$$p\text{-}ClC_6H_4CO_2H \xrightarrow[CH_3CN]{SiHCl_3;\ (C_3H_7)_3N} p\text{-}ClC_6H_4CH_2SiCl_3\ (69\%)$$

$$3,5\text{-}(CH_3)_2C_6H_3CO_2H \xrightarrow[CH_3CN]{SiHCl_3;\ (C_3H_7)_3N} 3,5\text{-}(CH_3)_2C_6H_3CH_2SiCl_3\ (51\%)$$

Again, since the products of this new reaction are benzylic silanes, a procedure has been developed which allows their cleavage by base to the corresponding toluene derivative without isolation of the intermediate silicon compound.[50] If one interrupts these reactions

$$C_6H_5CO_2H \xrightarrow[CH_3CN]{SiHCl_3} \xrightarrow[\Delta]{R_3N} \xrightarrow[aq\ CH_3OH]{KOH} C_6H_5CH_3\ (78\%)$$

$$p\text{-}ClC_6H_4CO_2H \xrightarrow[CH_3CN]{SiHCl_3} \xrightarrow[\Delta]{R_3N} \xrightarrow[aq\ CH_3OH]{KOH} p\text{-}ClC_6H_4CH_3\ (94\%)$$

$$o\text{-}C_6H_4(CO_2H)_2 \xrightarrow[CH_3CN]{SiHCl_3} \xrightarrow[\Delta]{R_3N} \xrightarrow[aq\ CH_3OH]{KOH} o\text{-}(CH_3)_2C_6H_4\ (64\%)$$

after the first step (heating with trichlorosilane in acetonitrile) and distils the product, a good yield of the acid anhydride can be obtained. Although we have shown that aromatic anhydrides can be reduced[50] in a similar fashion to benzylic silanes, one is not justified in assuming that anhydrides are intermediates in these acid reductions, since we have also shown that tribenzoyloxysilanes $[(ArCO_2)_3SiH]$ and benzoyloxychlorosilanes $[(ArCO_2)_xSiHCl_y]$ are also reduced under similar conditions to the corresponding benzylic silanes. Since it is known that anhydrides can be formed thermally[51] from acyloxysilanes and certain aroyloxysilanes, it is possible that the anhydrides obtained are formed *during* the distillation of the aroyloxy intermediates and play no significant role as intermediates in the formation of the benzylic silanes.

It would not be justifiable at this time to write detailed mechanisms for the varied "reductive silylations" described above since the necessary supporting experimental data are not yet available. We feel that in many cases, if not all of them, the trichlorosilyl anion plays an important, but as yet undefined, role. The oxygen which is removed from carbon is incorporated into a siloxane polymer. It is quite significant, we believe, that trichlorosilane alone, or in combination with certain tertiary amines, as well as hexachlorodisilane, have been used to deoxygenate phosphine oxides and sulfoxides in what mechanistically appears to be a closely related reaction.[52]

I wish to express my appreciation for the dedication and enthusiasm of my coworkers, most of whose names are included among the references. In addition, I wish to thank the National Science Foundation whose financial assistance provided support for a considerable portion of the work.

(50) R. A. Benkeser, K. M. Foley, J. M. Gaul, and G. S. Li, *J. Amer. Chem. Soc.*, **92**, 3232 (1970).

(51) K. A. Andrianov, A. A. Zhdanov, and S. A. Pavlov, *Dokl. Akad. Nauk SSSR, Ser. Khim.*, **102**, 85 (1955); J. Acton and W. Gerrard, *Research (London), Suppl.*, **8**, S55 (1955).

(52) H. Fritzsche, U. Hasserodt, and F. Korte, *Chem. Ber.*, **97**, 1988 (1964); **98**, 171, 1681 (1965); L. Horner and W. D. Balzer, *Tetrahedron Lett.*, 1157 (1965); T. H. Chan, A. Melnyk, and D. N. Harpp, *ibid.*, 201 (1969); K. Naumann, G. Zon, and K. Mislow, *J. Amer. Chem. Soc.*, **91**, 2788, 7012 (1969); G. Zon, K. E. DeBruin, K. Naumann, and K. Mislow, *ibid.*, **91**, 7023 (1969).

Biosynthesis of the Hemlock and Related Piperidine Alkaloids

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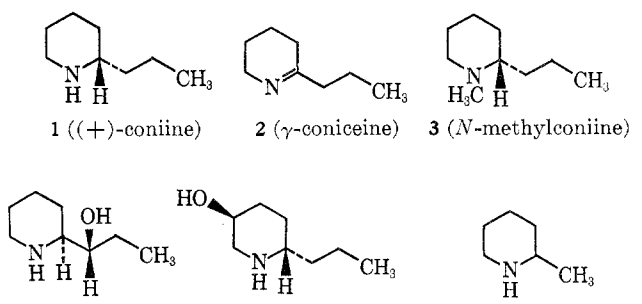
More than half of the known alkaloids contain piperidine rings.[1] However, in many of these compounds, the piperidine nucleus is fused to a carbocyclic or heterocyclic ring. Some of the simplest piperidine alkaloids are those found in the hemlock plant (*Conium maculatum*), and the structures of these are illustrated in Chart I. Most of these bases were identified a long time ago; in fact, coniine (**1**) was isolated in 1827[2] and was the first alkaloid to be synthesized.[3]

Modern methods for the isolation and separation of alkaloids (gas chromatography, thin-layer chromatography) have made it apparent that additional alkaloids of unknown structure are present in hemlock.[4-7] Also,

Chart I



(1) R. F. Raffauf, "A Handbook of Alkaloids and Alkaloid-Containing Plants," Wiley-Interscience, New York, N. Y., 1970.

(2) L. Giesecke, *Arch. Pharm. (Weinheim)*, **20**, 97 (1827).

(3) A. Ladenburg, *Ber.*, **19**, 439 (1886).

