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DETERMINATION OF THE 'STARTER' ACETATE UNIT IN THE BIOSYNTHESIS OF PINIDINE

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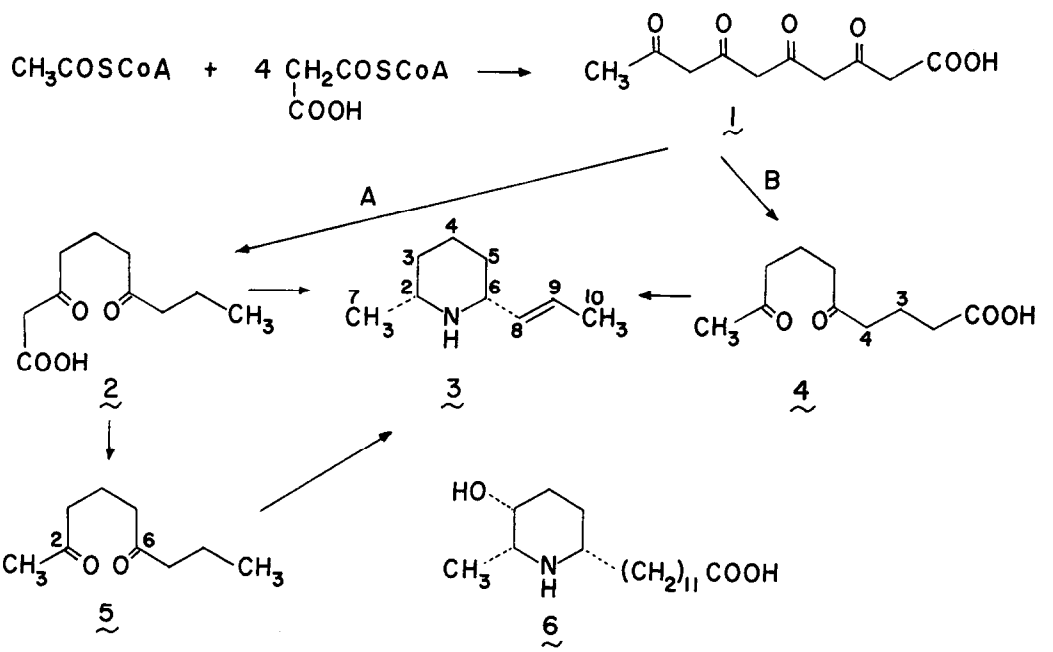
The alkaloid pinidine (3) is acetate-derived, the administration of [1-^{14}C]-acetate to Pinus jeffreyi plants affording pinidine which is labelled equally on alternate carbons[2]. It is presumed that the alkaloid is formed from a C_{10} poly-β-keto acid (1) produced by the condensation of a 'starter' acetyl-coenzyme A unit with subsequent units of malonyl-coenzyme A. This hypothetical intermediate can then yield pinidine by two alternate routes. In route A, 3,7-dioxodecanoic acid (2) or its decarboxylation product: 2,6-nonadione (5) are plausible intermediates. In route B, 5,9-dioxodecanoic acid (4) is the suggested intermediate. We have recently



tested [10-^{14}C]-5,9-dioxodecanoic acid as a precursor of pinidine and found negligible incorporation[3]. We have now found that [1-^{14}C]-2,6-nonadione also failed to label pinidine (Table I). In hemlock, Conium maculatum, octanoic acid is apparently oxidized to 5-oxo-octanoic acid prior to its conversion to coniine[4], another acetate-derived alkaloid. Thus [9-^{14}C]- and [10-^{14}C]-decanoic acids were administered to P. jeffreyi, in the hopes that analogous oxidations would occur ultimately yielding pinidine specifically labelled at one end or the other. However negligible incorporation into pinidine was observed.

The problem was finally solved by feeding diethyl [1-^{14}C]-malonate along with inactive acetate. A drop in the specific activity of the starter acetate unit was to be expected. A good incorporation of activity was obtained and systematic degradations (Figure 1) indicated that the activity at C-2 (9 %) was much lower than the activity at C-9 (ca. 30 %) (Table II). Thus C-2 and C-7 of pinidine are derived from the acetate 'starter' unit. The failure of (4) to serve as a precursor of pinidine may indicate that a double bond at C_3-C_4 is required for formation of the alkaloid.

Table I

Incorporation of Precursors (fed by the wick method) into Pinidine

Precursor	Duration of Feeding	% Incorporation[a]
Sodium [1-^{14}C]-acetate[2]	11 weeks	0.022
Diethyl [1-^{14}C]-malonate (0.1 mM) + inactive sodium acetate (0.5 mM)	1 week	0.013[b]
[10-^{14}C]-5,9-Dioxodecanoic acid[3]	5 weeks	0.0003
	10 weeks	0.004
[1-^{14}C]-2,6-Nonadione[5]	5 weeks	0.003
[9-^{14}C]-Decanoic acid[6]	5 weeks	0.004
[10-^{14}C]-Decanoic acid[6]	8 weeks	0.001

[a]Incorporation = total activity found in the isolated pinidine/total activity fed

[b]Calculated on the basis that half of the activity of the malonate is lost in the formation of the poly-β-keto acid.

