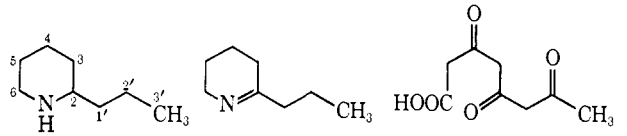
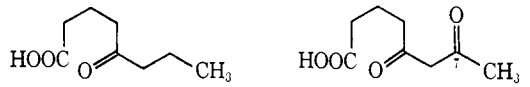
Biosynthesis of Coniine from Octanoic Acid in Hemlock Plants (*Conium maculatum*)[1]

Sir:

We have previously established[2] that coniine (**1**), one of the major alkaloids of hemlock,[3] is derived from acetic acid. Thus coniine, formed from acetic-1-^{14}C acid, was labeled mainly on the even-numbered carbons, activity being equally distributed between these four positions. This result favored the hypothesis that coniine is formed from an eight-carbon poly-β-keto acid (**3**) produced by the linear combination of four acetate units. γ-Coniceine (**2**) is apparently a precursor of coniine,[4-6] but nothing is known of the nature of the eight-carbon compound which is converted to



1 **2** **3**



4 **5**

the former alkaloid. We planned to test compounds such as 5-ketooctanoic acid (**4**) as precursors of the hemlock alkaloids. In preliminary work sodium octanoate-1-^{14}C (4.4 mg, 100 μCi)[7] was fed to hemlock plants[8] without any real expectation that it would be incorporated into coniine, since the formation of the piperidine ring would involve a cyclization at C-5, which has no apparent reactivity. To our surprise the alkaloids isolated 24 hr after feeding had high activity (9.8×10^5 dpm, 0.45% incorporation). Rigorously purified coniine hydrochloride (1.21×10^6 dpm/mmol) was degraded as previously described[2] affording C-6 as formaldehyde dimedone having an activity of 1.12×10^6 dpm/mmol. A Kuhn–Roth oxidation of the coniine yielded acetic acid (C-2′ + C-3′) having an activity of 1.0×10^4 dpm/mmol. Thus almost all the activity of the coniine was located at C-6 and octanoic acid served as a direct precursor of the alkaloid. Radioactive coniine isolated from plants which were allowed to grow for 7 days after feeding octanoic-**1**-^{14}C acid was similarly degraded and found to have 85% of its activity located at C-6. Sodium octanoate-8-^{14}C[9] (3.3 mg, 100 μCi) was also fed to hemlock plants and the alkaloids (9.8×10^5 dpm) were isolated 24 hr after the initial feeding. Coniine hydrochloride (9.5×10^5 dpm/mmol) was obtained, and degradation indicated that 48% of its activity was located at C-3′, with 6% at C-2′. Plants which were allowed to grow for 7 days after feeding octanoic-8-^{14}C acid afforded coniine having the following distribution of activity: C-2′ (9%), C-3′ (28), C-4 (9), C-5 (9), C-6 (10); *i.e.*, a significant excess of activity was still present at C-3′.

We interpret these results as follows: presumably some kind of oxidation is required at C-5 of octanoic acid to facilitate formation of the piperidine ring. We suggest that functionalization of the octanoic acid at C-5 facilitates further oxidation at C-7 to yield a compound such as **5** by the usual mechanism of β oxidation. Cleavage of this diketone by attack of coenzyme A at C-7 will yield acetyl-2-^{14}C-coenzyme A from octanoic-8-^{14}C acid. Passage of this methyl-labeled acetate through the Krebs cycle will yield acetic acid labeled on both carbons.[10] This acetate will then lead to coniine labeled uniformly. Indeed we have found that the administration of sodium acetate-2-^{14}C to hemlock yielded coniine which was labeled equally (12.5 ± 0.3%) on each of its carbons. If this hypothesis is correct the administration of octanoic-7-^{14}C acid should afford some acetate-1-^{14}C which would result in some general labeling of the even-numbered carbons of coniine. Another interpretation is that the coniine-3′-^{14}C produced from octanoic-8-^{14}C acid is metabolized in the plant, affording acetate-2-^{14}C which is then utilized for the biosynthesis of uniformly labeled coniine. These possibilities are being investigated.

Cromwell and Roberts[11,12] have obtained results which are apparently contradictory with ours. They reported extremely high incorporations (4–37%) of activity into γ-coniceine when Δ^1-piperideine-U-^{14}C or Δ^1-piperideine-2-carboxylic acid-U-^{14}C were fed to hemlock plants. No degradations were carried out on the isolated γ-coniceine to determine whether activity was localized in the piperidine ring, and it is possible that their γ-coniceine was contaminated with the administered ^{14}C compounds or their degradation products. We have now repeated some of their experiments with our variety of hemlock. Coniine isolated from plants which were fed Δ^1-piperideine-6-^{14}C hydrochloride[13] (100 mg, 82 μCi) for 2 weeks had negligible activity.

(1) This investigation was supported by Research Grant GM-13246 from the U. S. Public Health Service.

(2) E. Leete, *J. Amer. Chem. Soc.*, **86**, 2509 (1964).

(3) All of our tracer work has been carried out with a variety of *Conium maculatum* which has been cultivated for many years in the horticultural garden of the School of Pharmacy of our university. This "Minnesota" variety produces coniine as the major alkaloid in contrast to other varieties (*California*, *Chelsea*) which contain a larger proportion of γ-coniceine; *cf.* S. M. C. Dietrich and R. O. Martin, *Biochemistry*, **8**, 4163 (1969).

(4) J. W. Fairbairn and P. N. Suwal, *Phytochemistry*, **1**, 38 (1961).

(5) E. Leete and N. Adityachaudhury, *ibid.*, **6**, 219 (1967).

(6) S. M. C. Dietrich and R. O. Martin, *J. Amer. Chem. Soc.*, **90**, 1921 (1968), and the reference cited in footnote 3.

(7) Purchased from I. C. N. Corporation, Irvine, Calif.

(8) All feedings were carried out on plants (3–5 months old) growing in soil in a greenhouse. The tracers dissolved in water were administered *via* cotton wicks inserted into the stems near to ground level. In most experiments six plants were used, and had a fresh weight at the time of harvesting of 400–550 g. Coniine was isolated without dilution as its hydrochloride (50–80 mg) as previously described.[2] Conhydrine and γ-coniceine were also isolated from some of the feeding experiments, and degradations to determine the distribution of activity in these alkaloids will be reported in our complete publication.

(9) Purchased from Schwartz Bioresearch Inc., Orangeburg, N. Y. One referee suggested that the randomization of activity obtained after feeding octanoic-8-^{14}C acid to hemlock could be due to nonspecific labeling of the octanoic acid. However, a degradation of the administered octanoic-8-^{14}C acid (a Kuhn–Roth oxidation to yield acetic acid which was subjected to a Schmidt reaction) established that all the ^{14}C was located at C-8.

(10) *Cf.* J. H. Richards and J. B. Hendrickson in "The Biosynthesis of Steroids, Terpenes, and Acetogenins," W. A. Benjamin, New York, N. Y., 1964, p 142.

(11) B. T. Cromwell and M. F. Roberts, *Phytochemistry*, **3**, 369 (1964).

(12) B. T. Cromwell in "Biosynthetic Pathways in Higher Plants," J. B. Pridham and T. Swain, Ed., Academic Press, New York, N. Y., 1965, pp 147–157.

(13) E. Leete, *J. Amer. Chem. Soc.*, **91**, 1697 (1969).

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