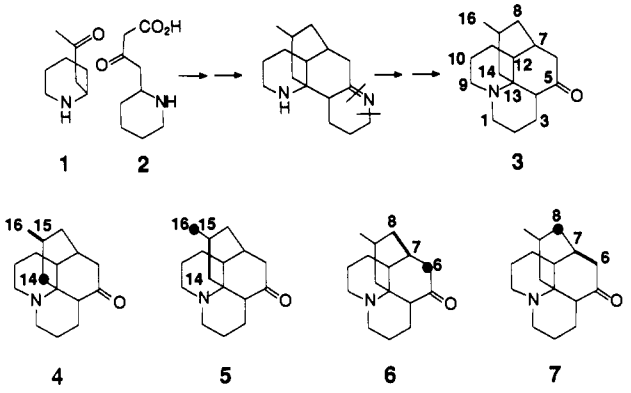
Biosynthesis of Lycopodine: Incorporation of Acetate via an Intermediate with C_{2v} Symmetry

Thomas Hemscheidt† and Ian D. Spenser*

Department of Chemistry, McMaster University
Hamilton, Ontario, Canada L8S 4M1
Department of Chemistry, University of Hawaii
Honolulu, Hawaii 96822

Received December 23, 1992

In continuation of our studies of biochemical diversity in the formation of acetate-derived C_3 units of alkaloids such as *N*-methylpelletierine[1] and tropine,[2] we have investigated the incorporation of [1,2-$^{13}C_2$]acetate into the two acetate-derived C_3 units of lycopodine (**3**). Lycopodine,[3] like pelletierine[4] (**1**), is derived from lysine and acetate, and the lycopodine skeleton can be formally constructed from two pelletierine units. Pelletierine does indeed enter lycopodine, but only one pelletierine unit is incorporated[5,6] into C-9–C-16. The other "half" of lycopodine, C-1–C-8, must be derived from a closely related molecule, possibly 4-(2-piperidyl)acetoacetate[6,7] (**2**).



1 **2** **3**

4 **5** **6** **7**

Incorporation of [$^{13}C_2$]acetate was anticipated to lead to one or the other of two labeling patterns: either pelletierine-like[1] regiochemistry (**4**) in C-14–C-16 of the pelletierine-derived segment of lycopodine (C-9–C-16) and cocaine-like[8] regiochemistry (**7**) in C-6–C-8 of the other segment (C-1–C-8), or cocaine-like regiochemistry in both acetate-derived segments, C-14–C-16 (**5**) and C-6–C-8 (**7**).

Contrary to expectation, administration of sodium [1,2-$^{13}C_2$]-acetate (99% $^{13}C_2$, 335 mg, MSD Isotopes, in admixture with 665 mg of unenriched acetate) in three repeat experiments, carried out during three growing seasons of *Lycopodium tristachyum* Pursh, led to an entirely different outcome. The 125-MHz ^{13}C NMR spectrum (Figure 1) of each of the three lycopodine samples, isolated[3] from these experiments (specific incorporation 0.4–0.7% above natural abundance), showed a doublet of satellites in each of the signals due to the two carbon atoms C-15 and C-16 (δ 25.4

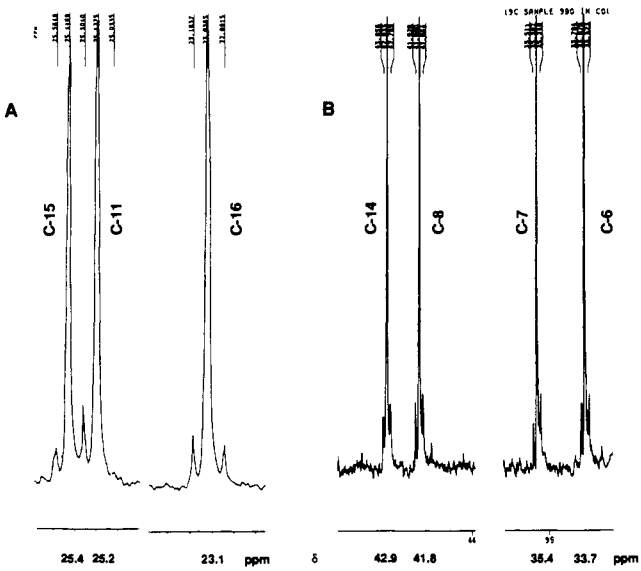


Figure 1. Proton noise decoupled ^{13}C NMR spectra (125 MHz) (**A**) of a sample of lycopodine (12 mg in 0.6 mL of $CDCl_3$) obtained from *L. tristachyum* to which sodium [1,2-$^{13}C_2$]acetate had been administered and (**B**) of dihydrolycopodine (10 mg in 0.6 mL of $CDCl_3$) derived from the labeled lycopodine by hydride reduction.

and 23.1, respectively,[9] $^1J_{15,16}$ = 35 Hz), in the pelletierine "half", C-9–C-16, of the molecule. After hydride reduction to dihydrolycopodine, a doublet of satellites was observed also in the signal due to C-14 (δ 42.9,[9] $^1J_{14,15}$ = 35 Hz) and in each of the signals due to C-6, C-7, and C-8 (δ 33.7, 35.4, and 41.8, respectively,[9] $^1J_{6,7}$ = $^1J_{7,8}$ = 35 Hz) in the other "half", C-1–C-8.[10] The specific incorporation, above natural abundance, was identical for each of the two C_2 regiochemistries in each of the two "halves" of the molecule in each of the three experiments.

