Biosynthesis and Metabolism of the Hemlock Alkaloids[1a]

Edward Leete* and John O. Olson[1b]

Contribution No. 116 from the Natural Products Laboratory, School of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455.
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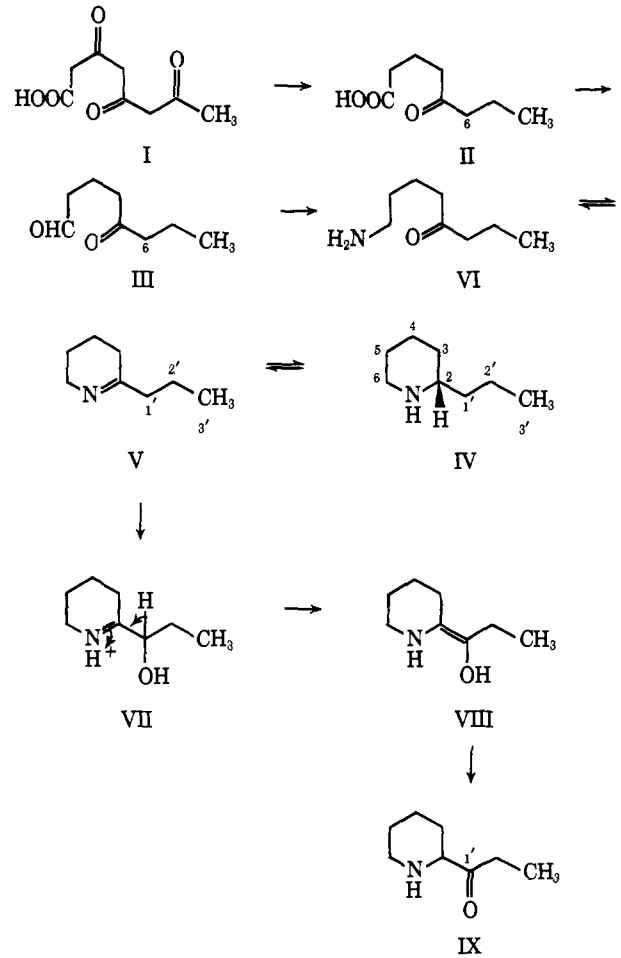
Abstract: The administration of 5-oxooctanal-6-^{14}C and 5-oxooctanoic-6-^{14}C acid to hemlock (*Conium maculatum*) resulted in the formation of coniine-1′-^{14}C and γ-coniceine-1′-^{14}C. The high incorporation of activity obtained with these compounds suggests that these are the immediate precursors of the alkaloids. The biochemical relationship of these precursors to other compounds (acetic-1- and -2-^{14}C acid, hexanoic-1-^{14}C acid, octanoic-1-, -7-, and -8-^{14}C acid) which serve as precursors of coniine, and the pattern of labeling obtained with these compounds are rationalized. (+)-Coniine-2′-^{14}C and (−)-coniine-2′-^{14}C were fed to hemlock, and it was found that the natural (+) isomer was dehydrogenated to γ-coniceine much more efficiently than its enantiomorph. By administering ^{15}N-labeled (+)-coniine and γ-coniceine to hemlock it was established that the nitrogen in these alkaloids is metabolically stable. A new alkaloid, (±)-conhydrinone (1′-oxoconiine), was isolated from hemlock, and its structure confirmed by synthesis. γ-Coniceine was shown to be a precursor of conhydrinone.

We discovered in 1963[2] that coniine (IV) is formed from acetic acid, and suggested that the alkaloids of hemlock (*Conium maculatum*), which are all related to 2-propylpiperidine,[3] are derived from an eight-carbon polyketo acid I produced by the linear condensation of four acetate units. Thus, the administration of acetate-1-^{14}C to hemlock yielded radioactive coniine labeled equally on the four even numbered carbons. Although there are many natural products which are acetate derived,[4] few attempts have been made to isolate or characterize the intermediates between acetic acid and the final natural product. We considered that 5-oxooctanoic acid (II) and 5-oxooctanal (III) would be plausible precursors of γ-coniceine (V) which is apparently the first alkaloid to be formed in hemlock.[5-7] 5-Oxooctanoic-6-^{14}C acid and 5-oxooctanol-6-^{14}C were prepared by the following method.[8] Reaction between propyl-1-^{14}C magnesium bromide and cyclopentanone yielded 1-propylcyclopentanol, which on oxidation with potassium dichromate in sulfuric acid afforded 5-oxooctanoic-6-^{14}C acid. Dehydration of the 1-propylcyclopentanol yielded 1-propylcyclopentene[9] which was treated with osmium tetroxide to give 1-propylcyclopentane-1,2-diol. Cleavage of this diol with sodium metaperiodate yielded 5-oxooctanal-6-^{14}C.[10] These compounds were administered to hemlock plants[11] by means of cotton wicks inserted into the stems of the plant near ground level. The coniine and γ-coniceine isolated from these plants were radioactive, excellent incorporations of activity being obtained (see Table I). Degradations were carried out on these alkaloids as previously described,[2,7] and it was established that essentially all the activity was located at C-1′, indicative of the direct incorporation of these compounds into the alkaloids. The incorporation of 5-oxooctanal-6-^{14}C into coniine was significantly higher than that of 5-oxooctanoic-6-^{14}C acid, a result which is consistent with the former

Scheme I



(1) (a) Presented in part at the 162nd National Meeting of the American Chemical Society, Washington D. C., Sept 1971. (b) Based in part on the Ph.D. Thesis of J. O. O., University of Minnesota, 1971.