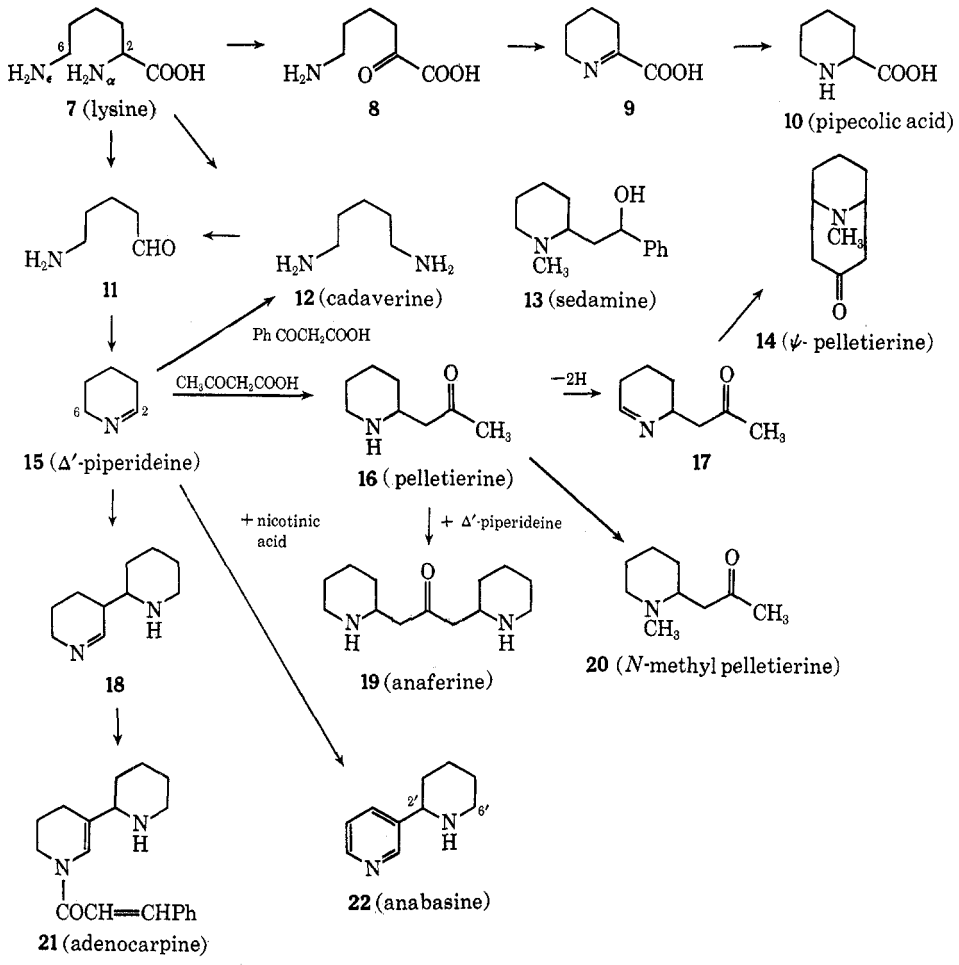
(4) B. T. Cromwell, *Biochem. J.*, **64**, 259 (1956).

(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) J. W. Fairbairn and A. A. E. R. Ali, *ibid.*, **7**, 1593 (1968).

Chart II



the proportion of the known alkaloids depends on the age of the plant,[4,6,8] the environment,[9] and the variety of *Conium maculatum*.[5] Thus the Chelsea and California varieties examined by Fairbairn[6,7] and Martin[5] contain a much higher percentage of γ-coniceine (**2**) than the Minnesota variety which we have used in most of our experiments. Coniine and γ-coniceine are relatively toxic to humans. The use of an extract of hemlock by the ancient Greeks for permanently silencing radical-liberal antiestablishmentarians, such as Socrates, is well known.

Lysine as a Precursor of Piperidine Alkaloids[10]

Robinson in his classical paper published in 1917[11] suggested that the piperidine rings of alkaloids are derived from lysine. Tracer experiments with labeled precursors have established that this biosynthetic route is correct for several alkaloids. Thus the administration of lysine-*2*-^{14}C to *Nicotiana glauca* plants led to the formation of radioactive anabasine (**22**), labeled exclusively at C-2′.[12] The nitrogen of the piperidine ring of anabasine is derived preferentially from the ε-amino group of lysine.[13] The immediate precursor of the piperidine ring seems to be Δ^1-piperideine (**15**), and anabasine formed from Δ^1-piperideine-*6*-^{14}C was labeled at C-6′.[14] Cadaverine (**12**) also served as a precursor of the piperidine ring of anabasine.[15] However, since lysine-*2*-^{14}C is incorporated into anabasine without randomization of activity between C-2′ and C-6′, cadaverine cannot be an intermediate. It is considered that the oxidation of cadaverine to Δ^1-piperideine is catalyzed by nonspecific enzymes in the plant, a transformation which has been observed in several biological systems.[16]

Other piperidine alkaloids which have been shown to arise from lysine or cadaverine are illustrated in Chart II. Tracer results are consistent with the formation of sedamine (**13**) by a condensation between Δ^1-piperideine and benzoylacetic acid (derived from phenylalanine).[17,18] Similarly pelletierine (**16**) and its *N*-methyl derivative (**20**) are plausibly formed from Δ^1-piperideine and acetoacetic acid.[19-21] Condensa-

(8) J. W. Fairbairn, *Abh. Deut. Akad. Wiss. Berlin, Kl. Chem., Geol. Biol.*, **3**, 141 (1966).
(9) We found that the amounts of conhydrine (**4**) and ψ-conhydrine (**5**) varied with the cultivation conditions: E. Leete and N. Adityachaudhury, *Phytochemistry*, **6**, 219 (1967).
(10) *Cf.* also a recent review: R. N. Gupta, *Lloydia*, **31**, 318 (1968).
(11) R. Robinson, *J. Chem. Soc.*, **111**, 876 (1917).
(12) E. Leete, *J. Amer. Chem. Soc.*, **78**, 3520 (1956).
(13) E. Leete, E. G. Gros, and T. J. Gilbertson, *ibid.*, **86**, 3907 (1964).
(14) E. Leete, *ibid.*, **91**, 1697 (1969).
(15) E. Leete, *ibid.*, **80**, 4393 (1958).
(16) K. Hasse and G. Schmid, *Biochem. Z.*, **337**, 69 (1963).
(17) R. N. Gupta and I. D. Spenser, *Can. J. Chem.*, **45**, 1275 (1967).
(18) R. N. Gupta and I. D. Spenser, *J. Biol. Chem.*, **244**, 88 (1969).

