggested that trachelanthamidine, and its diastereoisomer (**10**), should be tested as intermediates in the biosynthesis of the more complex necines retronecine (**2**) and rosmarinecine (**5**). This communication presents evidence that trachelanthamidine (**11**) is indeed an efficient precursor for retronecine (**2**), whereas rosmarinecine (**5**) is formed from isoretronecanol (**10**).

Pizzorno and Albonico synthesized (±)-trachelanthamidine and (±)-isoretronecanol from L-proline.[5] This route was adapted to produce ^{3}H-labelled samples of each necine. [5-^{3}H]Isoretronecanol (**13**) was prepared by 1,3-dipolar cycloaddition of *N*-formyl-[5-^{3}H]-L-proline with ethyl propiolate, followed by hydrogenation of the pyrrole and reduction of the ester (**12**) (Scheme 2). [5-^{3}H]Trachelanthamidine (**14**) was formed after epimerisation of the saturated ester (**12**), followed by reduction of the ester group. From ^{1}H and ^{13}C n.m.r. spectra of the two ^{3}H-labelled necines (**13**) and (**14**) it was estimated that each racemate contained less than 3% diastereoisomeric material.

The ^{3}H-labelled necines (as their hydrochlorides) were mixed with [1,4-^{14}C]putrescine dihydrochloride to give an initial ^{3}H/^{14}C ratio of 10.0. The two mixtures of isotopically labelled species were fed to *S. pleistocephalus* plants by the wick method, and to *S. isatideus* by the xylem-pricking technique.[6] After one week, rosmarinine (**4**) and retrorsine (**1**) were extracted and recrystallised to constant specific radioactivity.

The ^{3}H specific incorporation for rosmarinine (**4**) was 2.4% with a ^{3}H/^{14}C ratio of 17.0 after feeding with isoretronecanol (**13**) and it was < 0.1% with a ^{3}H/^{14}C ratio < 0.5 after feeding with trachelanthamidine (**14**). These results indicate that isoretronecanol is incorporated into rosmarinine more than 34 times more efficiently than is trachelanthamidine. Furthermore, isoretronecanol is a more efficient precursor for rosmarinine than two molecules of putrescine [*ca.* 3.4 times better, if it is assumed that only one enantiomer of the racemate (**13**) is utilised in the biosynthesis].

In the case of retrorsine (**1**), the ^{3}H specific incorporation was 0.3% with a ^{3}H/^{14}C ratio of 0.7 after feeding isoretronecanol (**13**) and it was 2.8% with a ^{3}H/^{14}C ratio of 14.3 after feeding trachelanthamidine (**14**). These results indicate that trachelanthamidine is a much better precursor for retrorsine biosynthesis than isoretronecanol (*ca.* 20 times more efficient). Trachelanthamidine is also incorporated more efficiently into retrorsine than putrescine, supporting the later position of trachelanthamidine in the biosynthetic pathway. The small apparent utilisation of isoretronecanol in retrorsine biosynthesis could arise from epimerisation of this base in the biosynthetic pathway, but is more likely to be due to a small amount of impurity (**13**) in the sample of (**14**).

The distribution of ^{3}H labels in the alkaloid samples was determined after feeding [5-^{3}H]isoretronecanol (**13**) to *S.*

pleistocephalus and [5-^{3}H]trachelanthamidine (**14**) to *S. isatideus*. Retrorsine was hydrolysed to give retronecine (**3**) containing 95% of the specific radioactivity of retrorsine and the acid portion with 3% of the activity. Further degradation of retronecine (**3**) with chromic acid yielded β-alanine (Scheme 3),[6] corresponding to carbons (5 + 6 + 7), which had 92% of the specific activity of retronecine. Similar hydrolysis of rosmarinine afforded rosmarinecine (**6**) with 97% of the original specific activity and the acid portion containing 4% of the activity. The β-alanine obtained by degradation of rosmarinecine contained 95% of the specific activity of rosmarinecine. Thus, each precursor is incorporated into the necines without significant breakdown occurring.

The incorporation of trachelanthamidine into retronecine is consistent with results obtained by pulse labelling young *Heliotropium spathulatum* plants with $^{14}CO_2$.[7] These plants produce trachelanthamidine (**11**), supinidine (the corresponding 1,2-unsaturated base), and retronecine (**2**). After short exposure to $^{14}CO_2$, the highest specific activity was observed in trachelanthamidine (**11**), and the lowest in retronecine (**2**). Continuation of the experiment led to increased activity in retronecine and less in trachelanthamidine.

Trachelanthamidine may be converted into retronecine by two hydroxylation processes followed by a dehydration, whereas formation of rosmarinecine from isoretronecanol requires two hydroxylations. It does not appear that epimerisation of the two bases (**10**) and (**11**) takes place to any appreciable extent during the biosynthesis of the two necines. The pathways apparently diverge prior to the formation of the alcohols (**10**) and (**11**) either during the cyclisation of the dialdehyde (Scheme 1) or at the aldehyde (**9**) stage. It is likely that the 8α-enantiomers of the bases [as drawn in (**10**) and (**11**)] are the actual biosynthetic intermediates. This could be tested after synthesis of labelled samples of these bases in optically active form.[8]

We thank the S.E.R.C. for a research assistantship (to E. K. K.).

Received, 17th October 1985; Com. 1478

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