Biosynthesis of the Lycopodium Alkaloids: the Origin of Cernuine

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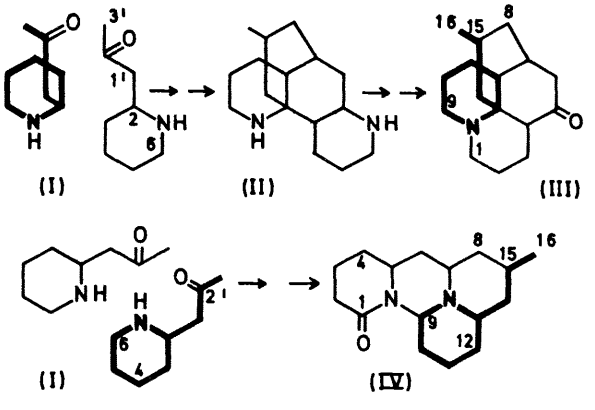
Summary Even though lysine and pelletierine (I) serve as precursors of the Lycopodium alkaloid, cernuine (IV), the alkaloid is not a modified dimer of pelletierine, as might have been anticipated on the basis of structural relations, since only one pelletierine unit is incorporated.

WE have shown that lysine[1] and acetate[2] serve as specific precursors of lycopodine (III), the major alkaloid of many Lycopodium species,[3] and have advanced the idea[1] that lycopodine and related Lycopodium alkaloids[3] are modified dimers of pelletierine[4] (I) whose nucleus is derived from lysine by way of a symmetrical intermediate[5] and whose side-chain originates from acetate.[5b] The structural correspondence of pelletierine (I) to the lycodine skeleton (II) and hence to lycopodine (III) is shown.

Lycopodium cernuum, a subtropical Lycopodium species, is exceptional in that it does not contain lycopodine. The major alkaloids found in this species are cernuine (IV) and lycocernuine (12-hydroxycernuine),[6] whose ring skeleton, even though entirely different from that of lycopodine, can also be thought to arise from two pelletierine units. We have tested the mode of incorporation of pelletierine into cernuine, in *Lycopodium cernuum* growing in Jamaica.

[6-^{14}C]Pelletierine, [4-^{3}H]pelletierine, and [2′-^{14}C]pelletierine (I) were prepared[7] from [6-^{14}C]-DL-lysine (Commissariat à l'Energie Atomique, France), [4-^{3}H]-DL-lysine (Commissariat à l'Energie Atomique, France), and ethyl [3-^{14}C]acetoacetate (New England Nuclear), respectively. An intermolecularly triply-labelled sample, [4-^{3}H,6,2′-^{14}C$_2$]-pelletierine (^{3}H : ^{14}C 12·4 ± 0·1; 6-^{14}C 59 ± 1%, 2′-^{14}C 41 ± 1% of the specific activity of intact pelletierine), obtained by uniting these samples, was administered to plants of *Lycopodium cernuum* L.† The plants were kept in contact with the tracer for 2 days. Cernuine (45 mg) and lycocernuine (89 mg), isolated[6] from the dried plant material (164 g), were purified to constant radioactivity by column chromatography on alumina, followed by crystallization. Whereas lycocernuine was inactive, cernuine contained radioactivity. The latter alkaloid, after sublimation at 110° and 5 × 10^{-3} mm, showed a ^{3}H : ^{14}C ratio (^{3}H : ^{14}C 12·2 ± 0·1) identical with that of the precursor. This result clearly indicates that, consistent with the hypothesis, pelletierine is incorporated intact into the alkaloid. To establish the distribution of ^{14}C the alkaloid was diluted with inactive carrier and the resulting sample [specific activity (1·99 ± 0·03) × 10^{4} counts min^{-1} mmole^{-1}] was subjected to Kuhn–Roth oxidation. The acetic acid, so obtained from C-15,-16 of cernuine and purified as the α-naphthylamide [specific activity (0·81 ± 0·01) × 10^{4} counts min^{-1} mmole^{-1}], contained 41 ± 1% of the activity of the intact cernuine, a result incompatible with the dimerization hypothesis.

The two results indicate clearly that only one intact



† A voucher specimen of the species used in these experiments is deposited in the herbarium of the University of the West Indies, Mona, Jamaica. We are greatly indebted to Dr. C. D. Adams for authenticating the sample.

pelletierine unit entered cernuine, supplying the C_8-unit, C-9 to -16. Entry of two intact pelletierine moieties into the alkaloid would have led to a Kuhn–Roth acetate containing only 20% of the activity of the intact alkaloid. Incorporation of one, rather than two pelletierine units into cernuine in *Lycopodium cernuum* parallels the finding[8] that only one pelletierine unit enters lycopodine (III) in *Lycopodium tristachyum*, supplying the corresponding C_8-unit, C-9 to -16. Since only one of the two C_8 units in the skeletons of cernuine and lycopodine is generated from pelletierine, the alkaloids do not arise by dimerization of the precursor.

Incorporation of lysine into cernuine was also investigated. Radioactive cernuine (12·5 mg), obtained from *L. cernuum* plants (77 g dry weight) to which [2-^{14}C]-DL-lysine (New England Nuclear) had been administered, was diluted with inactive carrier. Lithium aluminium hydride reduction[6]‡ of the resulting cernuine [specific activity $(2{\cdot}02 \pm 0{\cdot}06) \times 10^4$ counts min^{-1} mmole^{-1}], followed by chromic acid oxidation, gave β-alanine‡ [specific activity $(0{\cdot}48 \pm 0{\cdot}01) \times 10^4$ counts min^{-1} mmole^{-1}] ($24 \pm 1\%$ of the specific activity of intact cernuine) and γ-aminobutyric acid‡ [specific activity $(0{\cdot}51 \pm 0{\cdot}03) \times 10^4$ counts min^{-1} mmole^{-1}] ($25 \pm 2\%$ of the specific activity of cernuine). These data suggest that two C_5N units derived from lysine enter cernuine, and that incorporation takes place by way of a symmetrical intermediate. It thus appears that the modes of incorporation into cernuine of pelletierine and of lysine are similar to their manner of entry into lycopodine.[1,8]

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‡ Lithium aluminium hydride reduction of cernuine (IV) leads to a mixture of products, formed by the conversion of the carbonyl function into a methylene group, and/or by the hydrogenolysis of an N–C-9 bond. Chromic acid oxidation of the mixture of reduction products then yields β-alanine from the fragments N, C-1 to -3 and N, C-9 to -11, and γ-aminobutyric acid from the fragments N, C-1 to -4 and N, C-9 to -12.

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[2] M. Castillo, R. N. Gupta, D. B. MacLean, and I. D. Spenser, unpublished results.
[3] D. B. MacLean, in "The Alkaloids," ed. R. H. Manske, Academic Press, New York, 1968, vol. 10, p. 305.
[4] 2-Piperidylpropanone (I), known for many years as isopelletierine, is now referred to as pelletierine, following the suggestion of R. E. Gilman and L. Marion, *Bull. Soc. chim. France*, 1961, 1993.
[5] This suggested symmetrical mode of incorporation of lysine into the putative intermediate, pelletierine (I), differs from that actually shown to occur in *Sedum sarmentosum*, where lysine enters *N*-methylpelletierine in a non-symmetrical manner: (a) R. N. Gupta and I. D. Spenser, *Chem. Comm.*, 1968, 85; (b) R. N. Gupta and I. D. Spenser, *Phytochemistry*, 1969, **9**, 1937.
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