A Classical Paradigm of Alkaloid Biogenesis Revisited: Acetonedicarboxylic Acid as a Biosynthetic Precursor of Lycopodine

Thomas Hemscheidt[†] and Ian D. Spenser*[,‡]

Departments of Chemistry
University of Hawaii at Manoa
Honolulu, Hawaii 96822
McMaster University
Hamilton, Ontario, Canada L8S 4M1

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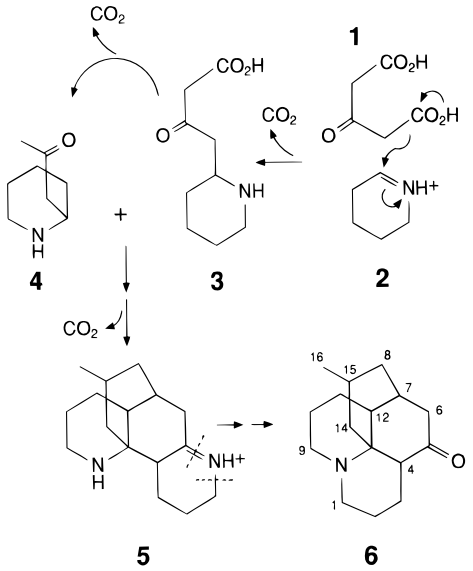
As part of our investigations[1,2] of the mechanism of assembly of acetate-derived C_3 units that form part of the skeleton of a large number of alkaloids, we recently reported[3] that the acetate-derived C_3 fragments, C-6,-7,-8 and C-14,-15,-16, of lycopodine (**6**) were derived in a manner that differs from either of the two modes of incorporation of [1,2-$^{13}C_2$]acetic acid for which precedent exists. These two previously established incorporation patterns are exemplified, on the one hand, by that found in the case of *N*-methylpelletierine,[1] which was interpreted as arising by Mannich-type condensation of C-2 of acetoacetate with an iminium ion, and, on the other hand, by that observed in the cases of cocaine[4] and the tropane alkaloids,[2,5] which was interpreted in terms of a stepwise introduction of two acetate units. Each of these pathways generates a distinct and specific labeling pattern within the acetate-derived multicarbon unit of the alkaloids when label from sodium [1,2-$^{13}C_2$]acetate is incorporated. The incorporation pattern resulting from the entry of label from sodium [1,2-$^{13}C_2$]acetate into lycopodine was more complex than either of these and, in fact, represents a superposition of the two simpler patterns on one another. This result, together with the finding that [1,2,3,4-$^{13}C_4$]acetoacetic acid did not supply an intact C_3 unit but was incorporated only after cleavage to [1,2-$^{13}C_2$]acetic acid, led us to suggest[3] that the acetate-derived C_3 fragments, C-6,-7,-8 and C-14,-15,-16, of lycopodine were introduced into the alkaloid *via* acetonedicarboxylic acid (**1**). We now provide direct experimental evidence in support of this proposal.



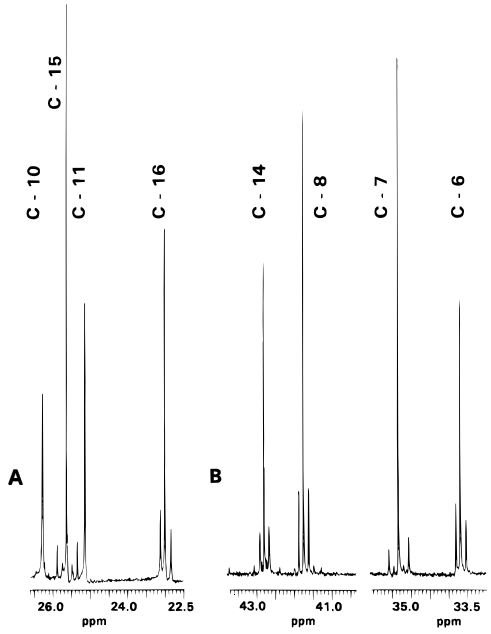


Figure 1. Portion of the proton noise decoupled ^{13}C NMR spectra (125 MHz) (A) of a sample of lycopodine (15 mg in 165 μL of [$^2H_5$]-pyridine) obtained from *L. tristachyum* to which sodium [1,2,3,4-$^{13}C_4$]-acetonedicarboxylate had been administered and (B) of a sample of dihydrolycopodine (12 mg in 150 μL of $CDCl_3$) derived from the labeled lycopodine by hydride reduction.