tions of this type have been achieved *in vitro*.[22] ψ-Pelletierine (**14**) is considered to arise *via* a dehydropelletierine (**17**), and recent tracer results are consistent with this hypothesis.[21,23] Anaferine (**19**) results from the condensation of pelletierine with a second molecule of Δ^1-piperideine.[21] Adenocarpine (**21**) is formed by the dimerization of Δ^1-piperideine to tetrahydroanabasine (**18**), followed by cinnamoylation of its enamine tautomer.[24]

Pipecolic acid (**10**) is formed from lysine in *Phaseolus vulgaris* (French kidney bean) and other species[18,25,26] *via* ε-amino-α-ketocaproic acid (**8**) and Δ^1-piperideine-2-carboxylic acid (**9**). The claim[27] that the nitrogen of pipecolic acid is formed preferentially from the α-amino group of lysine is apparently not substantiated by this recent work and needs further investigation. Until recently it was generally assumed that Δ^1-piperideine was formed by the decarboxylation of Δ^1-piperideine-2-carboxylic acid. However Spenser[25] has now discovered that lysine-*6*-^{14}C,*2*-*t* is incorporated into anabasine, *N*-methylpelletierine, and sedamine with retention of most of the tritium relative to the carbon-14, which makes this hypothesis untenable. We suggest that the retention of tritium at C-2 can be rationalized by postulating that 5-aminopentanal (**11**) is formed directly from lysine by a concerted decarboxylation and deamination of the α-amino group. Spenser has suggested an alternate hypothesis[25] in which the lysine is incorporated *via* N^{ϵ}-methyllysine and *N*-methylcadaverine.

Our initial experiments on the biosynthesis of the hemlock alkaloids were influenced by the ideas of Robinson, and it seemed plausible that coniine would be formed by reduction of pelletierine. However the feeding[28] of lysine-*2*-^{14}C,[29] cadaverine-*1*,*5*-$^{14}C_2$,[29] or Δ^1-piperideine-*6*-^{14}C[30] to hemlock plants failed to yield alkaloids containing a significant amount of radioactivity.[31]

(19) R. N. Gupta and I. D. Spenser, *Chem. Commun.*, 75 (1968); *Phytochemistry*, **8**, 1937 (1969).

(20) D. G. O'Donovan and M. F. Keogh, *Tetrahedron Lett.*, 265 (1968).

(21) M. F. Keogh and D. G. O'Donovan, *J. Chem. Soc. C*, 1792 (1970).

(22) C. Schöpf, F. Braun, and A. Komzak, *Ber.*, **89**, 1821 (1956).

(23) H. W. Liebisch, N. Marekov, and H. R. Schütte, *Z. Naturforsch. B*, **23**, 1116 (1968).

(24) H. R. Schütte, K. L. Kelling, D. Knofel, and K. Mothes, *Phytochemistry*, **3**, 249 (1964).

(25) R. N. Gupta and I. D. Spenser, *ibid.*, **9**, 2329 (1970); also private communication from Professor Spenser, McMaster University.

(26) J. A. Grove, T. J. Gilbertson, R. H. Hammerstedt, and L. M. Henderson, *Biochim. Biophys. Acta*, **184**, 329 (1969).

(27) H. R. Schütte and G. Seelig, *Z. Naturforsch. B*, **22**, 824 (1967).

(28) In our initial experiments hemlock plants were grown in hydroponics and the labeled compounds were added to the aqueous aeriated nutrient solution in which the roots of the plants were growing. All of our recent feedings have been carried out by the "wick method." A cotton wick is threaded through the stem of the plant near ground level and the ends are dipped into a small beaker containing an aqueous solution of the tracer. The uptake of the solution into the plant is rapid, and usually less than 3% of the labeled compound remains in the beaker after 24 hr.

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

(30) E. Leete, *ibid.*, **92**, 3835 (1970).

(31) B. T. Cromwell and M. F. Roberts, *Phytochemistry*, **3**, 369 (1964), reported an extremely high (4–37%) incorporation of Δ^1-piperideine-*U*-^{14}C and Δ^1-piperideine-2-carboxylic acid-*U*-^{14}C (**9**) into γ-coniceine. However Dr. Roberts, in a private communication, has informed me that these observations were not reproducible, and when the γ-coniceine, isolated from plants fed Δ^1-piperideine-*6*-^{14}C, was carefully purified, it had no activity.

Acetic Acid as a Precursor of Piperidine Alkaloids

Since we were unable to obtain the incorporation of lysine or its metabolites into coniine, we were led to consider another hypothesis for the origin of coniine. It was proposed[32] that coniine is formed from the β-polyketo acid **23** produced by the linear combination of four acetate units, as illustrated in Chart III. This hypothesis was tested by feeding sodium acetate-*1*-^{14}C to hemlock plants. A significant amount of activity was found in coniine and conhydrine, and a systematic degradation of these alkaloids indicated that almost all the activity was indeed located on the even numbered, alternate, carbon atoms and was equally divided between these four positions.[29]

Pinidine (**25**) was another alkaloid which was plausibly derived from a poly-β-keto acid such as **24**, formed from five acetate units. The feeding of acetate-*1*-^{14}C to *Pinus jeffreyi* resulted in the formation of labeled pinidine which was labeled on alternate carbons, as illustrated in Chart III.[33] We also found that lysine-*2*-^{14}C served as a precursor of pinidine; however it was a less efficient precursor of the alkaloid than acetate-*1*-^{14}C. We thus considered that the lysine was being catabolized to acetate,[34] as illustrated in Chart III, prior to its incorporation into pinidine. A partial degradation of the pinidine derived from lysine *2*-^{14}C confirmed this, the pattern of labeling being the same as that obtained with acetate-*1*-^{14}C.

