Quinolizidine Alkaloid Biosynthesis: Incorporation of [1-*amino*-^{15}N,1-^{13}C]Cadaverine into Sparteine

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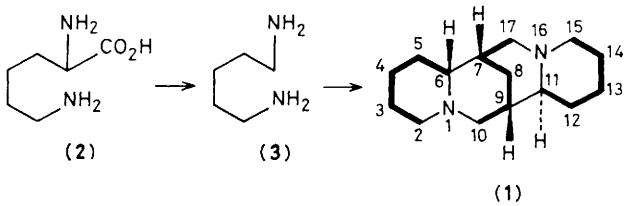
Three [1-*amino*-^{15}N,1-^{13}C]cadaverine (**4**) units are incorporated to about the same extent into sparteine (**5**); the presence of only two ^{13}C–^{15}N doublets in the ^{13}C{^{1}H} n.m.r. spectrum of sparteine indicates that two of these units are transformed into the outer rings of sparteine in a specific fashion.

Sparteine (**1**) is one of the most common C_{15} tetracyclic quinolizidine alkaloids;[1] it is present in a number of species of the plant family Leguminosae. The biosynthesis of sparteine is known to proceed from lysine (**2**) *via* cadaverine (**3**). Thus, [1,5-$^{14}C_2$]cadaverine was shown to be incorporated into sparteine with about 1/6th of the radioactivity at C-2, C-15, and C-17.[2] The remainder of the radioactivity was assumed to be at C-6, C-10, and C-11, leading to the proposed mode of incorporation of the three cadaverine units shown (Scheme 1). We report the use of a ^{13}C–^{15}N doubly labelled precursor to establish, for the first time, a complete labelling pattern in sparteine, and to demonstrate that two C–N bonds from two of the cadaverine units remain intact in sparteine.

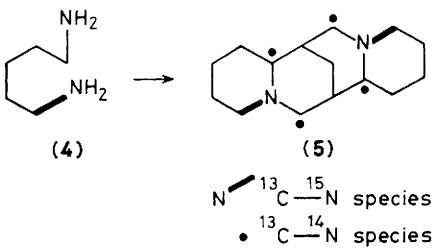
Treatment of the *N*-phthaloyl derivative of 1-amino-4-bromobutane with K^{13}C^{15}N (B.O.C. Prochem Ltd., London, containing 90.6% ^{13}C and 99.4% ^{15}N) gave the corresponding nitrile [^{13}C{^{1}H} n.m.r. spectrum ($CDCl_3$) δ 119.2 p.p.m. (d, *J* 7.1 Hz)]. Catalytic hydrogenation of this nitrile followed by acid hydrolysis yielded [1-*amino*-^{15}N,1-^{13}C]cadaverine (**4**), isolated and recrystallised as its dihydrochloride (34% overall yield) [^{13}C{^{1}H} n.m.r. spectrum (D_2O) δ 39.9 p.p.m. (d, *J* 4.9 Hz)]. Pulsed feeding of this ^{13}C–^{15}N doubly labelled precursor (**4**) (60 mg) together with [1,5-$^{14}C_2$]cadaverine dihydrochloride (5 μCi, New England Nuclear, Boston, Mass.) was carried out on eight *Lupinus luteus* plants by the method described previously.[3] Sparteine[4] was extracted and purified by column chromatography on basic alumina, with a specific ^{14}C incorporation of 3.8% per C_5 unit of cadaverine.†

