Pyrrolizidine Alkaloid Biosynthesis; Incorporation of ^{13}C-Labelled Putrescines into Retronecine

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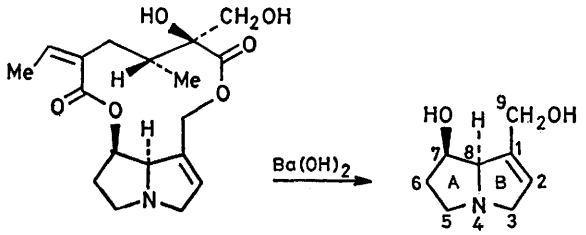
Summary [1,4-$^{13}C_2$]- and [2,3-$^{13}C_2$]-Putrescine are incorporated into retronecine (**2**) in *Senecio isatideus* plants, giving labelling patterns consistent with the formation of a C_4–N–C_4 intermediate in retronecine biosynthesis.

RETRONECINE is the most common base-portion of the pyrrolizidine alkaloids. Ornithine,[1–4] arginine,[5] putrescine,[1,3] spermidine, and spermine[1] have all been demonstrated to be specific precursors of retronecine (**2**). Degrada-

tions of retronecine derived biosynthetically from these precursors specifically labelled with ^{14}C (*i.e.* [2-^{14}C]- and [5-^{14}C]-ornithine and [1,4-^{14}C]putrescine, *etc.*), have shown that, in each case, *ca.* 25% of the total radioactivity is located at C-9 of retronecine (**2**), suggesting the intermediacy of a symmetrical C_4 fragment, such as putrescine (**3**), during the formation of ring B of retronecine. Further degradation of retronecine has proved difficult and conflicting results have been obtained for the distribution of radioactivity in ring A of retronecine.[1,4] Retrorsine (**1**) is the major pyrrolizidine alkaloid in *Senecio isatideus* plants and yields retronecine (**2**) on alkaline hydrolysis.[6] High total incorporations of ^{14}C-labelled precursors into retrorsine (up to 5·2%) have recently been obtained,[1] suggesting that the use of suitable ^{13}C-labelled precursors would resolve this conflict. We report the use of ^{13}C-labelled putrescines to establish, for the first time, the complete labelling pattern in retronecine.



(1) (2)

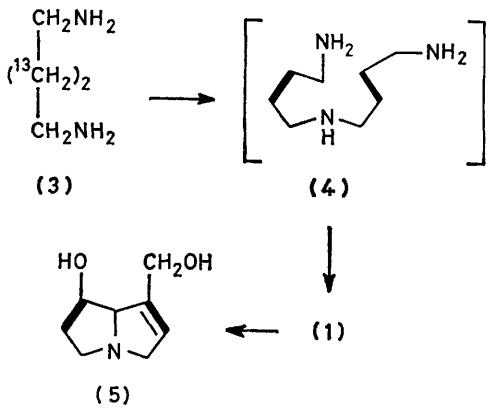
1,2-Dibromoethane was heated in aqueous ethanol under reflux for 5 h with K^{13}CN (B.O.C. Prochem Ltd., containing 93% ^{13}C) to yield [1,4-$^{13}C_2$]succinonitrile (containing 87% $^{13}C_2$, 12·5% $^{13}C^{12}C$, and 0·5% $^{12}C_2$), which was reduced with borane in tetrahydrofuran to yield [1,4-$^{13}C_2$]putrescine, isolated and recrystallised as its dihydrochloride (30% overall yield) [{^1H} ^{13}C n.m.r. (D_2O) δ 39·8 p.p.m. (s)].

In a similar fashion, [1,2-$^{13}C_2$]-1,2-dibromoethane (B.O.C. Prochem Ltd., containing 81% $^{13}C_2$, 18% $^{13}C^{12}C$, and 1% $^{12}C_2$) was converted into [2,3-$^{13}C_2$]putrescine dihydrochloride [{^1H} ^{13}C n.m.r. (D_2O) δ 24·7 p.p.m. (s)].

Pulsed feeding of the precursors was achieved by the direct absorption, on alternate days during one week, of sterile, aqueous solutions into the xylems of *S. isatideus* plants through stem punctures. Each ^{13}C-labelled precursor was 'spiked' by the addition of [1,4-^{14}C]putrescine dihydrochloride. After a further week, the plants were harvested and retrorsine (**1**) isolated and recrystallised to constant specific activity. Specific incorporations of 0·6—3·0% ^{14}C were obtained for retrorsine and in every experiment >95% of the specific radioactivity was retained in the retronecine hydrochloride, obtained by the alkaline hydrolysis of retrorsine. In a series of four experiments with [1,4-$^{13}C_2$]putrescine hydrochloride, enrichment factors† of 0·2—1·0% ^{13}C were found in retronecine (**2**) for C-5, C-9, C-3, and C-8, obtained from the corresponding‡ {^1H} ^{13}C n.m.r. (D_2O) signals at δ 54·9, 58·5, 62·2, and 79·6 p.p.m., respectively. In each separate experiment, the enhancements of these four signals were nearly equal.

Where ^{13}C enrichments are low (owing to dilution with appreciable quantities of endogenous retrorsine), it is desirable to use ^{13}C–^{13}C doubly labelled precursors to establish the labelling patterns. Accordingly, [2,3-$^{13}C_2$]putrescine dihydrochloride was incorporated into retrorsine (0·5% ^{14}C specific incorporation). The {^1H} ^{13}C n.m.r. spectrum of the derived retronecine (**5**) hydrochloride showed a pair of doublets at δ 137·4 and 122·1 p.p.m. (*J* 71 Hz), corresponding to C-1 and C-2 of retronecine (enrichment factor 0·16% ^{13}C), and a pair of doublets at 35·9 and 70·1 p.p.m. (*J* 34 Hz) corresponding to C-6 and C-7 (enrichment factor 0·18% ^{13}C).

The nearly equal enrichment factors observed for all four ^{13}C-labelled sites in each ^{13}C-labelled sample of retronecine suggest that two molecules of putrescine combine together to form a symmetrical intermediate, such as (**4**), which is then converted into retronecine (**2**) (see the Scheme).



(3) (4) (1) (5)

SCHEME

^{13}C-Labelled putrescines are thus readily available for further biosynthetic studies. More insight into retronecine biosynthesis is likely from use of other ^{13}C-labelled precursors.

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† The enrichment factor for a specific site is the excess of ^{13}C-label above natural abundance and is calculated from [intensity of labelled site—natural abundance intensity/(natural abundance intensity)] × 1·1%.

‡ ^{13}C N.m.r. data (δ p.p.m.) for retronecine hydrochloride (D_2O), assigned using the off-resonance and single-frequency decoupled spectra: 137·4 (C-1), 122·1 (C-2), 79·6 (C-8), 70·1 (C-7), 62·2 (C-3), 58·5 (C-9), 54·9 (C-5), and 35·9 (C-6) [*cf.* E. J. Barreiro, A. De Lima Pereira, L. Nelson, L. F. Gomes, and A. J. R. Da Silva, *J. Chem. Res.*, 1980 (*S*) 330].

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