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BIOSYNTHESIS OF THE HEMLOCK ALKALOIDS—II.

THE CONVERSION OF γ-CONICEINE TO CONIINE AND Ψ-CONHYDRINE

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Abstract—γ-Coniceine-1'-^{14}C was fed to 8-month-old *Conium maculatum* (hemlock) plants growing in a greenhouse. Radioactive coniine and Ψ-conhydrine were isolated from the plants 8 days later, the specific incorporations of tracer being 9·9 and 4·15% respectively. Systematic degradation of these alkaloids established that all the activity was located at C-1' of their propyl side chains, indicating that γ-coniceine can serve as a direct precursor of coniine and Ψ-conhydrine. The biosynthesis of the hydroxy-coniines is apparently very sensitive to environmental conditions since plants grown out of doors contained mainly conhydrine as a minor alkaloid, whereas the same variety of *C. maculatum* cultivated in a greenhouse contained the isomeric Ψ-conhydrine.

THE hemlock plant (*Conium maculatum* L.) contains the piperidine alkaloids: coniine (I), γ-coniceine (II), conhydrine (III), Ψ-conhydrine (IV), and N-methylconiine (V). It has been suggested[1] that γ-coniceine plays a central role on the formation of the other alkaloids, and support for this hypothesis was obtained by examination of the alkaloid content of hemlock plants at various stages of growth.[2, 3] The results indicated that there was a rapid interchange of coniine and γ-coniceine in the developing pericarp. We have now tested this hypothesis by feeding radioactive γ-coniceine to hemlock plants. γ-Coniceine-1'-^{14}C was prepared by Lukeš method.[4] Propyl bromide-1-^{14}C was obtained from commercially available propanol-1-^{14}C by treatment with hydrobromic acid. The Grignard reagent from the bromide was

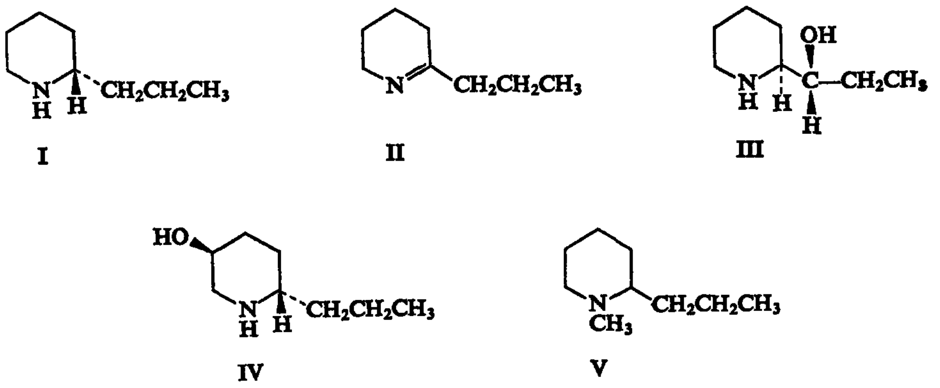
I II III IV V

FIG. 1. THE HEMLOCK ALKALOIDS.

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[1] J. W. FAIRBAIRN and S. B. CHALLEN, *Biochem. J.* **72**, 556 (1959).

[2] J. W. FAIRBAIRN and P. N. SUWAL, *Phytochem.* **1**, 38 (1961).

[3] J. W. FAIRBAIRN, *Abhandl. Deut. Akad. Wiss. Berlin Kl. Chem. Geol. Biol.* **3**, 141 (1966).

[4] R. LUKEŠ, F. ŠORM and Z. ARNOLD, *Coll. Czech. Chem. Commun.* **12**, 641 (1947).

condensed with 2-piperidone in boiling xylene yielding γ-coniceine-1′-^{14}C which was purified by distillation and crystallization of its hydrochloride.

The radioactive γ-coniceine hydrochloride was fed to 8-month-old hemlock plants growing in a greenhouse, via the petioles after removal of the leaf blade.[5] The crude alkaloids were isolated and we proceeded to separate coniine (I) from what we thought was conhydrine (III) by our previously described method.[6] To our surprise the solid obtained on fractional sublimation of the crude alkaloids proved to be almost pure Ψ-conhydrine (IV). In our previous work[6] coniine and conhydrine were the two main alkaloids isolated from an identical variety of *C. maculatum*. The main difference in the two experiments was that the present work was carried out with plants grown in a greenhouse, while our previous investigation utilized plants which were grown out of doors. The plants in the two experiments were certainly subjected to quite different temperature and humidity conditions and this surprising change in alkaloid content may be due to these factors. The plants in the earlier work were fed tracers in June, while the feeding experiments in the present work were carried out in August and October. We are currently investigating the relationship between alkaloid content and time of year in our variety of *C. maculatum*.[7] The effect of external conditions on alkaloid production has been reviewed by Mothes[8] and it is clear that much more work needs to be done on this subject.