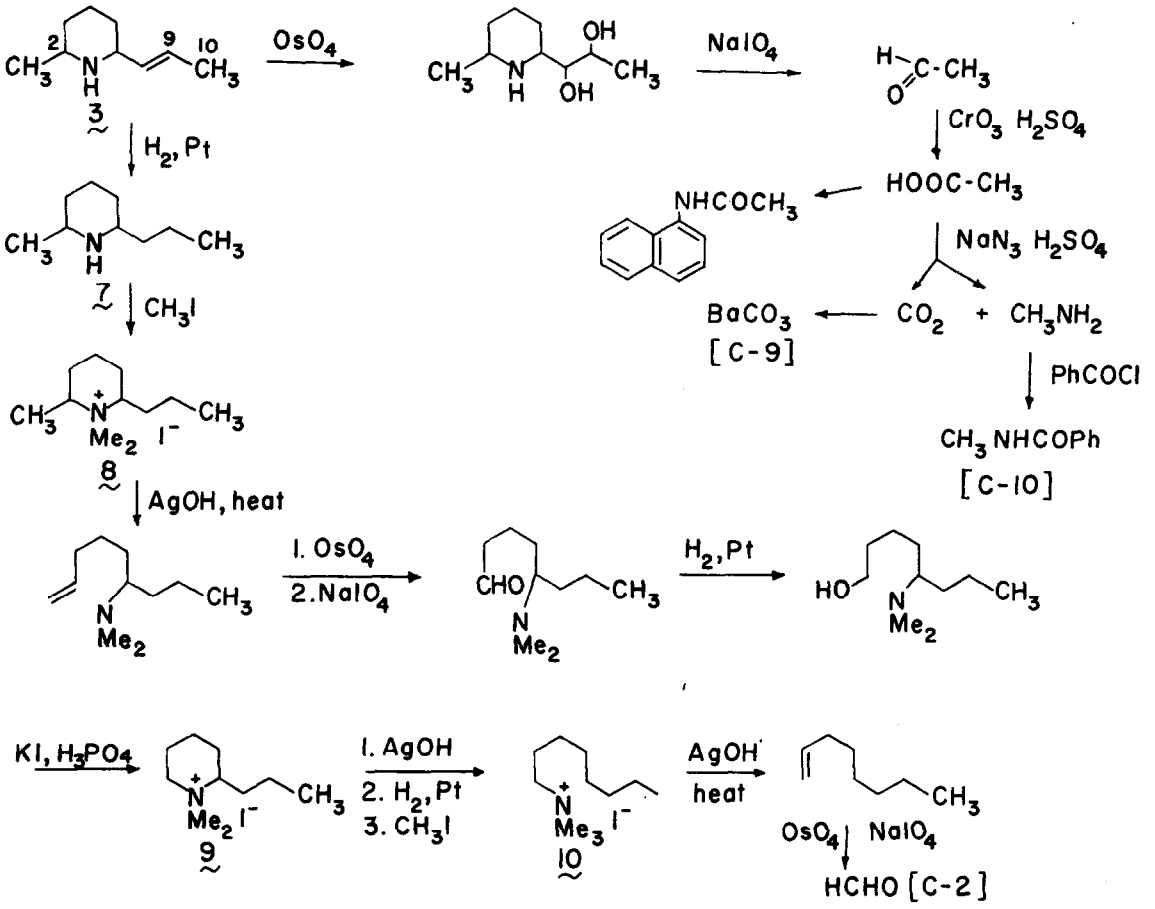


Figure 1. Degradation[2,7] of the Pinidine Derived from [1-^{14}C]-Malonate

Table II

	Specific Activity dpm/mM x 10^{-4}	Relative Specific Activity
Pinidine hydrochloride (3)	7.80	100
Acetyl-α-naphthylamine [C-9 + C-10]	2.40	31
Barium carbonate [C-9]	2.20	28
N-Methylbenzamide [C-10]	<0.05	<1
Dihydropinidine hydrochloride (7)	7.83	100
N-Methyldihydropinidine methiodide (8)	7.75	99
N-Methylconiine methiodide (9)	7.83	100
1-Dimethylamino-octane methiodide (10)	7.80	100
Formaldehyde dimedone [C-2]	0.71	9

The alkaloid spicigerine (6) obtained[8] from Prosopis spicigera (Leguminosae) is possibly formed by a similar biosynthetic route as pinidine. However with this compound no ambiguity exists in its biosynthesis, since the terminal carboxyl group is retained in the alkaloid.

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References and Notes

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5. Prepared by the sequence: [^{14}C]-methyl Mg iodide + 2-propylcyclopentanone ⟶ 1-methyl-2-propylcyclopentanol $\xrightarrow{P_2O_5}$ 1-methyl-2-propyl-1-cyclopentene $\xrightarrow{OsO_4}$ 1-methyl-2-propylcyclopentane-1,2-diol $\xrightarrow{NaIO_4}$ [1-^{14}C]-2,6-nonadione.
6. Prepared from [1-^{14}C]-ethyl iodide by conversion to diethyl cadmium, which on reaction with ethyl 7-chloroformylheptanoate yielded, after hydrolysis, [9-^{14}C]-8-oxodecanoic acid. Wolff-Kishner reduction of this compound then afforded [9-^{14}C]-decanoic acid. [10-^{14}C]-Decanoic acid was prepared by a similar sequence starting with [^{14}C]-methyl iodide and methyl 8-chloroformyl-octanoate.
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