(2) E. Leete, *J. Amer. Chem. Soc.*, **85**, 3523 (1963); **86**, 2509 (1964).

(3) One possible exception is 2-methyl-Δ′-piperideine, which B. T. Cromwell (*Biochem. J.*, **64**, 259 (1956)) has claimed, on the basis of chromatographic evidence, to be a minor alkaloid of hemlock.

(4) *Cf.* J. H. Richards and J. B. Hendrickson, "The Biosynthesis of Steroids, Terpenes and Acetogenins," W. A. Banjamin, New York, N. Y., 1964.

(5) S. M. C. Dietrich and R. O. Martin, *Biochemistry*, **8**, 4163 (1969).

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

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

(8) A preliminary account of this work has appeared as a communication: E. Leete and J. O. Olson, *Chem. Commun.*, 1651 (1970).

(9) F. Eisenlohr and G. Gorr, *Fortschr. Chem. Phys. Phys. Chem.*, **18**, 528 (1925).

(10) E. Leete, H. V. Isaacson, and H. D. Durst, *J. Label. Compounds*, **7**, 313 (1971).

(11) Tracer experiments were carried out with both the "Minnesota" variety of *Conium maculatum*, which contains coniine as the major alkaloid, and the "Chelsea" variety which has a larger proportion of γ-coniceine. We thank Professor J. W. Fairbairn, School of Pharmacy, University of London, for seeds of the latter variety.

Table I

Expt no.	Compd fed to hemlock (activity (μCi),[a] amount (mmol))	Variety of hemlock[b]	Duration of feeding, days	Absolute % inc of ^{14}C into total alkaloids	Specific % inc into named alkaloid[c]	Distribution of activity in the alkaloid,[d] % Carbon no. 6	5	4	3	2	1′	2′	3′
1	Acetic-*1*-^{14}C acid (420, 0.25)	M	1	0.035	Coniine, 0.009	24.5						24.4	0.7
2	Acetic-*2*-^{14}C acid (500, 0.25)	M	1	0.17	Coniine, 0.04	10.1						9.8	15.1
3	Acetic-*2*-^{14}C acid (500, 0.25)	M	14	0.15	Coniine, 0.035	12.3	13.0	12.5				12.2	13.2
4	Hexanoic-*1*-^{14}C acid (100, 0.1)	M	7	0.34	Coniine, 0.024	1.4	0.9	92				1.6	0.9
5	Hexanoic-*1*-^{14}C acid (200, 0.25)	C	1	0.36	γ-Coniceine, 0.05	0.6	0.7	95				1.0	0.6
6	Octanoic-*1*-^{14}C acid (100, 0.01)	M	1	0.45	Coniine, 0.01	93						0.6	0.3
7	Octanoic-*1*-^{14}C acid (100, 0.01)	M	7	0.89	Coniine, 0.01	85						3.2	1.1
8	Octanoic-*7*-^{14}C acid (100, 0.25)	M	1	0.30	Coniine, 0.07	0.6						93	1.0
9	Octanoic-*8*-^{14}C acid (100, 0.02)	M	1	0.45	Coniine, 0.01	6	9					6	48
10	Octanoic-*8*-^{14}C acid (100, 0.02)	M	7	0.89	Coniine, 0.01	10	9					9	28
11	5-Oxooctanoic-*6*-^{14}C acid (5.5, 0.25)	M	1	3.6	Coniine, 0.61	<2					95	<2	
12	5-Oxooctanoic-*6*-^{14}C acid (0.64, 0.029)	C	5	1.1	Coniine, 0.06 γ-Coniceine, 0.05	<2					94 98	<2	
13	5-Oxooctanal-*6*-^{14}C (6.4, 0.25)	M	1	6.8	Coniine, 1.1			2			95	2	
14	(+)-Coniine-*2′*-^{14}C (0.89, 0.026)	C	7	54	Coniine, 0.27 γ-Coniceine, 0.23							99 98	<1 <2
15	(−)-Coniine-*2′*-^{14}C (0.84, 0.025)	C	7	55	γ-Coniceine, 0.019							98	<1
16	(+)-Coniine-^{15}N,*1′*-^{14}C (0.50, 0.092)	M	1	40	Coniine, ^{14}C, 5.03; ^{15}N, 5.04						99		
17	(+)-Coniine-^{15}N,*1′*-^{14}C (0.50, 0.092)	M	9	47	Coniine, ^{14}C, 4.58; ^{15}N, 3.07								
18	(+)-Coniine-^{15}N,*1′*-^{14}C (0.25, 0.046)	C	9	57	Coniine, ^{14}C, 9.43; ^{15}N, 9.11								
19	γ-Coniceine-^{15}N,*1′*-^{14}C (0.75, 0.084)	M	1	47	Coniine, ^{14}C, 1.44; ^{15}N, 1.48 γ-Coniceine, ^{14}C, 51.6; ^{15}N, 48.5						>99		
20	γ-Coniceine-^{15}N,*1′*-^{14}C (0.75, 0.084)	M	9	25	Coniine, ^{14}C, 3.28; ^{15}N, 3.32 Conhydrinone, ^{14}C, 3.50; ^{15}N, 3.36						98	<1	
21	γ-Coniceine-^{15}N,*1′*-^{14}C (0.75, 0.084)	C	9	34	γ-Coniceine, ^{14}C, 1.99; ^{15}N, 2.00								