Some time ago Schiedt and Hoss[35] reported that L-lysine-*U*-^{14}C, when fed to hemlock, yielded radioactive coniine. However no degradations were carried out to determine the distribution of activity in the alkaloid, and their results can be rationalized by postulating catabolism of the lysine to acetate.[36] Another piperidine alkaloid whose biosynthesis has been investigated with tracers is carpaine (**26**),[37] and it was found that acetate

(32) Our first thoughts on this subject were written in 1959 in a chapter entitled, "The Use of Isotopes in the Study of Alkaloid Biogenesis," which was to appear in a book, "The Biogenesis of Natural Substances," edited by M. Gates and references to this material (which was widely circulated) have even appeared in the literature: N. J. Leonard and S. W. Blum, *J. Amer. Chem. Soc.*, **82**, 503 (1960). This book was abandoned because some of the contributors failed to come forth with promised manuscripts. Our hypothesis on the biosynthesis of coniine from acetate thus first appeared in "Biogenesis of Natural Compounds," P. Bernfeld, Ed., Pergamon Press, 1963, p 751. A similar scheme was suggested by Büchi for the origin of muscopyridine (K. Biemann, G. Büchi, and B. H. Walker, *J. Amer. Chem. Soc.*, **79**, 5558 (1957)). A. R. Battersby, *Quart. Rev., Chem. Soc.*, **15**, 259 (1961), also considered the formation of coniine from acetate plausible.

(33) E. Leete and K. N. Juneau, *J. Amer. Chem. Soc.*, **91**, 5614 (1969).

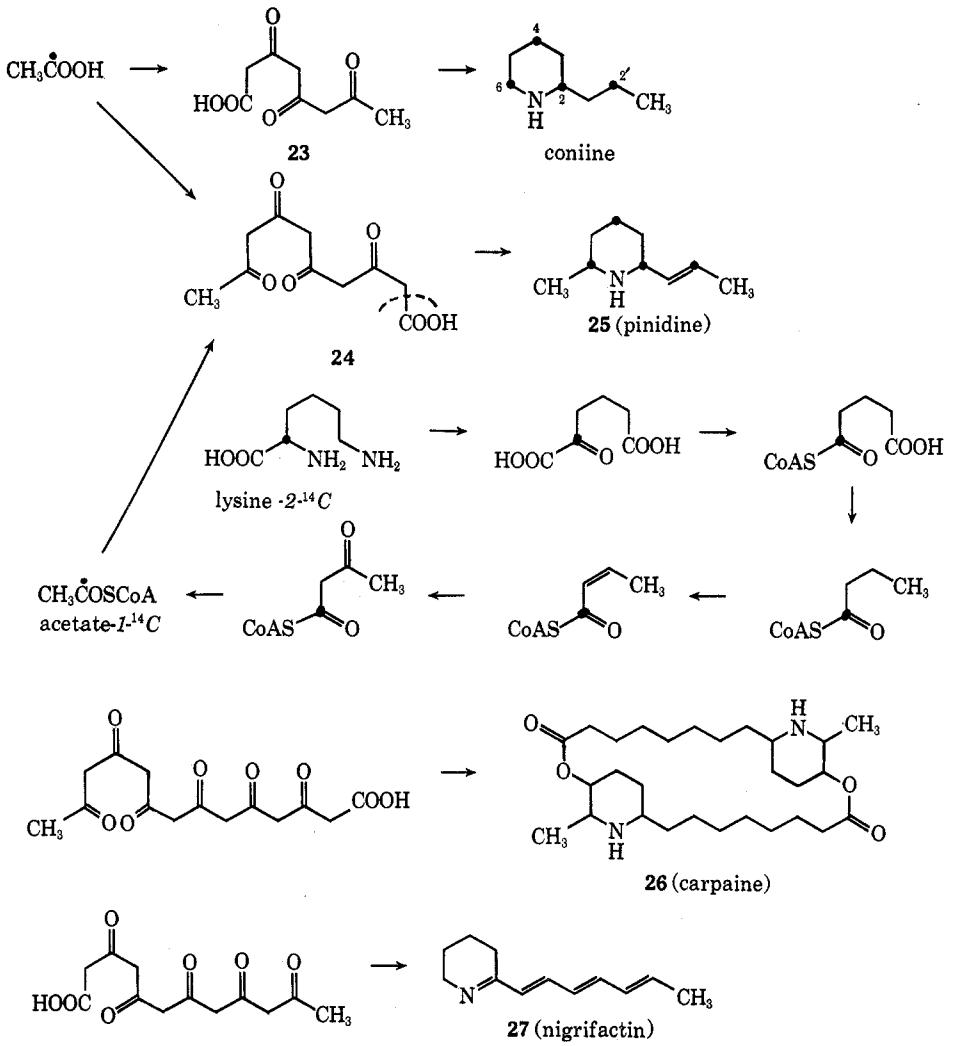
(34) *Cf.* I. D. Spenser, *Compr. Biochem.*, **20**, 256 (1968).

(35) U. Schiedt and H. G. Hoss, *Z. Naturforsch. B*, **13**, 691 (1958); *Z. Physiol. Chem.*, **330**, 74 (1962).

(36) At the 7th International Symposium on the Chemistry of Natural Products, Riga, USSR, June 21–28, 1970, H. W. Liebisch reported during a discussion of our paper (Abstracts, p 542) that he and his colleagues in Halle, DDR, had obtained radioactive coniine from lysine-*2*-^{14}C. The alkaloid was degraded and found to have activity in the propyl group. The distribution of activity between the piperidine ring and the side chain was consistent with the catabolism of lysine to acetate prior to incorporation of activity into coniine. By the use of lysine labeled with ^{15}N on the α or ε position it was established that both amino groups were equally efficient as precursors of the nitrogen in coniine.

(37) C. W. Bevan and A. U. Ogan, *Phytochemistry*, **3**, 591 (1964).

Chart III



was a more efficient precursor than lysine, consistent with its formation from two 14-carbon poly-β-keto acids, as illustrated in Chart III. It also seems likely that the alkaloid nigrifactin (**27**), produced by a *Streptomyces* species,[38] is derived from acetate.

Having discovered the specific incorporation of acetate-*1*-^{14}C into coniine, we then turned our attention to the later stages of the biosynthesis of the alkaloid. The work of Fairbairn[6,8] indicated that γ-coniceine played a major role in the biosynthesis of the other hemlock alkaloids; his results suggested that γ-coniceine was a precursor of coniine. We confirmed this by feeding γ-coniceine-*1'*-^{14}C to hemlock when it was converted to coniine (9.9% specific incorporation) and ψ-conhydrine (4.15% specific incorporation). These transformations were direct since it was shown that the compounds were labeled specifically at their C-1′ positions.[9]

The biosynthesis of the hemlock alkaloids has been investigated using carbon-^{14}C dioxide.[5] By comparing the specific activities of the alkaloids with time periods of 0 to 7 days after exposure of the plants to $^{14}CO_2$, Martin found that the sequence for the formation of the alkaloids was: γ-coniceine → coniine → *N*-methylconiine. The hydroxylated alkaloids were produced at a much slower rate.