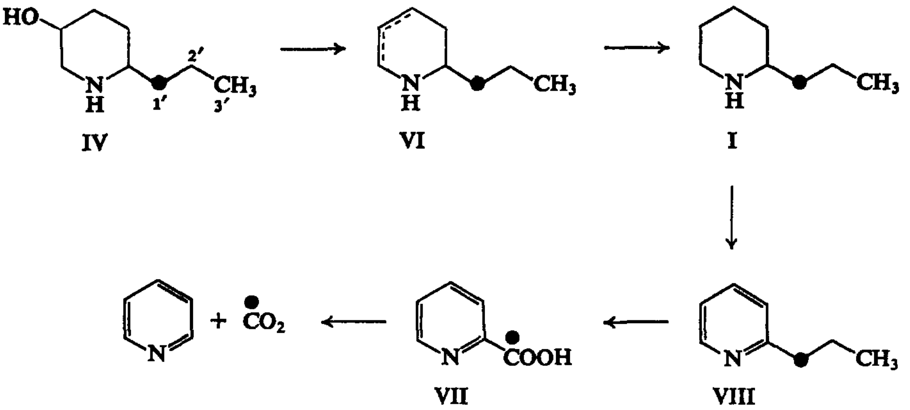

FIG. 2. DEGRADATION OF Ψ-CONHYDRINE AND CONIINE (CARBON-14 INDICATED WITH HEAVY DOT).

The coniine hydrochloride and Ψ-conhydrine were crystallized to constant activity and high specific incorporation of tracer (9·9 and 4·15 % respectively) was found in these alkaloids, strongly suggesting that they were derived directly from γ-coniceine without any prior breakdown of the administered alkaloid. The coniine and Ψ-conhydrine were degraded by the scheme illustrated in Fig. 2. Ψ-Conhydrine was dehydrated by heating with phosphorus pentoxide.[9] The resultant dehydro-coniine (VI) was hydrogenated affording coniine which

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[5] B. T. CROMWELL and M. F. ROBERTS, *Phytochem.* **3**, 369 (1964).

[6] E. LEETE, *J. Am. Chem. Soc.* **86**, 2509 (1964); this paper is regarded as Part I of this series on the biosynthesis of the hemlock alkaloids.

[7] Our variety of *Conium maculatum* apparently differs from that used by Professor Fairbairn,[1, 2] and in preliminary experiments he has found that our variety contains coniine as the major alkaloid in the vegetative parts of the plant, whereas the "Chelsea variety" he worked with contains γ-coniceine as the major alkaloid (private communication).

[8] K. MOTHES, *J. Pharm. Pharmacol.* **11**, 193 (1959).

[9] K. LÖFFLER, *Chem. Ber.* **42**, 116 (1909).

was dehydrogenated to 2-propylpyridine (VIII) by heating with silver acetate in dilute acetic acid at 180°.[10] Oxidation of VIII with alkaline potassium permanganate yielded α-picolinic acid (VII) which was decarboxylated by refluxing in quinoline in the presence of copper chromite. In a separate experiment the α-picolinic acid was heated with calcium oxide when pyridine was obtained and collected as its oxalate. Kuhn-Roth oxidations on coniine and Ψ-conhydrine yielded acetic acid which was assayed as its α-naphthylamide derivative.[11] The activities of conine, Ψ-conhydrine and their degradation products are recorded in Table 1, and it is apparent that essentially all the activity of both alkaloids was located at C-1′ of their propyl side chains.

TABLE 1. ACTIVITIES OF CONIINE, Ψ-CONHYDRINE AND THEIR DEGRADATION PRODUCTS

	Specific activity (dpm/mM × 10^{-5})	
	From coniine	From Ψ-conhydrine
Coniine hydrochloride	2·78	—
Ψ-Conhydrine	—	1·17
α-Picolinic acid	2·68	1·10
Barium carbonate [C-1′]	2·60	1·08
Pyridine oxalate	<0·01	<0·05
1-Acetamidonaphthalene	<0·03	<0·03

The *in vivo* hydrogenation of γ-coniceine to coniine requires little comment, however this reduction must be fairly stereospecific since natural coniine exists mainly in the dextrorotary form (I).[12] The formation of Ψ-conhydrine from γ-coniceine is of considerable interest and it is suggested that an intermediate in this transformation is compound IX (Fig. 3) formed by a tautomeric shift of the double bond. Position 5 is now allylic and would be susceptible to oxidation affording X, which on reduction yields Ψ-conhydrine (IV).