[a] The amount of activity actually absorbed by the plants is recorded. In general, the residual activity in the beakers used as reservoirs for the cotton wicks was less than 5%. All the carboxylic acids were fed as their sodium salts. The alkaloids were fed as their hydrochlorides. [b] C = Chelsea variety, M = Minnesota variety. [c] Specific incorporation is defined as the dpm/mmol, or percentage excess of ^{15}N found in the alkaloids, divided by the dpm/mmol, or percentage excess of ^{15}N in the compound administered to the hemlock. [d] The absence of a number indicates that the activity was not specifically determined at that position (see Scheme I).

compound being the immediate precursor of the alkaloids. We suggested[12] that the 5-oxooctanal undergoes transamination at the aldehyde group yielding 5-oxooctylamine (VI) which then cyclizes nonenzymatically to γ-coniceine. Recently, Roberts[13] has been able to isolate from hemlock leaves (Chelsea variety) an enzyme which catalyzes a transamination between 5-oxooctanal and L-alanine yielding γ-coniceine and pyruvic acid.

Prior to our work with 5-oxooctanal we serendipitously discovered that the administration of octanoic-*1*-^{14}C acid to hemlock yielded coniine which had almost all its activity located at C-6.[14] The coniine derived from octanoic-*7*-^{14}C was labeled mostly at C-2′. On the other hand the coniine isolated from hemlock plants one day after feeding octanoic-*8*-^{14}C had only 48% of its activity at C-3′ with considerable randomization of activity on the other carbons. The randomization of activity was even greater in the coniine isolated 7 days after feeding octanoic-*8*-^{14}C acid (only 28% at C-3′). We rationalized these apparently paradoxical results in the following way.[12] It is considered that the labeled octanoic acids are incorporated intact into coniine *via* 5-oxooctanoic acid. Evidence in favor of this was obtained by adding nonradioactive 5-oxooctanoic acid to an extract of hemlock plants which had been fed octanoic-*1*-^{14}C acid. The reisolated 5-oxooctanoic acid was radioactive, and degradation established that essentially all the activity was located on the carboxyl group, indicative of a direct formation from octanoic acid. The labeled octanoic acids are also considered to undergo β oxidation affording labeled acetic acid. Thus, octanoic-*1*- and -*7*-^{14}C acid would yield acetate-*1*-^{14}C. Entrance of this acetate-*1*-^{14}C into the Krebs cycle should result in labeling of the compounds in this cycle. This proved to be the case. The fumaric acid isolated from hemlock plants which had been fed octanoic-*1*-^{14}C for 1 day was highly radioactive (4.4% incorporation), with all the activity located on the carboxyl groups (see Table II). The β oxidation of octanoic-*8*-^{14}C leads ultimately to acetate-*2*-^{14}C. The greater randomization of activity which occurred when octanoic-*8*-^{14}C acid was fed to hemlock, compared with the rather specific incorporation of octanoic-*1*- and -*7*-^{14}C acid, is explained by postulating that the carbon-14 in acetate-*2*-^{14}C is more efficiently utilized for the formation of coniine than the label in acetate-*1*-^{14}C. Direct comparison of the incorporation of acetate-*1*-^{14}C and acetate-*2*-^{14}C (see Table I) proved that this was the case, the latter compound being four to five times more efficient as a source of labeled coniine. Few investigators have commented on the more efficient utilization of the carbon-14 of acetate-*2*-^{14}C compared with the label in acetate-*1*-^{14}C, although several examples have been reported for acetate-derived natural products.[15] This phenomenon is readily understood when one considers the fate of the carbons of acetic acid when it enters the Krebs cycle. The carboxyl carbon of acetate-*1*-^{14}C is never incorporated into the central carbons of the four-carbon dicarboxylic acids such as fumaric, and is ultimately lost as carbon dioxide. Thus, the pyruvate formed by the decarboxylation of oxaloacetate is labeled only on its carboxyl group. The acetyl coenzyme A formed from this pyruvate will thus be unlabeled. On the other hand passage of acetate-*2*-^{14}C around the cycle several times will yield fumaric acid which is labeled on both the carboxyl groups and the central carbons.[16] Thus, the acetic acid derived from pyruvate emanating from the Krebs cycle which has been supplied with acetate-*2*-^{14}C will be labeled on both carbons. It was indeed found that coniine, obtained from plants which had been allowed to metabolize acetate-*2*-^{14}C for 14 days, was essentially uniformly labeled (Table I, experiment 3). Furthermore, the fumaric acid isolated from plants which had been fed acetate-*2*-^{14}C for 1 day had activity on both the carboxyl groups (35%) and the central carbons (65%), indicative of rapid equilibration of the carbons of acetic acid *via* the Krebs cycle.