Sodium [1,2,3,4-$^{13}C_4$]acetonedicarboxylate (33% $^{13}C_4$), obtained by alkaline hydrolysis of a mixture of diethyl [1,2,3,4-$^{13}C_4$]acetonedicarboxylate[6−8] (165 mg, 99% $^{13}C_4$) and unlabeled diethyl acetonedicarboxylate (330 mg), was administered in five equal portions over 5 days to 40 shoots of *Lycopodium tristachyum* Pursh, growing in its natural habitat. After a further 3 days, the plants were harvested, and the alkaloid was extracted and purified by standard procedures.[9] A section of the 125 MHz ^{13}C NMR spectrum of the sample of lycopodine from this experiment, recorded in [$^2H_5$]pyridine,[10] is shown in Figure 1A. Satellites, indicating ^{13}C-enrichment, are evident in the signals of those carbon atoms that are known[3] to be derived from acetate. Thus, the signal due to the C-methyl group, C-16, at 23.0 ppm shows a pair of satellites (d, $^1J_{15,16} = 34$ Hz), as does the signal due to the adjacent tertiary carbon atom, C-15, at 25.6 ppm (dd, $^1J_{14,15} = {}^1J_{15,16} = 34$ Hz) (Figure 1A).[11] The satellites that straddle the signal for C-15 are approximately half as intense as those surrounding the signal due to C-16. Even though enrichment was discernible in several other signals (not shown), the analysis of the coupling pattern of these was hampered by second-order effects. Characterization of the ^{13}C incorporation pattern was facilitated when lycopodine was

[†] University of Hawaii.
[‡] McMaster University.

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(6) Prepared by modification of a published procedure[7] from ethyl [1,2,3,4-$^{13}C_4$]acetoacetate (Isotec Inc., Miamisburg, OH) and diethyl carbonate. Lithium tetramethylpiperidide (1.1 equiv) and *n*-butyllithium[8] (2 equiv) were used as base.

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(10) When the sample of lycopodine from this experiment was dissolved in $^2HCCl_3$, the ^{13}C NMR signals due to C-15 and C-16 were not first order. The assignment of the ^{13}C NMR spectrum in pyridine rests on COSY, HMQC, and HMBC experiments.

(11) In addition, a weak set of satellites ($^1J = 34$ Hz) was discernible in each of the signals for C-7 and C-15. Presumably these resulted from low-level incorporation into lycopodine of [1,2-$^{13}C_2$]acetic acid, generated from [1,2,3,4-$^{13}C_4$]acetonedicarboxylic acid by decarboxylation and retro-Claisen reaction, either by a biological process within the plant or chemically during the hydrolysis of diethyl [1,2,3,4-$^{13}C_4$]acetonedicarboxylate in preparation for feeding.

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reduced to the corresponding carbinol, dihydrolycopodine, by means of $LiAlH_4$ in ether/THF.

The 125 MHz ^{13}C NMR spectrum of the dihydrolycopodine sample in $CDCl_3$ (Figure 1B) permitted analysis of the coupling pattern of the remaining four acetate-derived carbon atoms, C-6, C-7, C-8, and C-14. Thus, the signal due to C-14 at 42.8 ppm, *i.e.*, the remaining carbon atom of the triad C-14,-15,-16, showed satellites (d, $^1J_{14,15} = 34$ Hz). Furthermore, the signals due to C-6 and C-8 at 33.7 and 41.7 ppm, respectively, were straddled by satellites (d, $^1J = 34$ Hz), as was the signal due to C-7 at 35.3 ppm (dd, $^1J_{6,7} = {}^1J_{7,8} = 34$ Hz) (Figure 1B).[11] The satellites around the signal for C-7 were approximately half as intense as those of the signals of C-6 and C-8.