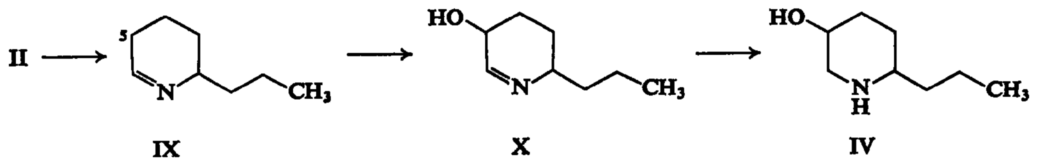

FIG. 3. HYPOTHETICAL BIOSYNTHESIS OF Ψ-CONHYDRINE.

EXPERIMENTAL

γ-Coniceine-1′-^{14}C Hydrochloride

A mixture of 1-propanol-1-^{14}C[13] (10 g, 0·5 mc) and 48% HBr (77·5 ml) was distilled slowly to half volume. The distillate was washed with conc. HCl, water, $NaHCO_3$ solution, and finally water. After drying ($CaCl_2$) the product was distilled, affording n-propyl bromide-1-^{14}C (11·45 g), b.p. 71° (760 mm). Mg turnings (2·75 g) were suspended in dry ether (42 ml) under N_2, and 1 ml of the radioactive propyl bromide added along with a crystal of I_2. After

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[10] J. TAFEL, *Chem. Ber.* **25**, 1619 (1892).
[11] E. LEETE, H. GREGORY and E. G. GROS, *J. Am. Chem. Soc.* **87**, 3475 (1965).
[12] N. J. LEONARD and W. J. MIDDLETON, *J. Am. Chem. Soc.* **74**, 5776 (1952).
[13] Purchased from Nuclear Research Chemicals Inc., Orlando, Florida.

warming to start the reaction, the rest of the propyl bromide was added to the stirred mixture during 40 min. When all the Mg had dissolved, dry xylene (42 ml) was added and the ether removed by raising the temperature to 120°. A solution of 2-piperidone (2·5 g) in xylene (5 ml) was added and the mixture refluxed for 3 hr at 135°. After cooling, the reaction mixture was added to ice (100 g). BaO (60 g) was added and the mixture steam distilled into 20 ml of N HCl. When 1 l. of distillate had been collected, it was evaporated to small volume *in vacuo*, made alkaline with 30% NaOH, and extracted with ether which was then dried (NaOH). The dried residue was distilled using a short fractionating column, γ-coniceine being obtained as a colorless oil (0·482 g), b.p. 63–64° (13·5–14 mm). Dry HCl was passed into a solution (0·48 g) in dry ether (20 ml) for 10 min when colorless crystals of γ-coniceine-1'-^{14}C hydrochloride separated (0·420 g), m.p. 135–138° (lit.[14] m.p. 143°), which was dried (P_2O_5). It had an activity of $1{\cdot}74 \times 10^4$ d.p.m./mg, $2{\cdot}82 \times 10^6$ d.p.m./mM. Radioactivity measurements were carried out in a Nuclear Chicago liquid scintillation spectrometer, Model 724, using as solvents either toluene or dioxane–water with the usual scintillators.[15] Paper chromatography of the γ-coniceine hydrochloride[16] indicated that it was radiochemically homogeneous, activity all being located at a spot coincident with the alkaloid.

Administration of γ-Coniceine-1'-^{14}C to Conium maculatum *and Isolation of the Alkaloids*

The hemlock plants, *C. maculatum* (Minnesota variety) were grown from seed and cultivated in a greenhouse. At the time of feeding (October, 1965) they were 8 months old. γ-Coniceine-1'-^{14}C hydrochloride (163·2 mg, $2{\cdot}84 \times 10^6$ d.p.m.) was dissolved in 20 ml of water and distributed equally between twenty plants. One of the leaf blades of each plant was cut off with a razor blade and the petiole dipped into the tracer solution. On subsequent days distilled water was given to wash the tracer into the plant. After 8 days the plants were harvested and dried in air (2 kg). The plants were macerated with a mixture of chloroform (4 l.) and 15 N ammonia (300 ml). After standing for several days, the mixture was filtered through cloth and the two layers separated. The aqueous layer had a total activity of $3{\cdot}42 \times 10^4$ d.p.m. (1·2% of the activity fed). The chloroform layer was concentrated to 200 ml in the presence of 50 ml of 2 N H_2SO_4. The mixture was then diluted with 200 ml of ether and extracted several times with 2 N H_2SO_4. The acidic layer was made basic with 20% NaOH and extracted with chloroform which was then dried (K_2CO_3). Excess ethanolic HCl was added to the dried extract which on evaporation yielded the alkaloid hydrochlorides having an activity of $1{\cdot}41 \times 10^6$ d.p.m. (49·6% of the activity fed). The crude hydrochlorides were dissolved in water, made alkaline with NaOH, and extracted with chloroform. Evaporation of the dried (K_2CO_3) extract yielded a dark brown oil which was distilled (100°, 0·1 mm) in a hot air bath. Coniine and other relatively volatile alkaloids were collected in a U-tube cooled in dry ice–acetone. A solid was deposited near the exit from the hot air bath. This material was crystallized from light petroleum (b.p. 60–70°) affording hair-like needles of Ψ-conhydrine (34 mg), m.p. 103–105°, having a specific activity of $1{\cdot}17 \times 10^5$ d.p.m./mM. Its i.r. spectrum was identical with a specimen of synthetic ($\pm$)-Ψ-conhydrine.[17] A small amount of this Ψ-conhydrine (2·14 mg) was diluted with inactive (+)-conhydrine (98·79 mg) and the mixture crystallized from light petroleum. The recovered conhydrine had negligible activity indicating that there was no radioactive conhydrine contaminating the Ψ-