Lycopodine, derived from [1,2-$^{13}C_2$]acetate, thus consists of an equimolar mixture of eight labeled species, four of which are bond-labeled, at C-15,-16, at C-14,-15, at C-7,-8, and at C-6,-7, respectively, and four are singly labeled, enriched at C-14, at C-16, at C-6, and at C-8, respectively. These eight species are schematically represented as **4**, **5**, **6**, and **7**.

One possible interpretation of the observed incorporation pattern calls for the formation of acetoacetate from acetate, followed by concomitant, simultaneous, and equimolar incorporation by two different routes (e.g., Routes A plus C or Routes A plus B, ref 1, Scheme II) of acetoacetate into the precursors of both "halves" of lycopodine.

To test this idea, [1,2,3,4-$^{13}C_4$]acetoacetate (49% $^{13}C_4$, prepared[1] by alkaline hydrolysis of ethyl [1,2,3,4-$^{13}C_4$]acetoacetate, 98% $^{13}C_4$, Isotec Inc., 500 mg, in admixture with unlabeled ethyl acetoacetate, 500 mg) was administered to *L. tristachyum*. The ^{13}C incorporation pattern in the lycopodine from this experiment (specific incorporation 0.3% above natural abundance) was identical with that from the acetate experiments. Thus, the spectrum indicates that acetoacetate had been cleaved to acetate prior to incorporation. Even though it is conceivable that this is the fortuitous consequence of the relative rates of two competing reactions, a retro-Claisen cleavage to acetate and a condensation with Δ^1-piperideine, we consider this result to be significant, since in another plant species incorporation of an intact C_3-unit from acetoacetate into *N*-methylpelletierine was indeed observed.[1]

Conclusive evidence that acetate was not incorporated by either of the routes for which precedent exists came from an experiment

* Address correspondence to this author at McMaster University.
† University of Hawaii.

(1) Hemscheidt, T.; Spenser, I. D. *J. Am. Chem. Soc.* **1990**, *112*, 6360.
(2) Hemscheidt, T.; Spenser, I. D. *J. Am. Chem. Soc.* **1992**, *114*, 5472.
(3) Castillo, M.; Gupta, R. N.; MacLean, D. B.; Spenser, I. D. *Can. J. Chem.* **1970**, *48*, 1893.
(4) Gupta, R. N.; Spenser, I. D. *Phytochemistry* **1969**, *8*, 1937.
(5) Castillo, M.; Gupta, R. N.; Ho, Y. K.; MacLean, D. B.; Spenser, I. D. *J. Am. Chem. Soc.* **1970**, *92*, 1074.
(6) Castillo, M.; Gupta, R. N.; Ho, Y. K.; MacLean, D. B.; Spenser, I. D. *Can. J. Chem.* **1970**, *48*, 2911.
(7) Braekman, J.-C.; Gupta, R. N.; MacLean, D. B.; Spenser, I. D. *Can. J. Chem.* **1972**, *50*, 2591.
(8) Leete, E.; Kim, S. H. *J. Am. Chem. Soc.* **1988**, *110*, 2976.

(9) Assignments of the signals in the ^{13}C NMR spectra of lycopodine and dihydrolycopodine are based on 2 D INADEQUATE spectra. For published assignments, cf. Nakashima, T. T.; Singer, P. P.; Browne, L. M.; Ayer, W. A. *Can. J. Chem.* **1975**, *53*, 1936.
(10) A doublet of doublets was not detectable in the signals of C-7 and C-15.

0002-7863/93/1515-3020$04.00/0

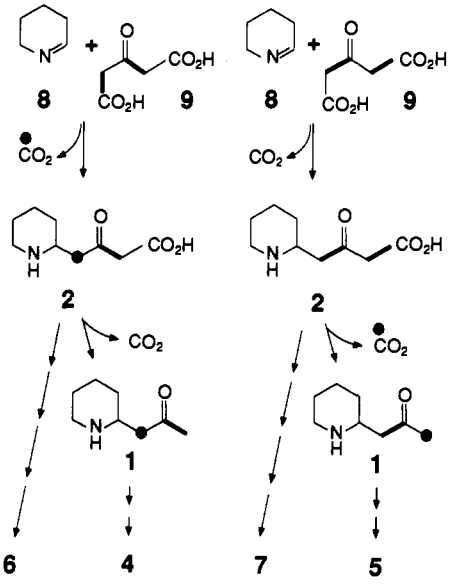
with [1,2-$^{13}C_2$,2,2,2-$^2H_3$]acetate (99% $^{13}C_2$, 99% ^2H, 500 mg, MSD Isotopes). Enrichment was clearly detectable in the ^{13}C-{^1H,^2H} NMR spectrum of the alkaloid from this experiment (specific incorporation 0.4% above natural abundance). Unexpectedly, however, the sets of satellites were centered *symmetrically* around the natural abundance signals of C-6, C-7, and C-8 as well as those of C-14, C-15, and C-16. No trace of any one-bond isotope-shifted component was detectable, either by 1D or by 2D NMR,[11,12] for any of the four carbon atoms, C-6, C-8, C-14, or C-16.