Thus, two $^{13}C_3$ units, each derived from acetonedicarboxylic acid by loss of both carboxyl groups, had been incorporated intact into lycopodine. Loss of only one carboxyl group would have yielded acetoacetic acid, and it should be recalled at this point that incorporation of label from [$^{13}C_4$]acetoacetic acid did not occur directly but only after cleavage to [$^{13}C_2$]acetic acid (see above[3]).

Moreover, the two C_3 fragments, C-6,-7,-8 and C-14,-15,-16, each a portion of one of the two "halves", C-1 to C-8 and C-9 to C-16, of lycopodine, showed identical ^{13}C enrichment within the limits of accuracy of the measurement (1.05% specific incorporation of ^{13}C above natural abundance).

The mode of incorporation into lycopodine of label from acetonedicarboxylic acid here reported, together with our earlier results[3] on the incorporation pattern from acetic acid and acetoacetic acid and those on the mode of entry of pelletierine (**4**),[12,13] supports the notion[12−14] that lycopodine (**6**) is generated by modification of an intermediate (**5**) that is formed by the condensation of 4-(2-piperidyl)acetoacetic acid (**3**) with pelletierine (**4**), the latter arising by decarboxylation of the former.[15] This hypothesis can now be extended by the proposal that **3** arises by condensation of Δ^1-piperideine (**2**) with acetonedicarboxylic acid (**1**).[16] Such a model is consistent with all reported evidence based on earlier incorporation studies with radioactive [9,12−14,17,18] as well as stable isotopes.[3] None of the other biogenetic schemes that have been advanced[19−21] to account for the origin of lycopodine are consistent with all the available data.

The observation that acetonedicarboxylic acid serves as an intermediate in the biosynthesis of lycopodine is the first experimental evidence in support of a biogenetic idea that was put forward some 80 years ago by the late Sir Robert Robinson.[22] Modeled on his one-pot synthesis of tropinone from succindialdehyde, methylamine, and acetonedicarboxylic acid,[23] he advanced the idea, on the basis of structure alone, that the key step in the biological derivation of the alkaloids of the hygrine, tropane, cocaine, and pelletierine groups (including the hemlock alkaloids) was the condensation of a "pseudobase" (*i.e.*, a carbinolamine), with acetonedicarboxylic acid.[24] Later, Robinson expanded the idea to encompass the derivation of other alkaloid skeletons that, on the basis of structural analogy, might also originate from acetonedicarboxylic acid.[25,26]

When results of experiments with radioactive tracers accumulated, it became apparent in several instances that such a biogenetic derivation did not correspond to reality. Acetonedicarboxylic acid does not play the central role in alkaloid biosynthesis that had been assigned to it by biogenetic theory and does not appear to be implicated even in the biosynthesis of the tropane alkaloids, whose synthesis had provided the impetus for the formulation of the theory.[29] Sir Robert would have appreciated the paradox that the first direct evidence for the participation of acetonedicarboxylic acid in alkaloid biosynthesis has now surfaced in connection with the biosynthesis of an alkaloid whose structure had not even been established when he first put forth his hypothesis.

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(16) While the possibility cannot be entirely ruled out that **3** owes its formation within the plant to the nonbiological condensation of Δ^1-piperideine (**2**) with acetonedicarboxylic acid (**1**) when large quantities of the latter are administered, it must be kept in mind that the unique labeling pattern observed in the incorporation of [1,2-$^{13}C_2$]acetic acid demands the intermediacy of a compound with C_{2v} symmetry,[3] such as **1**.
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(24) It has been repeatedly stated that Robert Robinson had modeled his 1917 synthesis of tropinone[22] on his proposal of the biogenetic origin of the tropa alkaloids.[21] According to Sir Robert himself, this is not so: On his last visit to McMaster University, in 1968, he told one of us that his biogenetic proposal was inspired by his tropinone synthesis, rather than the reverse.
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