Comparison of the normalised signal integrals in the 50 MHz ^{13}C{^{1}H} n.m.r. spectrum[5] of the labelled sparteine taken in benzene with those of unlabelled material run under the same conditions showed enrichment factors‡ of 1.7, 1.9, 1.7, 1.6, 1.9, and 1.7% ^{13}C for the signals at δ 66.5 (C-6), 64.4 (C-11), 62.2 (C-10), 56.5 (C-2), 55.7 (C-15), and 53.9 p.p.m. (C-17), respectively. The average enrichment factor per C_5 unit of sparteine is 3.5%, corresponding to a specific ^{13}C incorporation of 3.9%, which compares well with the observed specific ^{14}C incorporation. The approximately equal levels of enhancement of ^{13}C at each of the six labelled positions in sparteine (**5**) confirms that it is formed from three units of cadaverine. Furthermore, the resolution enhanced ^{13}C{^{1}H} n.m.r. spectrum of sparteine (Figure 1) showed the presence of doublets at δ 56.5 (*J* 3.7) and 55.7 p.p.m. (*J* 3.4 Hz) due to ^{13}C–^{15}N species, flanking signals at natural abundance intensity. The lack of enhancement of these central signals shows that there is no detectable breakdown of the ^{13}C–^{15}N bonds in the cadaverine molecules producing the C-2, N-1 and C-15, N-16 bonds in sparteine (**5**). These results demonstrate that two of the molecules of cadaverine are incorporated in a specific manner into the two outer rings of sparteine.§

It has been shown that crude enzyme preparations from cell suspension cultures of *Lupinus polyphyllus* are able to catalyse the conversion of cadaverine into 17-oxosparteine, in the presence of pyruvic acid.[6] This suggests that transamination reactions are occurring ($CH_2NH_2 \rightarrow CHO$), with pyruvic acid acting as a receptor for the amino groups in cadaverine. The pattern of incorporation of the labelled cadaverine (**4**) observed in this work indicates that the two outer rings of sparteine are derived from two 5-aminopentanal equivalents, while the central portion is produced from a glutaric dialdehyde equivalent. However, no intermediates were detected during the enzymic conversion, and a series of enzyme-linked intermediates on an enzyme complex were postulated.[6] The specific pattern of incorporation of the labelled cadaverine (**4**) observed in this work is consistent with the proposed series of enzyme-linked intermediates.[6] Further insight into quinolizidine alkaloid biosynthesis is likely from the use of other ^{13}C-labelled precursors.



Scheme 1



† Specific ^{14}C incorporation of cadaverine per C_5 unit into sparteine (**5**) is calculated as [(molar activity of sparteine × 1/3)/(molar activity of cadaverine)] × 100%.

‡ The enrichment factor for a specific site in sparteine is the excess of ^{13}C label above natural abundance and is calculated from [(integral of labelled site − natural abundance integral)/(natural abundance integral)] × 1.1%.

§ Because of the high level of ^{13}C incorporation obtained in this experiment, further couplings are visible in the ^{13}C{^{1}H} n.m.r. spectrum of sparteine (**5**). The signals for C-6 and C-11 each show coupling to both C-10 and C-17. In addition the signals for C-10 and C-17 display further coupling to ^{15}N and C-2 and C-15 respectively.

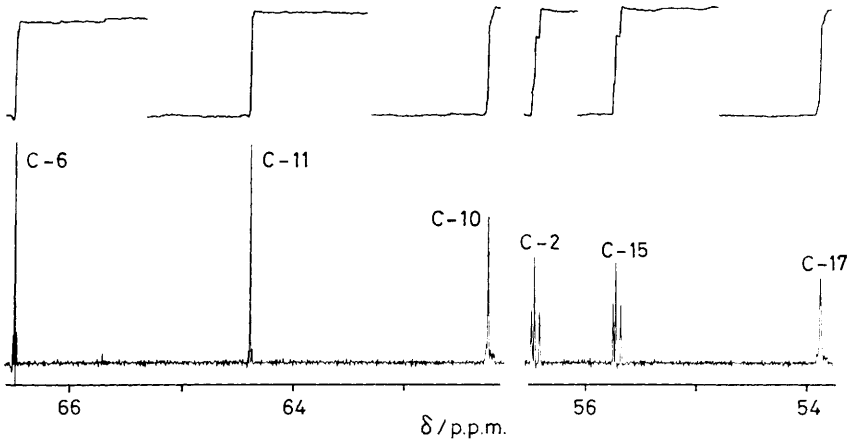


Figure 1. 50.32 MHz $^{13}C\{^{1}H\}$ N.m.r. spectrum of sparteine (**5**) (15 mg) in benzene derived from [1-*amino*-^{15}N,1-^{13}C]cadaverine (**4**).

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