In preliminary work, when it was not known whether the octanoic-*1*-^{14}C acid was undergoing β oxidation to yield acetate-*1*-^{14}C in hemlock, hexanoic-*1*-^{14}C acid was also fed to both the Minnesota and Chelsea varieties. The formation of fumaric acid specifically labeled with ^{14}C on its carboxyl groups (Table II) indicated that β oxidation of the hexanoic acid was occurring. However, the alkaloids were labeled mostly at C-4. Thus, chain elongation of hexanoic acid is apparently possible in hemlock.[17]

Having established that 5-oxooctanal is the immediate precursor of the hemlock alkaloids we turned our attention to the interconversion of the alkaloids. Fairbairn[6] previously showed that coniine and γ-coniceine are apparently rapidly interconverted, dramatic fluctuations in the amounts of these two alkaloids occurring during a single day. Fairbairn and Ali[18] fed coniine-*U*-^{14}C (obtained biosynthetically from a $^{14}CO_2$ feeding experiment) to hemlock and observed its transformation to γ-coniceine-^{14}C. We have investigated this

Table II. Incorporation of Activity in Fumaric Acid and Distribution of the Label

Expt no.	Compd fed (duration of feeding, days)	Absolute inc of activity, %	Distribution of activity in fumaric acid, % Carboxyls	C-2 + C-3
1	Acetic-*1*-^{14}C acid (1)	0.91	99	<1
2	Acetic-*2*-^{14}C acid (1)	0.72	35	65
6	Octanoic-*1*-^{14}C acid (1)	4.4	100	<0.2
7	Octanoic-*1*-^{14}C acid (7)	0.94	98.5	1.2
9	Octanoic-*8*-^{14}C acid (1)	0.51	30	71
4	Hexanoic-*1*-^{14}C acid (7)	0.82	97.9	1.5

(12) E. Leete, *Accounts Chem. Res.*, **4**, 100 (1971).
(13) M. F. Roberts, *Phytochemistry*, **10**, 3057 (1971).
(14) A preliminary account of this work appeared as a communication: E. Leete, *J. Amer. Chem. Soc.*, **92**, 3835 (1970).
(15) Acetate-*2*-^{14}C was a significantly better precursor than acetate-*1*-^{14}C of the Ergot alkaloids (S. Bhattacharji, A. J. Birch, A. Brack, A. Hofmann, H. Kobel, D. C. C. Smith, H. Smith, and J. Winter, *J. Chem. Soc.*, 421 (1962)), dictamnine (I. Monkovic, I. D. Spenser, and A. O. Plunket, *Can. J. Chem.*, **45**, 1935 (1967)), and glauconic acid (J. L. Bloomer, C. E. Moppett, and J. K. Sutherland, *Chem. Commun.*, 619 (1965); *J. Chem. Soc. C*, 588 (1968)).
(16) *Cf.* I. D. Spenser, *Compr. Biochem.*, **20**, 256 (1968), for a more comprehensive discussion of the fate of the carbons of acetic acid in the Krebs cycle.
(17) *Cf.* ref 12 for other examples of the somewhat rare observation of the chain elongation of medium length fatty acids in nature.
(18) J. W. Fairbairn and A. A. E. R. Ali, *Phytochemistry*, **7**, 1599 (1968).

conversion, feeding both (+)-coniine-*2'*-^{14}C (the naturally occurring alkaloid) and (−)-coniine-*2'*-^{14}C to the Chelsea variety of hemlock (Table I, experiments 14 and 15). It was found that the label from the (+)-coniine was incorporated into γ-coniceine about 13 times more efficiently than the carbon-14 in the (−)-coniine. The observed small apparent conversion of (−)-coniine to γ-coniceine (0.02% specific incorporation) could possibly have been due to a small amount of residual (+)-coniine in the (−)-coniine. Degradation of the γ-coniceine derived from (+)-coniine-*2'*-^{14}C indicated that essentially all its activity was located at C-2'. The ready interconversion of (+)-coniine and γ-coniceine in the hemlock plant could indicate that these alkaloids are involved in an oxidation–reduction system which is of biological importance in the plant.

In order to determine whether the transamination of 5-oxooctanal to 5-oxooctylamine is reversible, ^{15}N-labeled alkaloids were administered to hemlock. Coniine-^{15}N was obtained by the reductive amination of 5-oxooctanal with sodium cyanoborohydride and ammonium-^{15}N bromide.[10] Carbon-14 was also introduced into the coniine at C-1' by using 5-oxooctanal-*6*-^{14}C in this synthesis. The coniine was resolved and the unnatural (−)-coniine used to prepare γ-coniceine-^{15}N,*1'*-^{14}C by the method of Grundon and Reynolds.[19] These ^{15}N-labeled alkaloids were administered to both the Minnesota and Chelsea varieties of hemlock. The alkaloids were isolated from the plants 1 and 9 days after the initial feeding. Even after 9 days there was little, if any, loss of ^{15}N relative to the ^{14}C. Degradation of the alkaloids indicated that all the activity was located at C-1'. Thus, apparently the nitrogen of these alkaloids is not removed by a reversal of their biosynthesis back to 5-oxooctanal, or by other metabolic reactions.