We have investigated the dehydrogenation of coniine to γ-coniceine in hemlock and found that it is stereospecific.[39] Coniine-*2'*-^{14}C was prepared by the catalytic reduction of 2-propylpyridine obtained by reaction of the lithium salt of α-picoline with ethyl-*1*-^{14}C bromide. The (±)-coniine-*2'*-^{14}C was readily resolved with mandelic acid.[40] The incorporation of the natural (+)-coniine-*2'*-^{14}C into γ-coniceine (isolated as *N*-benzoyl-5-oxooctylamine) was about ten times more efficient than the incorporation of (−)-coniine-*2'*-^{14}C. Degradation of the radioactive γ-coniceine derived from (+)-coniine-*2'*-^{14}C indicated that essentially all the activity was located at the C-2′ position. The recovered radioactive (+)-coniine from the plant had also suffered no randomization of activity. Fairbairn[41] has also observed that coniine-*U*-^{14}C is metabolized to γ-coniceine.

(38) T. Terashima, Y. Kuroda, and Y. Kaneko, *Tetrahedron Lett.*, 2535 (1969).

(39) E. Leete and J. O. Olson, unpublished work.

(40) J. C. Craig, University of California, San Francisco, private communication.

(41) J. W. Fairbairn and A. A. E. R. Ali, *Phytochemistry*, **7**, 1599 (1968).

Table I

Compd fed to *Conium maculatum* var. "Minnesota"	Duration of feeding, days	Abs incorpn of act. into alkaloids, %	Spec incorpn of act. into coniine,[a] %	% distribution of act. in coniine 6	5	4	3	2	1′	2′	3′	Ref
Sodium acetate-*1*-^{14}C	1	0.035	0.009							24.4	0.7	49
	8	0.035		24	1	21	1.6	26	1.3	22	1	29
Sodium acetate-*2*-^{14}C	1	0.17	0.04							9.8	15.1	49
	14	0.15		12.3	13.0	12.5				12.2	13.2	30
Octanoic-*1*-^{14}C acid	1	0.45		93						0.60	0.33	30
	7	0.23		85						3.2	1.1	30
Octanoic-*7*-^{14}C acid	1	0.30	0.07	0.6						93	1.0	49
Octanoic-*8*-^{14}C acid	1	0.45		6	9					6	48	30
	7	0.89		10	9					9	28	30
Hexanoic-*1*-^{14}C acid	7	0.34		1.4	0.9	92				1.6	0.9	49
5-Oxooctanoic-*6*-^{14}C acid	1	3.6	0.61	←		<2		→	>95	←	<2 →	51
5-Oxooctanal-*6*-^{14}C	1	6.8	1.1	←		<1		→	>95	←	<2 →	51

[a] The specific incorporation (specific activity of coniine/specific activity of precursor) of activity into coniine is not a meaningful measure of the efficiency of a certain compound to serve as a precursor, unless equivalent molar amounts of the precursors are fed to same weights of plants. The specific incorporations reported in the table satisfied these conditions, *i.e.*, 0.25 m*M* of the labeled precursor was fed to six hemlock plants which had a fresh weight at the time of harvesting of 600–700 g.

Intermediates between Acetate and Coniine: Octanoic Acid

We then concerned ourselves with intermediates between acetic acid and γ-coniceine. There are a large number of natural products which are formally derived from poly-β-keto acids, and these have been referred to as polyketides[42] or acetogenins.[43] It has been established by means of tracer experiments that many of these compounds are in fact derived from acetic acid, but only in a few cases have poly-β-keto acids been isolated as intermediates.[44] It is generally assumed that these poly-β-ketones do not exist in the free state, but are protected possibly by reaction with SH groups in proteins yielding thioketals.[43]

5-Oxooctanoic acid[45] was a likely candidate as an intermediate between acetate and the hemlock alkaloids. However, prior to the synthesis of this compound labeled with ^{14}C, it was decided to feed octanoic-*1*-^{14}C acid to hemlock.[30] It is hard to give a rational justification for even considering this as a precursor of coniine. However, it is commercially available, and at the very worst one might learn something about the metabolism of fatty acids in hemlock. It was thus very exciting to discover that octanoic-*1*-^{14}C acid served as an excellent precursor of coniine (*cf.* Table I); furthermore the alkaloid isolated 24 hr after the feeding was labeled almost exclusively (93%) at C-6, suggesting that the acid was incorporated intact, without any prior degradation to two-carbon units. Even when the plants were allowed to grow for 7 days after feeding octanoic-*1*-^{14}C acid most of the activity of the isolated coniine was still located at C-6.

In order to substantiate this surprising result we fed octanoic-*8*-^{14}C acid to hemlock and obtained less specific labeling of the coniine. Coniine isolated 24 hr after feeding this precursor had only 48% of its activity located at C-3′, the position expected to be derived from C-8 of octanoic acid. The rest of the activity was apparently fairly uniformly distributed through the rest of the molecule. Plants allowed to grow for 7 days after feeding octanoic-*8*-^{14}C acid yielded coniine which was even more uniformly labeled; however there was still a significant excess of activity at C-3′ (28%). When we obtained these results we offered two explanations of the labeling patterns obtained with octanoic-*1*- and -*8*-^{14}C acid.[46] We suggested that the octanoic-*8*-^{14}C acid was somehow being degraded from its methyl end, affording an acetate unit which would be labeled on its methyl group. This acetate-*2*-^{14}C could then enter the Krebs cycle (see Chart V) affording oxaloacetate labeled at C-2 and C-3.[47] The acetate formed by oxidative decarboxylation of pyruvate derived from this oxaloacetate will thus be labeled equally on both carbons. Incorporation of this acetate into coniine would then afford a uniformly labeled alkaloid. Coniine

(42) A. J. Birch, *Proc. Chem. Soc., London*, 3 (1962); *Annu. Rev. Plant Physiol.*, **19**, 321 (1968).

