Pyrrolizidine Alkaloids: Evidence for *N*-(4-Aminobutyl)-1,4-diaminobutane (Homospermidine) as an Intermediate in Retronecine Biosynthesis

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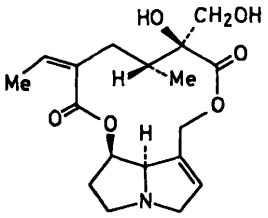
Summary [1-*amino*-^{15}N; 1-^{13}C]Putrescine (**3**) is incorporated into retronecine (**2**) in *Senecio isatideus* plants with a labelling pattern consistent with the formation of a symmetrical C_4–N–C_4 intermediate; the intermediate is shown to be homospermidine (**4**) by ^{14}C-labelling experiments.

PUTRESCINE [as (**3**)] is the most efficient precursor so far found for retronecine (**2**),[1] the base portion of many pyrrolizidine alkaloids. Retronecine is formed by alkaline hydrolysis of retrorsine (**1**), the major alkaloid present in *Senecio isatideus* plants. ^{13}C-Labelled putrescines have recently been used to establish the complete labelling pattern in retronecine (**2**).[2] The results indicate that two putrescine molecules combine to form retronecine with

nearly equal labelling in both halves of the molecule. This suggests, but does not prove, that a later C_4–N–C_4 symmetrical intermediate is involved in retronecine biosynthesis.[3] We believed that the use of [^{13}C–^{15}N]-labelled putrescine (**3**) would provide this proof. Biosynthesis of



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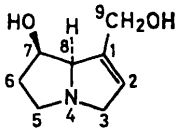
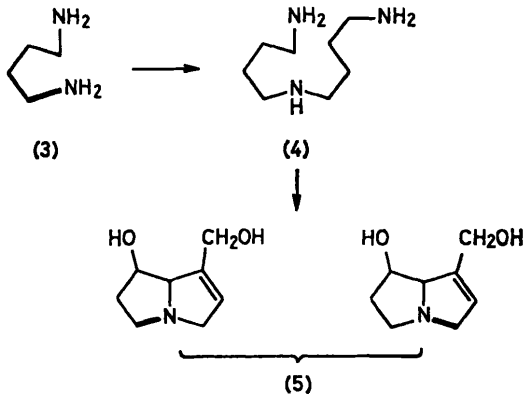


(2)

retronecine (**5**) from (**3**) *via* a symmetrical intermediate [such as (**4**)] should produce two ^{13}C–^{15}N couplings in the {^{1}H}-^{13}C n.m.r. spectrum of retronecine (Scheme).

The *N*-benzyloxycarbonyl derivative of 1-amino-3-bromopropane was treated with K^{13}C^{15}N (B.O.C. Prochem Ltd., containing 90·6% ^{13}C and 99·4% ^{15}N) to give the corresponding nitrile [{^{1}H}-^{13}C n.m.r. spectrum ($CDCl_3$)



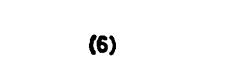
(3) (4) (5)

SCHEME

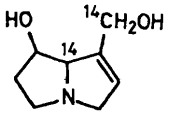
δ 119·0 p.p.m. (d, J 17 Hz)], which was catalytically hydrogenated to give [1-*amino*-^{15}N; 1-^{13}C]putrescine (**3**) isolated and recrystallised as its dihydrochloride (28% overall yield) [{^{1}H}-^{13}C n.m.r. spectrum (D_2O) δ 39·6 p.p.m. (d, J 5·1 Hz)].

Introduction of the [^{13}C–^{15}N]-labelled precursor (**3**), together with [1,4-^{14}C]putrescine dihydrochloride (5 μCi) into two three-month old *Senecio isatideus* plants was carried out as described previously.[1] Retrorsine (**1**) was extracted and recrystallised to constant specific activity (2·2% specific incorporation). Alkaline hydrolysis of retrorsine gave retronecine (**2**) isolated and recrystallised as its hydrochloride with the same specific activity.

Comparison of the 25 and 90 MHz {^{1}H}-^{13}C n.m.r. spectra of labelled retronecine hydrochloride[2] taken in D_2O, with unlabelled material run under the same conditions, showed enrichment factors of 0·4% for the signals at δ 55·4 (C-5) and 80·6 p.p.m. (C-8), and 0·5% for those at δ 62·6 (C-3) and 59·2 p.p.m. (C-9). This corresponds to a total enrichment factor of 1·8%, and a specific ^{13}C incorporation of *ca.* 2%. In addition, the resolution-enhanced spectra showed the presence of doublets at δ 55·4 (J 4·5 Hz) and 62·6 p.p.m. (J 5 Hz) with enrichment factors of 0·2—0·25%. The presence of ^{13}C–^{15}N species in retronecine hydrochloride was confirmed by observation of the 36·5 MHz {^{1}H}-^{15}N n.m.r. spectrum taken in D_2O, which showed ^{13}C–^{15}N satellites (J *ca.* 5 Hz) in addition to the natural-abundance signal at δ 311·2 p.p.m. upfield from external nitromethane. The fact that C-3 and C-5 of retronecine are both enriched approximately equally with ^{13}C–^{15}N species [as in (**5**)] provides strong evidence for the involvement of a symmetrical, C_4–N–C_4, intermediate in retronecine biosynthesis.



(6)



(7)

A reasonable possibility for this symmetrical intermediate is *N*-(4-aminobutyl)-1,4-diaminobutane (homospermidine) (**4**), a known plant constituent.[4] Accordingly ^{14}C-labelled homospermidine (**6**) was synthesized as follows. The *N*-benzyloxycarbonyl derivative of 4-aminobutanoic acid was condensed with 3-bromopropylamine. Treatment of the protected bromoamide with K^{14}CN, followed by hydrogenation and reduction with borane in tetrahydrofuran, yielded ^{14}C-labelled homospermidine (**6**), isolated and recrystallised as its trihydrochloride (20% yield from the bromoamide). The feeding experiment was carried out as usual.[1] Retrorsine (**1**) was isolated, diluted with inactive alkaloid, and recrystallised to constant specific activity (0·5% total incorporation, 0·3% specific incorporation). Retronecine (**7**), derived from retrorsine by base hydrolysis, had the same specific activity. Treatment of retronecine with OsO_4–HIO_4 gave formaldehyde (from C-9),[5] isolated as its dimedone derivative, containing 44 $\pm$ 4% of the retronecine activity. Modified Kuhn–Roth oxidation of retronecine gave β-alanine [from C-(5 + 6 + 7)], isolated as its *N*-2,4-dinitrophenyl derivative, with 2 $\pm$ 2% of the retronecine activity. These results are consistent with the intact incorporation of homospermidine (**6**) into retronecine (**7**). Moreover, homospermidine was isolated in a radioactive form after feeding DL-[5-^{14}C]ornithine to an *S. isatideus* plant. After 24 h, the plant was macerated in 0·4 M aqueous trichloroacetic acid containing inactive homospermidine trihydrochloride (35 mg). The *N'*-substituted *N*-phenylthiourea derivative of homospermidine was prepared and purified as described for other polyamines.[6] Recrystallisation to constant specific activity gave a radioactive derivative containing 0·5% of the original activity fed, thus indicating that homospermidine is a normal intermediate in retronecine biosynthesis.

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