During these investigations on the biosynthesis and metabolism of the hemlock alkaloids we observed the presence of an unknown alkaloid on thin-layer chromatography plates. Fortuitously the hydrochloride of this alkaloid (mp 249–250°) separated out essentially pure on crystallization of the hemlock alkaloid hydrochlorides from ethanol. Elementary analysis and mass spectra indicated a molecular formula for the free base of $C_8H_{15}NO$. Its infrared spectrum had no absorption in the OH region, but a strong absorption at 1715 cm^{-1} indicative of a carbonyl group. Its nmr spectrum was consistent with the presence of an ethyl ketone. Hydrogenation in the presence of Adams catalyst yielded *dl*-conhydrine (1'-hydroxyconiine). The unknown alkaloid was thus considered to be conhydrinone (IX). Confirmation of this structure was obtained by synthesis. Ethyl-2-pyridyl ketone was ketalized with ethylene glycol and then hydrogenated in acetic acid in the presence of Adams catalyst. The product, 2-(1',1'-ethylenedioxypropyl)piperidine, was hydrolyzed with hydrochloric acid yielding the hydrochloride of (±)-conhydrinone, identical in every respect with the unknown alkaloid isolated from hemlock. Conhydrinone was described by Hess[20] who obtained it by the oxidation of (+)-conhydrine with chromium trioxide in acetic acid. However, he reported that its hydrobromide salt had a melting point of 146°. The hydrobromide of our new alkaloid had a melting point of 228–229°. The large difference in melting points may be due to the fact that Hess obtained optically active conhydrinone from his oxidation, reporting an $[\alpha]D$ of −10.6°. We have repeated Hess's oxidation of (+)-conhydrine; however, the only product isolated was (±)-conhydrinone, identical with the new alkaloid from hemlock. Racemization of optically active conhydrinone would be expected to be rapid since the chiral center is adjacent to the ketone group. Inactive conhydrinone was isolated from both varieties of hemlock, and no change in the composition of the alkaloids was observed when strongly alkaline conditions were avoided during the isolation of the alkaloids. γ-Coniceine-^{15}N,*1'*-^{14}C was an excellent precursor of conhydrinone (Table I, experiment 20) and a degradation established that essentially all the carbon-14 was located at C-1'. It is suggested that γ-coniceine undergoes allylic oxidation affording 1'-hydroxy-γ-coniceine (VII). A tautomeric shift would then yield the enol of conhydrinone (VIII).

(19) M. F. Grundon and B. E. Reynolds, *J. Chem. Soc.*, 2445 (1964).
(20) K. Hess and R. Grau, *Justus Liebigs Ann. Chem.*, **441**, 101(1925).

Experimental Section

A Nuclear Chicago Model 724 or Mark II liquid scintillation counter was used for assay of the radioactive compounds, using either toluene or dioxane–ethanol with the usual scintillators.[21] Assays were carried out in duplicate and were reproducible to 5%. ^{15}N-Labeled compounds were assayed by mass spectrometry as previously described.[22] Elementary analyses were carried out by Mr. Luke Lam at the University of Minnesota.

Compounds Fed to Hemlock. The following compounds were obtained commercially from the indicated sources: sodium acetate-*1*- and -*2*-^{14}C (Nuclear Chicago), sodium hexanoate-*1*-^{14}C (ICN, California), sodium octanoate-*1*-^{14}C (ICN), and octanoic-*8*-^{14}C acid (Schwarz Bioresearch).

Octanoic-*7*-^{14}C acid was obtained by the Wolff–Kishner reduction[23] of ethyl 6-oxooctanoate-*7*-^{14}C produced by the reaction of diethylcadmium-*1*-^{14}C with the acid chloride of the monoethyl ester of adipic acid.[24,25]

5-Oxooctanal-*6*-^{14}C. Propyl-*1*-^{14}C bromide (The Radiochemical Centre, Amersham) (0.492 g, 4 mmol, nominal activity 0.1 mCi) was added to a magnetically stirred suspension of magnesium (0.096 g, 4 mg-atom) in ether (4 ml) containing a small amount of iodine. After 40 min all the magnesium had dissolved and the mixture was cooled to 0° when a solution of cyclopentanone (0.336 g, 4 mmol) in ether (2 ml) was slowly added. The temperature of the reaction mixture was allowed to rise to 25° and stirred for 18 hr. A saturated aqueous ammonium chloride solution (10 ml) was added and the ether layer separated. The ether extract was washed with 10% Na_2CO_3 and dried over $MgSO_4$. The ether was removed at 0° and the residue distilled (120°, 0.01 mm) collecting 1-propylcyclopentanol in a U tube cooled in Dry Ice–acetone. The contents of the U tube were washed into a round-bottomed flask with ether (5 ml), phosphorus pentoxide (1.5 g) was added, and the mixture was refluxed on a metal bath maintained at 130° for 1 hr. The contents of the flask and ether washings from the condenser were then distilled (100° (0.01 mm)), again collecting the volatile products in a Dry Ice cooled U tube. The contents of the U tube were washed out with ether (50 ml), and osmium tetroxide (1.0 g) was added, together with a few drops of pyridine. After standing overnight the ether was evaporated and the residue refluxed with sodium sulfite (2 g) dissolved in 50% methanol (40 ml) for 2 hr. The filtered mixture was evaporated to dryness and the residue suspended in a little water and extracted with ether (four 15-ml portions). The dried ($MgSO_4$) extract was evaporated and the residue distilled (180° (0.01 mm)) affording 1-propylcyclopentane-1,2-diol as a colorless viscous oil (185 mg, 32%) having an

(21) A. R. Friedman and E. Leete, *J. Amer. Chem. Soc.*, **85**, 2141 (1963).
(22) E. Leete and J. N. Wemple, *ibid.*, **91**, 2698 (1969).
(23) L. F. Fieser and J. Szmuszkovicz, *ibid.*, **70**, 3352 (1948).
(24) E. E. Blaise and A. Koehler, *Bull. Soc. Chim.*, **7**, 215 (1910).
(25) J. Cason, *Chem. Rev.*, **40**, 15 (1947).