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

(44) 3,4-Diketohexanoic acid (triacetic acid) has been obtained by the enzyme-catalyzed combination of 1 mole of acetyl coenzyme A with 2 moles of malonyl coenzyme A: J. D. Brodie, G. Wisson, and J. W. Porter, *J. Biol. Chem.*, **239**, 1346 (1964). Triacetic lactone (4-hydroxy-6-methyl-2*H*-pyran-2-one) has been isolated from cultures of *Penicillium patulum*, a mold which also produces 6-methylsalicylic acid: T. M. Harris, C. M. Harris, and R. J. Light, *Biochim. Biophys. Acta*, **121**, 420 (1966); P. Dimroth, H. Walter, and F. Lynen, *Eur. J. Biochem.*, **13**, 98 (1970).

(45) Interestingly this compound was first obtained by the oxidation of coniine with hydrogen peroxide: R. Wolffenstein, *Ber.*, **28**, 1459 (1895).

(46) One referee of our manuscript[30] had the audacity to suggest that the octanoic-*8*-^{14}C acid was not radiochemically pure, and this was the reason for the randomization of activity found in the coniine. However it was established by degradation that all the ^{14}C was at C-8 in the administered octanoic acid.

(47) This is the pattern of labeling obtained after operation of the Krebs cycle for one time. Subsequent cycles lead to labeling of the carboxyl groups of the four-carbon acids of the cycle, with an ultimate steady-state distribution of activity: 66.7% on the central carbons and 33.3% on the carboxyl groups.[34]

isolated from plants which had been fed acetate-*2*-^{14}C for 2 weeks was in fact uniformly labeled. We also proposed that coniine-3′-^{14}C derived from octanoic-*8*-^{14}C acid was metabolized in the plant, yielding acetate-*2*-^{14}C from the side chain. This latter explanation was shown to be untenable, since the radioactive coniine reisolated (after 5 days) from a plant which had been fed (+)-coniine-*2*′-^{14}C was labeled only at the C-2′ position.[39]

In order to test our first explanation we fed octanoic-*7*-^{14}C acid to hemlock, fully expecting some general labeling of the even numbered carbons of coniine. Actually almost all the activity was located at C-2′ (93%)! The first clue to what was happening was obtained by an examination of the aqueous alkaline extract[48] of the plants which had been fed octanoic-*1*-^{14}C acid. This alkaline solution was acidified with hydrochloric acid and extracted in a continuous extractor with ether. One of the major components of this extract was found to be fumaric acid (*ca.* 100 mg from 100 g of fresh plant). The incorporation of activity into fumaric acid isolated from hemlock plants 24 hr after feeding octanoic-*1*-^{14}C acid was very high (4.1%). Degradation of the fumaric acid indicated that essentially all (99.8%) of the activity was located on the carboxyl groups. Fumaric acid isolated 7 days after feeding octanoic-*1*-^{14}C acid had lower activity (0.94% incorporation), but activity was still almost all on the carboxyl groups (only 1.2% at C-2 + C-3). Thus, contrary to our initial thoughts, the octanoic-*1*-^{14}C acid does undergo β oxidation in the plant to afford acetate-*1*-^{14}C which then enters the Krebs cycle, resulting in labeling of the carboxyl groups of fumaric acid. Octanoic-*8*-^{14}C acid by consecutive β oxidations ultimately affords acetate-*2*-^{14}C.

We can explain our results if we postulate 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. An examination of Table I indicates that this is indeed the case. Both the specific and absolute incorporation of acetate-*2*-^{14}C into coniine is from four to five times greater than that of acetate-*1*-^{14}C.[49] The absolute incorporation of acetate-*2*-^{14}C into the total alkaloids (0.15–0.17%) was not much less than that of the octanoic-^{14}C acids (0.23–0.89%); thus acetate-*2*-^{14}C derived from octanoic-*8*-^{14}C acid will be able to compete effectively with the octanoic acid as a source of the carbons of coniine. On the other hand the acetate-*1*-^{14}C formed by the β oxidation of octanoic-*1*- or -*7*-^{14}C acid is a much poorer precursor of coniine and results in less general labeling of the alkaloid. We can rationalize the lower efficiency of acetate-*1*-^{14}C as a precursor of coniine by the fact that its entrance into the Krebs cycle results in loss of the labeled carbon as carbon dioxide. On the other hand the entrance of acetate-*2*-^{14}C into the cycle ultimately affords uniformly labeled acetate as previously described, both carbons being then available for the formation of coniine.

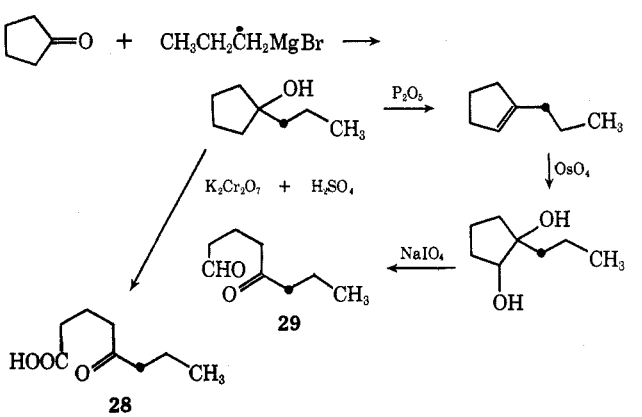
Examination of the fumaric acid isolated from hemlock plants which had been fed acetate-*1*- and -*2*-^{14}C for 24 hr substantiated these ideas. The fumaric acid isolated from the acetate-*2*-^{14}C feeding experiment had 35% of its activity on the carboxyl groups and 65% on the central carbons. The coniine isolated from this particular experiment had approximately the same kind of distribution of activity on alternate carbons (C-2′ and C-3′) derived from the carboxyl and methyl groups of acetate, respectively. As expected, the fumaric acid obtained after feeding acetate-*1*-^{14}C for 24 hr had all its activity on the carboxyl groups.