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[14] H. C. BEYERMAN, M. VAN LEEUVAN, J. SMIDT and A. VAN VEEN, *Rec. Trav. Chim.* **80**, 513 (1961).
[15] A. R. FRIEDMAN and E. LEETE, *J. Am. Chem. Soc.* **85**, 2141 (1963).
[16] B. T. CROMWELL, *Biochem. J.* **64**, 259 (1956).
[17] W. GRUBER and K. SCHLÖGL, *Monatsh. Chem.* **80**, 499 (1949).

conhydrine. The rest of the Ψ-conhydrine was diluted with inactive (±)-Ψ-conhydrine for subsequent degradations. The U-tube which contained coniine and γ-coniceine (paper chromatography) was dissolved in ethanol and dry HCl passed into the solution. The resultant hydrochloride (261 mg) was crystallized from a mixture of ethanol and acetone affording (+)-coniine hydrochloride (150 mg), m.p. 222–223°, having an activity of $2 \cdot 78 \times 10^5$ d.p.m./mM. The mother liquors which contained some γ-coniceine could not be crystallized. The coniine hydrochloride was diluted with inactive (±)-coniine hydrochloride for further degradations.

A preliminary feeding experiment was carried out with a smaller amount of γ-coniceine-1'-^{14}C hydrochloride (25 mg) in August 1965. Active coniine and Ψ-conhydrine were isolated from the plant, and essentially no conhydrine.

Degradation of the Alkaloids

Ψ-Conhydrine (311 mg) was intimately mixed with P_2O_5 (2·0 g) and heated at 150° in N_2 for 6 hr. The mixture was added to ice, made alkaline (KOH) and was extracted with CH_2Cl_2 which was then dried ($MgSO_4$). The residue was dissolved in ethanol (50 ml) and hydrogenated in the presence of Pt_2O (0·1 g) for 12 hr. The filtered mixture was acidified (ethanolic HCl), evaporated to dryness, and residual coniine hydrochloride (204 mg) heated in a sealed tube with 10% acetic acid (4 ml), hydrated sodium acetate (0·4 g) and silver acetate (4·0 g) at 180° for 6 hr. The contents of the tube were made alkaline with 10% NaOH, and the mixture steam distilled until 50 ml of distillate had been collected. $KMnO_4$ (1·0 g) was added to the distillate and the mixture heated on a steam bath for 24 hr. Residual permanganate was destroyed by the addition of a few drops of ethanol, the filtrate adjusted to pH 3·2 (HCl), evaporated to dryness and the residue extracted with boiling benzene. α-Picolinic acid (27 mg) was obtained on evaporation of the benzene, and was purified by sublimation (160°, 0·01 mm) and crystallization from benzene–light petroleum. It had m.p. 137–138°, not depressed on admixture with an authentic specimen.

The radioactive coniine hydrochloride was dehydrogenated with silver acetate in the same way. Kuhn–Roth oxidations on coniine and Ψ-conhydrine were carried out as previously described.[6]

Decarboxylation of α-Picolinic Acid

(*a*) α-Picolinic acid (62 mg) was intimately mixed in a mortar with CaO (0·5 g) and heated in a stream of N_2 at 360°. Volatile products were collected in ether cooled in dry ice–acetone. Oxalic acid (60 mg) in acetone was added to the ether solution and crystallization of the resulting white precipitate from acetone yielded colorless needles of pyridine oxalate ($C_5H_5N.C_2H_2O_4$) (42 mg), m.p. 149–150°, not depressed on admixture with an authentic specimen.

(*b*) α-Picolinic acid (30 mg) was refluxed in quinoline (1 ml) in the presence of copper chromite catalyst (30 mg) for 2 hr in a stream of N_2. The evolved CO_2 was passed into a solution of $Ba(OH)_2$ affording $BaCO_3$ (33 mg).

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