activity of 5.6×10^7 dpm/mmol. Since it was anticipated that 5-oxooctanal would be unstable it was prepared just prior to feeding by the following method. The diol (36 mg, 0.25 mmol) was dissolved in water (3 ml) containing a drop of acetic acid. Sodium metaperiodate (54 mg, 0.25 mmol) in water (2 ml) was added and the mixture shaken for 20 min. A solution of barium chloride dihydrate (30.5 mg, 0.125 mmol) was added and the precipitated barium iodate (60 mg, 99%) removed by filtration. The aqueous solution containing 5-oxooctanal-*6*-^{14}C was fed immediately to the hemlock plants. Working on a larger scale it was found possible to extract the 5-oxooctanal from the aqueous solution with ether and prepare a disemicarbazone, mp 185°.[10]

5-Oxooctanoic-*6*-^{14}C Acid. 1-(Propyl-*1'*-^{14}C)-cyclopropanol (2.16 g, 16.9 mmol) was prepared as described in the previous section from propyl-*1*-^{14}C bromide (3.07 g, 25 mmol, 0.5 mCi), and was stirred at 0° while a solution of potassium dichromate (4.73 g, 16.1 mmol) in a mixture of concentrated sulfuric acid (6 ml) and water (20 ml) was added. After stirring for 1 hr at 0° the mixture was allowed to warm up to room temperature and stirred for an additional 18 hr. Finally the mixture was heated on a steam bath for 1 hr. The cooled, dark green reaction mixture was extracted with ether (five 20-ml portions). Evaporation of this extract afforded crude 5-oxooctanoic-*6*-^{14}C acid (1.32 g, 34.9% yield from propyl bromide). This material was purified in small portions by tlc on 2-mm thick plates of Silica Gel PF-254 (Merck), developing with a mixture of chloroform–methanol–formic acid (80:20:1). With this system 5-oxooctanoic acid has an R_f of 0.6. The zone corresponding to this acid (detected by spraying a narrow strip with a solution of iodine in hexane) was extracted with 20% methanol in chloroform, evaporated, and distilled (110° (0.01 mm)) affording 5-oxooctanoic-*6*-^{14}C acid as a pale yellow oil, which crystallized on cooling, mp 32–34° (lit.[26] mp 35°). For assay it was converted to its semicarbazone, mp 198–197° (lit.[26] mp 195°), 4.8×10^7 dpm/mmol.

(+)-Coniine-^{15}N,*1'*-^{14}C. 1-(Propyl-*1'*-^{14}C)-cyclopentane-1,2-diol (288 mg, 2 mmol, 2.2×10^7 dpm/mmol) was cleaved with periodate as previously described to yield 5-oxooctanal-*6*-^{14}C which was reductively aminated with ammonium-^{15}N bromide (96% atom excess ^{15}N) and sodium cyanoborohydride[10] to afford (±)-coniine-^{15}N,*1'*-^{14}C hydrochloride (137 mg, 2.15×10^7 dpm/mmol). This coniine hydrochloride (130 mg, 0.78 mmol) was dissolved in water, made basic with 10% NaOH, and extracted with ether. D(−)-Mandelic acid (obtained from Fluka AG. Switzerland) (122 mg, 0.8 mmol) was added to the ether solution which was then evaporated to dryness. The residue was dissolved in methanol (0.5 ml) and ether (2 ml) was added. Since the (−)-mandelate salt of (+)-coniine failed to crystallize out in satisfactory yield, (+)-coniine (−)-mandelate[27] (70 mg) was added to the solution and the whole crystallized from a 1:2 mixture of methanol–ether. (+)-Coniine (−)-mandelate separated out as fine colorless needles (108 mg), mp 126–127° (lit.[27] mp 127.5°). This salt was dissolved in dilute HCl and extracted with ether. The residue obtained on evaporation of the aqueous solution was crystallized from a mixture of ethanol and ether affording colorless needles of (+)-coniine hydrochloride (54 mg), mp 222–223°, having an activity of 1.24×10^7 dpm/mmol and an ^{15}N atom % excess of 49.6%.

γ-Coniceine-^{15}N,*1'*-^{14}C. The mother liquors obtained after removal of the (+)-coniine (−)-mandelate were evaporated to dryness, made basic with 10% NaOH, and extracted with ether. The dried ($MgSO_4$) extract was evaporated and the residue distilled affording coniine (85 mg) which was dissolved in methylene chloride (5 ml) and stirred with *N*-chlorosuccinimide (90 mg) for 2 days. Water (5 ml) was then added and the aqueous layer extracted with more methylene chloride. The combined, dried ($MgSO_4$) extracts were evaporated and the residue distilled (100° (0.02 mm)) collecting the volatile products in a U tube cooled in Dry Ice. The contents of the trap were added to a solution of KOH (1 g) in methanol (10 ml) and stirred at room temperature for 5 hr. Concentrated HCl was then added and the mixture filtered. The filtrate was evaporated to dryness and made basic with NaOH and extracted with chloroform. Thin-layer chromatography on a portion of this extract indicated that all the radioactivity was located at an R_f value corresponding to γ-coniceine. No trace of activity was detected at a position where coniine would have appeared. The chloroform solution of γ-coniceine was treated with HCl gas and evaporated yielding γ-coniceine hydrochloride (41.2 mg), 1.48×10^7 dpm/mmol, 58.2% ^{15}N. The assay was carried out on *N*-benzoyl-5-oxooctylamine, prepared from the γ-coniceine as described later.