Another surprising result was obtained when hexanoic-*1*-^{14}C acid was fed to hemlock.[49] The resultant radioactive coniine had the major portion of its activity at C-4. Thus chain elongation of hexanoic acid is apparently possible in this higher plant, presumably by reaction of hexanoyl coenzyme A with malonyl coenzyme A. In other biological systems the utilization of fatty acids without prior breakdown to acetate is rare.[50]

5-Oxooctanoic Acid and 5-Oxooctanal: The Immediate Precursors of Coniine

We then returned to an examination of 5-oxooctanoic acid (**28**) and 5-oxooctanal (**29**) as possible precursors of coniine. They were prepared, labeled with ^{14}C at C-6 by the route illustrated in Chart IV. Equivalent amounts of these two compounds were fed to hemlock plants for 24 hr and the compounds were found to be excellent precursors of the alkaloids,[51] the incorporation of the keto aldehyde being significantly higher than that of the keto acid. This result is consistent with the keto aldehyde being the more immediate precursor of the

Chart IV



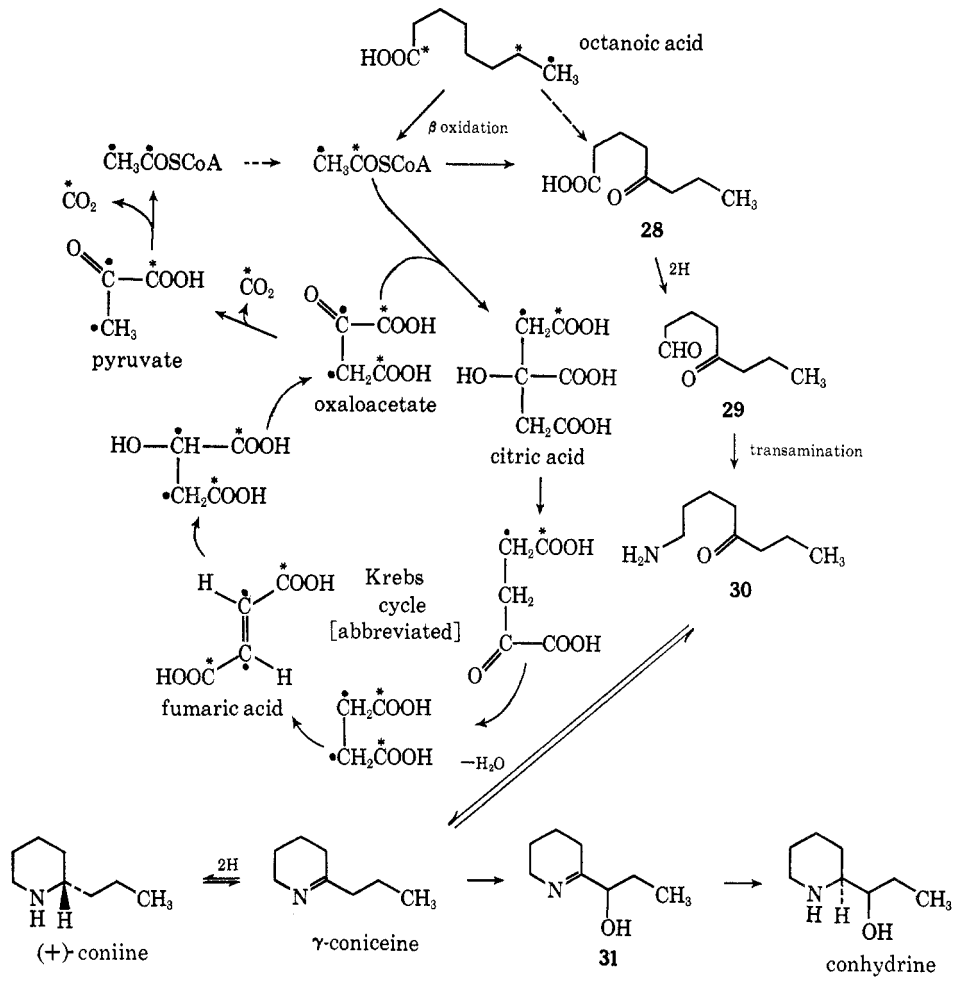
(48) The fresh plants are mascerated in a Waring Blendor with a mixture of chloroform and aqueous 5% sodium hydroxide. The alkaloids are found in the chloroform layer, and it is the aqueous layer which is referred to here.

(49) E. Leete, unpublished work.

(50) The chain elongation of fatty acids containing 8 to 14 carbons has been observed in barley seedlings: J. C. Hawke and P. K. Stumpf, *J. Biol. Chem.*, **240**, 4746 (1965). Another example is the utilization of butyric-*1*-^{14}C acid for the biosynthesis of the phloroglucinol derivative, margaspidin, in the fern, *Dryopteris marginalis:* P. G. Gordon, A. Penttila, and H. M. Fales, *J. Amer. Chem. Soc.*, **90**, 1376 (1968).

(51) E. Leete and J. O. Olson, *Chem. Commun.*, 1651 (1970).

Chart V



alkaloids. Degradation of the isolated coniine established that essentially all the activity was located at the expected position C-1′.

We consider that the next step in the biosynthesis of coniine involves a transamination of the aldehyde group of 5-oxooctanal to yield 5-oxooctylamine (**30**) which is the open-chain form of γ-coniceine. This is a reaction which we plan to study at the enzyme level in a cell-free system in the hope that we can learn something of the origin of the nitrogen in coniine. Very few of the reactions leading to the formation of alkaloids have been examined by enzymologists.[52]

We can now question the role of octanoic acid in the biosynthesis of the hemlock alkaloids. It does not seem reasonable that octanoic acid would be synthesized in the plant from acetate by steps which certainly must involve reductions, and then undergo an oxidation to 5-oxooctanoic acid prior to the formation of coniine. We feel that octanoic acid is not on the direct route to coniine; however, its observed good incorporation into coniine indicates that there are probably enzymes in the plant capable of catalyzing the oxidation at C-5. Evidence that this occurs was obtained by adding inactive 5-oxooctanoic acid to the aqueous alkaline extract[48] of hemlock plants which had been fed octanoic-*1*-^{14}C acid for 24 hr. The reisolated 5-oxooctanoic acid was radioactive (0.09% incorporation) and had most of its activity on the carboxyl group, strongly indicative of its direct formation from the octanoic-*1*-^{14}C acid.[51]

The apparent oxidation of octanoic acid at C-5 may seem a little unusual; however, it should be pointed out that oxidations remote from any activating groups occur quite often in biological systems, for example, the conversion of stearic to oleic acid.[53] Chemical oxidations at positions remote from activating groups recently have been achieved by Breslow.[54] The alkaloids tomatidine (**32**) and solanidine (**33**) contain piperidine rings, and it has been established that they are formed from cholesterol.[55,56] Here, also, it is clear that some kind of oxidation of the alkyl side chain of cholesterol must occur prior to the incorporation of the nitrogen.