Furthermore, the ^2H-{^{13}C,^1H} spectrum in $CHCl_3$ (10 000 scans) displayed no deuterium signals apart from the natural abundance signal of the solvent.[13] If an acetoacetate-derived C_3 unit had been incorporated by the pelletierine route, the methyl carbon C-16, in particular, would have been expected to bear deuterium and show a ^{13}C NMR signal with isotope-shifted satellites, since this carbon atom lies in the pelletierine-derived "half" of the molecule and since, of the molecules bond-labeled in this half (**4**, **5**), 50% show pelletierine-like regiochemistry (**4**).

Any explanation of the observed incorporation pattern not only must provide a rationale for the 1:1 ratio of the two incorporation patterns, pelletierine-like (**4** and **6**) and cocaine-like (**5** and **7**), into each of the two acetate-derived C_3 units of lycopodine but must also account for the wash-out of deuterium from the methyl group of acetate on its route into the C-16 methyl group of the alkaloid.

The loss of deuterium suggests intermediacy, on the route to the pelletierine side chain, of a compound in which all acetate-derived protons are readily exchangeable due to enolization. The 1:1 pattern of distribution of the acetate C_2 unit suggests that this intermediate possesses C_{2v} symmetry. A compound that fulfils both of these conditions is 3-oxoglutaric acid[14] (acetonedicarboxylic acid) (**9**) (or its bis(coenzyme A) ester). The required distribution would result[15] if the compound were derived from acetate via acetyl CoA, by carboxylation to malonyl CoA, followed by Claisen-type condensation of two molecules of the latter, accompanied by decarboxylation, to the monoCoA ester of 3-oxoglutaric acid,[16] followed by thiol ester hydrolysis. An intact $^{13}C_2$ unit of acetate thereby enters one or the other of two distinct sites of the resulting dicarboxylic acid to yield an equimolar mixture of $HO_2C^{13}CH_2$-$^{13}COCH_2CO_2H$ plus HO_2CCH_2-$CO^{13}CH_2$-$^{13}CO_2H$ (represented as $HO_2C^{13}CH_2$-^{13}CO-$^{13}CH_2$-$^{13}CO_2H$ (**9**)). Mannich-type condensation of this diacid (**9**) with Δ^1-piperideine (**8**), accompanied by decarboxylation, yields 4-(2-piperidyl)acetoacetate (**2**) with two different bond-labeling patterns (Scheme I). In the absence of a significant isotope effect favoring ^{13}C–^{12}C over ^{13}C–^{13}C bond cleavage, this process, followed by a further decarboxylation, yields the observed 1:1 mixture of the two different ^{13}C–^{13}C regiochemistries within lycopodine.

Scheme I



Acknowledgment. We are grateful to Mr. D. Strickland, Head Biologist, and Mr. G. E. Martelle, Park Superintendent, Algonquin Park, Ontario, for their cooperation in this investigation and to Mr. W. Yoshida and Dr. W. Niemczura, Department of Chemistry, University of Hawaii, for determining the NMR spectra. This work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada.

(11) Trimble, L. A.; Reese, P. B.; Vederas, J. C. *J. Am. Chem. Soc.* **1985**, *107*, 2175.

(12) Reese, P. B.; Trimble, L. A.; Vederas, J. C. *Can. J. Chem.* **1986**, *64*, 1427.

(13) That retention of deuterium, at the level calculated from the observed incorporation of ^{13}C or at a level 1 order of magnitude lower, would have been easily detected was shown by spiking the solution with [$^2H_6$]acetone at the corresponding concentration and rerunning the spectrum under otherwise identical conditions.

(14) 3-Oxoglutaric acid has been identified as a metabolite in several bacterial strains (Donelly, M. I.; Chapman, P. J.; Dagley, S. *J. Bacteriol.* **1981**, *147*, 477–481) but has not hitherto been detected in higher plants. For another reported occurrence of the compound in an extract of natural origin, see: Kim, D. S.; Kim, Y. M.; Woo, S. G. *Han'guk Yongyang Siklyong Hakhoechi* **1990**, *19*, 305–310; Chem. Abstr. **1991**, *115*, 7210v.

(15) The required distribution of label from [1,2-$^{13}C_2$]acetate would not result if the 3-oxoglutaric acid were derived from citric acid by oxidative decarboxylation.

(16) The other possible intermediate with C_{2v} symmetry, the bisCoA ester of 3-oxoglutaric acid, may be formed from the monoCoA ester at this stage.