(+)- and (−)-Coniine-*2'*-^{14}C. 2-Propylpyridine-*2'*-^{14}C (conyrine) was obtained by reaction between ethyl-*1*-^{14}C iodide (Amersham-Searle) and the lithium salt of 2-methylpyridine.[28] The conyrine-*2'*-^{14}C was dissolved in ethanol; the solution was acidified with concentrated HCl and hydrogenated in the presence of Adams catalyst at 2 atm of pressure. Removal of the catalyst and evaporation yielded (±)-coniine-*2'*-^{14}C hydrochloride. The hydrochloride was converted to the free base which was resolved with mandelic acid.[27] The mandelate salts were converted to hydrochlorides as previously described.

General Method of Administering Radioactive Compounds to Hemlock and Isolation of the Alkaloids. At the time of feeding most of the hemlock plants were 3–5 months old, growing in soil in a greenhouse. The tracers were administered by means of cotton wicks inserted into the stems of the plants near ground level. In experiments 14 and 15 the plants were 14 months old, and aqueous solutions of the tracer were injected by means of a hypodermic syringe directly into the stems of the plant near to the position where they emerged from the carrot-like root. A typical extraction procedure was as follows. The fresh whole plants (500–800 g) were macerated in a Waring blendor with a mixture of chloroform (2 l.) and 5% NaOH (200 ml). The plant debris was removed by filtration and the aqueous layer (extract A) and chloroform separated. The chloroform layer was evaporated in the presence of 2 *N* HCl (200 ml). The residual pale green aqueous layer was filtered through Celite to remove tar and then made basic with sodium hydroxide and extracted with chloroform (five 200-ml portions). Hydrogen chloride gas was passed into the chloroform extract, which was then evaporated to dryness to yield the crude alkaloid hydrochlorides (0.3–0.5 g). This residue was made basic with NaOH, extracted with ether, dried ($MgSO_4$), and distilled (120° (0.001 mm)) into a U tube cooled in Dry Ice. Ethanolic HCl was added to the contents of the U tube and the solution evaporated to dryness. The residue was dissolved in hot ethanol (5 ml) and on standing at room temperature fine feathery needles of almost pure conhydrinone hydrochloride (20–30 mg), mp 249–250°, separated. The mother liquor was evaporated to dryness and subjected to thin-layer chromatography in Silica Gel PF-254. Development with a mixture of chloroform–ethanol–concentrated ammonia (50:28:4) gave distinct zones corresponding to the following alkaloids, with the indicated R_f values: conhydrine (0.3), coniine (0.55), conhydrinone (0.80), and γ-coniceine (0.95). The alkaloids were detected by spraying a thin strip at the side of the plate with a solution of iodine in hexane. The zones were extracted with a mixture of chloroform and methanol. Evaporation of these extracts in the presence of HCl afforded the alkaloid hydrochlorides.

Assay of the Alkaloids and Their Degradation to Determine the Location of Activity. Coniine and conhydrinone were assayed as their hydrochlorides. γ-Coniceine hydrochloride which is hygroscopic was benzoylated as follows. The crude hydrochloride (100 mg) was shaken with a mixture of benzoyl chloride (0.5 ml) and 5% NaOH (10 ml) for 3 hr. The mixture was extracted with ether, dried ($MgSO_4$), and evaporated to yield a semisolid residue which was crystallized from a mixture of acetone and hexane to afford colorless needles of *N*-benzoyl-5-oxooctylamine (76 mg), mp 52–53° (lit.[19] mp 58–59°). Reduction of γ-coniceine in ethanol with sodium borohydride yielded coniine which was degraded by methods previously described.[2,7] The conhydrinone derived from γ-coniceine-^{15}N,*1'*-^{14}C (experiment 20) was subjected to a Kuhn–Roth oxidation[2] yielding a mixture of acetic and propionic acids which was converted to their α-naphthylamides,[29] and separated by thin-layer chromatography.[30]

Properties of the (±)-Conhydrinone Isolated from Hemlock. The conhydrinone hydrochloride (mp 249–250°) had no significant absorption in the uv. Its nmr spectrum (in methanol-d_4) taken on a Varian XL-100 spectrometer had a triplet at 1.10 ppm (3 H) and a quartet at 2.6 ppm (2 H). Its mass spectrum had a parent peak at *m/e* 142 ($C_8H_{15}NO + H^+$) and a strong peak at *m/e* 84, characteristic of piperidines. *Anal.* Calcd for $C_8H_{15}NO \cdot HCl$: C, 54.08; H, 9.07; N, 7.88. Found: C, 54.33; H, 9.00; N, 7.68.

(26) A. Franke and A. Kroupa, *Monatsh. Chem.*, **69**, 167 (1936).
(27) J. C. Craig and A. R. Pinder, *J. Org. Chem.*, **36**, 3648 (1971).
(28) E. Bergman and W. Rosenthal, *J. Prakt. Chem.*, **135**, 267 (1932).
(29) E. Leete, H. Gregory, and E. G. Gros, *J. Amer. Chem. Soc.*, **87**, 3475 (1965).
(30) E. Leete and K. N. Juneau, *ibid.*, **91**, 5614 (1969).