Chart V summarizes our present thoughts on the biosynthesis of coniine. The reversibility of the final steps in this scheme have already been mentioned. We are examining the fate of coniine labeled with both ^{15}N and ^{14}C in order to discover whether the nitrogen can be

(52) E. Leete, *Advan. Enzymol.*, **32**, 373 (1969).

(53) D. K. Bloomfield and K. Bloch, *J. Biol. Chem.*, **235**, 337 (1960).

(54) R. Breslow and M. Winnik, *J. Amer. Chem. Soc.*, **91**, 3083 (1969); R. Breslow and S. W. Baldwin, *ibid.*, **92**, 732 (1970).

(55) E. Heftmann, E. R. Lieber, and R. D. Bennett, *Phytochemistry*, **6**, 225 (1967).

(56) R. Tschesche and H. Hulpke, *Z. Naturforsch. B*, **21**, 893 (1966); **22**, 791 (1967).

lost by a reversal of earlier steps in this biosynthetic pathway, *i.e.*, the transamination reaction involving 5-oxooctanal. 2-Methylpiperidine (**6**), a minor alkaloid of hemlock[4] and certain *Pinus* species,[57] is plausibly formed in an analogous manner from 5-oxohexanoic acid.

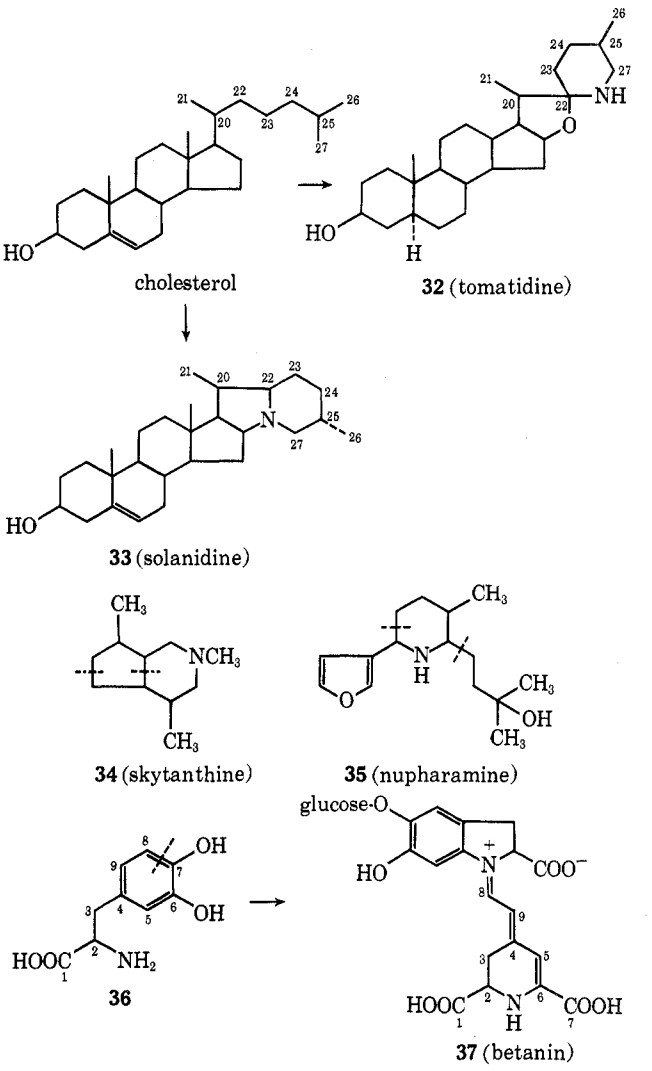
Little has been said about the origin of the hydroxylated alkaloids of hemlock, namely conhydrine and ψ-conhydrine. Compounds such as (**31**) may be intermediates between γ-coniceine and conhydrine. In fact the yield of conhydrine from hemlock is increased if the crude alkaloids are hydrogenated prior to separation,[49] favoring the presence of an unsaturated compound such as **31**.

Other Biosynthetic Routes to Piperidine Rings

In conclusion it should be mentioned that there are other known routes whereby piperidine rings are made in nature (Chart VI). Thus the alkaloids skytanthine (**34**) and nupharamine (**35**) are probably mono- and sesquiterpenes, respectively. It has been established that the former alkaloid is derived from mevalonic acid.[58] The dotted lines on the structures **34** and **35** indicate the dissection into isoprene units. The Δ^2-piperideine ring in betanin (**37**) is formed from dopa (**36**) by a sequence of reactions involving the opening of the catechol ring,[59] the atoms being numbered to illustrate the derivation of the carbons in betanin from dopa. A large group of alkaloids which contain the piperidine ring are those found in *Lycopodium* species, and it is considered that these are dimers of pelletierine.[60]

(57) W. H. Tallent, V. L. Stromberg, and E. C. Horning, *J. Amer. Chem. Soc.*, **77**, 6361 (1955).
(58) H. Auda, H. R. Juneja, E. J. Eisenbraun, G. R. Waller, W. R. Keys, and H. H. Appel, *ibid.*, **89**, 2476 (1967).
(59) H. E. Miller, H. Rösler, A. Wohlpart, H. Wyler, M. E. Wilcox, H. Frohfer, T. J. Mabry, and A. S. Drieding, *Helv. Chim. Acta*, **51**, 1470 (1968).
(60) R. N. Gupta, M. Castillo, D. B. MacLean, I. D. Spenser, and J. T. Wrobel, *J. Amer. Chem. Soc.*, **90**, 9360 (1968); M. Castillo, R. N. Gupta, D. B. MacLean, and I. D. Spenser, *Can. J. Chem.*, **48**, 1893 (1970); M. Castillo, R. N. Gupta, Y. K. Ho, D. B. MacLean, and I. D. Spenser, *J. Amer. Chem. Soc.*, **92**, 1074 (1970); *Can. J. Chem.*, **48**, 2911 (1970).

Chart VI



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Kinetic Studies of Hydrogen-Bonded Solvation Complexes of Amines in Water and Hydroxylic Solvents

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The familiar formula, NH_4OH, for ammonia in aqueous solution is correct in at least one respect: kinetic studies of proton exchange show clearly that amines form hydrates in aqueous solution. Thus, when the conjugate acid BH^+ of an amine B undergoes acid dissociation, the process is best represented by eq 1.