A Biogenetically Patterned Synthesis of the Pyrrolizidine Alkaloid Trachelanthamidine

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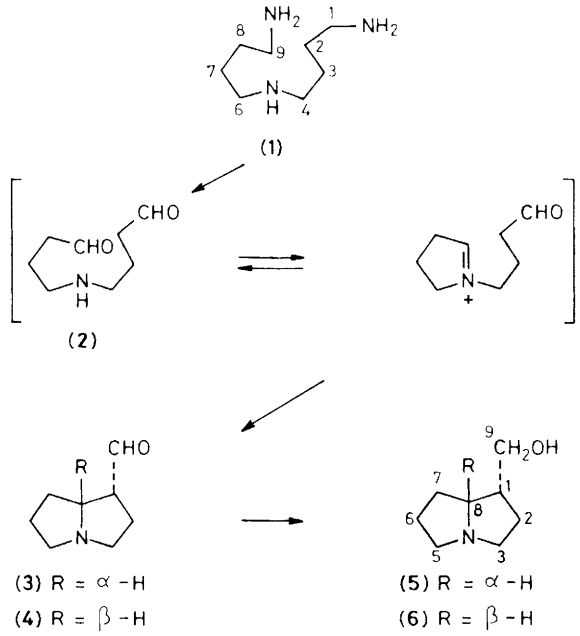
A synthesis of (±)-trachelanthamidine (**5**) is described using enzymes and physiological conditions; this synthesis is patterned on the likely biosynthetic pathway to pyrrolizidine alkaloids from homospermidine (**1**).

Experiments with isotopically labelled compounds have shown that the pyrrolizidine base, retronecine (**7**) is derived biosynthetically from two molecules of ornithine or arginine *via* putrescine and a symmetrical C_4–N–C_4 intermediate.[1] Evidence for homospermidine (**1**) as this intermediate in retronecine (**7**) biosynthesis in *Senecio isatideus* plants was obtained recently by ^{14}C-labelling experiments, and by demonstrating that homospermidine is present in these plants.[2] The initial steps in the conversion of homospermidine into pyrrolizidine alkaloids plausibly involve oxidation of the primary amino-groups by a diamine oxidase enzyme to generate the dialdehyde (**2**). This dialdehyde has been suggested[3] as an intermediate in the biosynthetic pathway, since Mannich-type condensation of (**2**) generates 1-formylpyrrolizidine, from which 1-hydroxymethylpyrrolizidine can be obtained by reduction (Scheme 1).[4] The feasibility of this proposed biosynthetic pathway from homospermidine has been demonstrated by a synthesis of (±)-trachelanthamidine (**5**) under physiological conditions using diamine oxidase to carry out the initial oxidation steps, and a coupled dehydrogenase enzyme system to complete the synthesis.

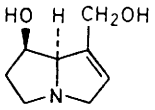
Homospermidine (**1**) was prepared in 60% yield by the reaction of benzylamine with 4-chlorobutanenitrile (2 equiv.), followed by catalytic hydrogenation.[5] The triamine (**1**) was incubated at 27 °C with pea seedling diamine oxidase[6] and catalase in phosphate buffer at pH 7.[7] After six days, the basified mixture was extracted with chloroform to yield a colourless oil. The presumed intermediates [(**3**) and (**4**)] were reduced immediately with sodium borohydride in methanol at 0 °C for 2 h. Examination of the products by thin layer chromatography and ^{1}H n.m.r. spectroscopy indicated that some starting material (**1**) was present. This was removed by formation of the derivative of (**1**) with isothiocyanatobenzene in ethanol.[2,8] G.l.c. analysis[9] of the remaining material, and comparison with authentic samples[10] indicated that the mixture was predominantly trachelanthamidine (**5**), [formed from



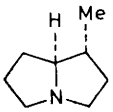
Scheme 1

the thermally more stable *exo*-aldehyde (**3**)] together with *ca.* 5% isoretronecanol (**6**). (±)-Trachelanthamidine (**5**) (40% yield) was characterised as its picrate, m.p. 169—170 °C (lit.[9] m.p. 170—172 °C), and as (±)-benzoyloxymethylpyrrolizidine hydrochloride, m.p. 185—186 °C (lit.[4] m.p. 185—185.5 °C).

The intact transformation of homospermidine (**1**) into (±)-trachelanthamidine (**5**) was demonstrated as follows. [1,9-$^{14}C_2$]-Homospermidine (specific activity 14.8 μCi mmol^{-1})[2] was



(7)



(8)

treated as described above to yield (±)-1-benzoyloxymethylpyrrolizidine hydrochloride (14.3 μCi mmol^{-1}). This material was converted into (±)-psuedoheliotridane (**8**) (14.4 μCi mmol^{-1}) by treatment with thionyl chloride followed by lithium aluminium hydride reduction.[4] Kuhn–Roth oxidation of (**8**) gave acetic acid (7.4 μCi mmol^{-1}) as its barium salt. Schmidt degradation of the barium acetate yielded inactive barium carbonate and methylamine (7.3 μCi mmol^{-1}), isolated as 5-methylamino-2,4-dinitrotoluene.[11] Thus, 51% of the original activity from [1,9-$^{14}C_2$]homospermidine is located at C-9 of trachelanthamidine (**5**), indicating that (**1**) is not broken down (*e.g.* to putrescine) before formation of trachelanthamidine.

Finally, a 'one-pot' conversion of homospermidine (**1**) into trachelanthamidine (**5**) was achieved. [1,9-$^{14}C_2$]-Homospermidine was incubated with diamine oxidase and catalase at 27 °C in phosphate buffer at pH 7.5. After six days, liver alcohol dehydrogenase, NADH, and ethanol (as hydride donor)[12] were added and the incubation was continued at 37 °C for 24 h. Unlabelled (±)-trachelanthamidine was added and the mixture of bases was isolated. (±)-1-Benzoyloxymethylpyrrolizidine hydrochloride was crystallised to constant specific activity which indicated a 21.8% conversion of homospermidine into (±)-trachelanthamidine.

Thus homospermidine can be converted into (±)-trachelanthamidine by the sequence of enzymic oxidations with diamine oxidase, non-enzymic cyclisation under physiological conditions, and reduction by a coupled dehydrogenase system. The facility of these transformations supports the suggestion that such reactions are involved in the biosynthesis of pyrrolizidine alkaloids.

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