Reduction of the alkaloid hydrochloride in ethanol with hydrogen in the presence of Adams catalyst yielded *dl*-conhydrine, mp 95–96°, identical (mixture melting point, ir, tlc) with authentic material.

(±)-Conhydrinone. (a) From Ethyl 2-Pyridyl Ketone. Ethyl 2-pyridyl ketone[31] (6.75 g, 0.05 mol), *p*-toluenesulfonic acid (14 g, 0.1 mol), ethylene glycol (12.4 g, 0.4 mol) and benzene (50 ml) were refluxed for 20 hr, separating water in a Dean-Stark trap. The cooled reaction mixture was added to an aqueous solution of K_2CO_3 and extracted with ether. The dried (K_2CO_3) extract was evaporated and the residue distilled (100° (0.01 mm)) affording 2-(1′,1′-ethylenedioxypropyl)pyridine as a colorless oil (5.6 g), which was analyzed as its picrate, mp 128–129°. *Anal.* Calcd for $C_{16}H_{16}N_4O_9$: C, 47.06; H, 3.95; N, 13.72. Found: C, 46.85; H, 3.98; N, 13.37.

This ketal (1.0 g) was dissolved in acetic acid (40 ml) and hydrogenated in the presence of Adams catalyst (0.2 g) at 3 atm of pressure for 18 hr. The filtered reaction mixture was added to a mixture of K_2CO_3 (70 g), ice, and water and extracted several times with ether. The dried (K_2CO_3) extract was evaporated and distilled (120° (0.01 mm)) yielding 2-(1′,1′-ethylenedioxypropyl)-piperidine (0.85 g) as a colorless viscous oil. This ketal (0.80 g) was stirred overnight at room temperature with 4% HBr (10 ml). The reaction mixture was evaporated to dryness and the residue crystallized from a mixture of ethanol and ether, affording fine colorless needles of (±)-conhydrinone hydrobromide (0.81 g), mp 228–229°. *Anal.* Calcd for $C_8H_{15}NO \cdot HBr$: C, 43.25; H, 7.26; N, 6.30. Found: C, 43.00; H, 7.10; N, 6.12. Hydrolysis of the ketal with HCl yielded the hydrochloride of conhydrinone, mp 249–250°, identical (mixture melting point, ir, tlc) with material obtained from hemlock.

(b) From Conhydrine. (+)-Conhydrine (1.43 g, 10 mmol) was dissolved in acetic acid (10 ml) and a solution of CrO_3 (0.70 g, 7 mmol) in water (10 ml) and 2 *N* sulfuric acid (5 ml) slowly added. After stirring for 2 days at room temperature the reaction mixture was dark green, and conhydrine was absent (established by tlc). The mixture was then added to K_2CO_3 solution and extracted several times with ether. The dried ($MgSO_4$) extract was evaporated and the pale yellow residue distilled (100° (0.01 mm)) affording a colorless oil which on treatment with HCl and crystallization from ethanol and ether yielded (±)-conhydrinone hydrochloride (0.71 g), mp 249–250°.

Examination of Extract A from Experiment 5. The brown aqueous extract from the hemlock plants which had been fed octanoic-*1*-^{14}C acid (for 1 day) had an activity of 9.6×10^7 dpm (44% of the activity fed). It was acidified with HCl and extracted with ether for 7 days. The ether extract (135 ml) had an activity of 2.5×10^7 dpm. Inactive 5-oxooctanoic acid (53.3 mg) was added to a portion (10 ml) of this extract, which was then evaporated to dryness yielding a semicrystalline residue (215 mg). This residue was suspended in chloroform and filtered. The residue (61 mg) was sublimed (170° (0.01 mm)) affording a white solid (40 mg), mp 293° (sealed capillary), identical (ir spectra) with fumaric acid. It had an activity of 1.8×10^4 dpm/mg. Thus, the total activity in the plant due to fumaric acid = $1.8 \times 10^4 \times 40 \times 13.5 = 9.7 \times 10^6$ (4.4% incorporation). This fumaric acid (1.95×10^6 dpm/mmol) was catalytically reduced to succinic acid (1.91×10^6 dpm/mmol) which was subjected to a Schmidt reaction[32] yielding barium carbonate (0.96×10^6 dpm/mol) and ethylenediamine collected and assayed as its *N*,*N*-dibenzoyl derivative (0.004×10^6 dpm/mmol).

The chloroform solution from which the fumaric acid had been removed was evaporated to small bulk and subjected to thin layer chromatography using the same system as that used for the purification of 5-oxooctanoic acid. The zone corresponding to this acid was removed and extracted, and the residual oil obtained on evaporation distilled (110° (0.01 mm)), yielding a pale yellow oil (35 mg) having a total activity of 1.0×10^5 dpm. Treatment of this oil with semicarbazide hydrochloride and sodium acetate in aqueous solution yielded the semicarbazone of 5-oxooctanoic acid, which had a constant specific activity, after repeated crystallization, of 4.1×10^4 dpm/mmol. On heating this semicarbazone (30 mg) with an equal weight of copper chromite in refluxing quinoline (1 ml) carbon dioxide was produced and collected as barium carbonate (21 mg) having an activity of 4.0×10^4 dpm/mmol.

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(31) A. Pinner, *Ber.*, **34**, 4234 (1901).

(32) E. F. Phares and M. V. Long, *J. Amer. Chem. Soc.*, 77, 2556 (1955).