Biosynthesis of Pinidine[1]

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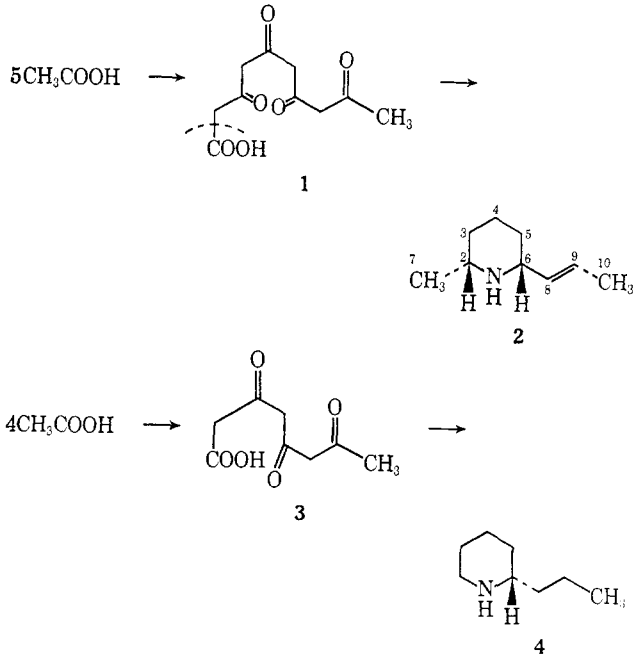
Abstract: The administration of sodium acetate-1-^{14}C to *Pinus jeffreyi* plants afforded radioactive pinidine (2-methyl-6-(2-*trans*-propenyl)piperidine). A systematic degradation of the alkaloid indicated that almost all the radioactivity was located on carbon atoms 2, 4, 6, and 9, and was equally distributed between these positions. This result favors the hypothesis that this piperidine alkaloid is derived from a ten-carbon chain formed by the linear combination of five acetate units. Pinidine isolated from plants which had been fed *dl*-lysine-2-^{14}C was also radioactive; however, a partial degradation of this labeled pinidine indicated that the activity was not confined to the piperidine ring. The pattern of labeling was consistent with catabolism of the lysine-2-^{14}C to acetic acid-1-^{14}C which was then incorporated as previously postulated.

Pinidine was isolated by Tallent, *et al.*,[2] from *Pinus sabiniana*, *jeffreyi*, *torreyana*, and some related species of pine in 1955. They established its structure as 2-methyl-6-(2-propenyl)piperidine[3] and later Hill, *et al.*,[4] determined the absolute stereochemistry to be 2-(*R*)-methyl-6(*R*)-(2-*trans*-propenyl)piperidine as illustrated in Scheme I, formula **2**. Tallent[2] pointed out that those species of pine which contain an appreciable amount of pinidine are unusual in that they do not contain bicyclic terpenes; both α- and β-pinenes and Δ^3-carene are absent from the turpentine fraction of these species. It was suggested that a biochemical relationship might exist between the formation of pinidine and the saturated aliphatic hydrocarbons, heptane, nonane, and undecane, found in these species.[5] Sanderman and Schweers[6] showed that the heptane found in *P. jeffreyi* was derived from acetic acid, the even-numbered carbons becoming labeled after the administration of sodium acetate-1-^{14}C to the plant. We suggested[7] that pinidine is formed by the linear combination of five acetate units as illustrated in Scheme I. Loss of the carboxyl group from the intermediate poly-β-keto acid **1**, reaction with a nitrogen source, and plausible reductions and dehydrations could afford pinidine. It has been established that the hemlock alkaloid, coniine (**4**), can be formed by the linear combination of four acetate units.[8] Nonane and heptane would be plausibly formed by reduction and decarboxylation of the hypothetical poly-β-keto acids **1** and **3**, respectively.

Scheme I. Biosynthetic Route to Pinidine and Coniine



We have now tested this hypothesis for the origin of pinidine by feeding acetate-1-^{14}C to *Pinus jeffreyi*. In our initial experiments the pine needles were removed from the plant and the cut ends placed in a solution of sodium acetate-1-^{14}C. Although the tracer was absorbed by the needles, negligible activity was detected in the pinidine isolated 2 weeks later. Lack of incorporation may possibly be due to the fact that the feeding was carried out in December when little growth was taking place. Subsequent feedings were carried out by the wick method. The highest incorporation (0.02%) of activity into pinidine was obtained when the plants were fed in August and allowed to grow for an additional 11 weeks before harvesting (see Table I, Experimental Section). The radioactive pinidine was degraded as illustrated in Scheme II in which activity was determined on all nine carbon atoms. Pinidine formed from acetate-1-^{14}C would be expected to have activity at C-2, 4, 6, and 9 if the hypothesis illustrated in Scheme I is correct. Oxidation of pinidine with potassium permanganate yielded acetic acid derived from C-9 and 10. The double bond of pinidine was also oxidized by treatment with osmium tetroxide to yield the 8,9-diol which was cleaved with sodium metaperiodate affording acetaldehyde. Attempts to isolate 6-methylpiperidine-2-

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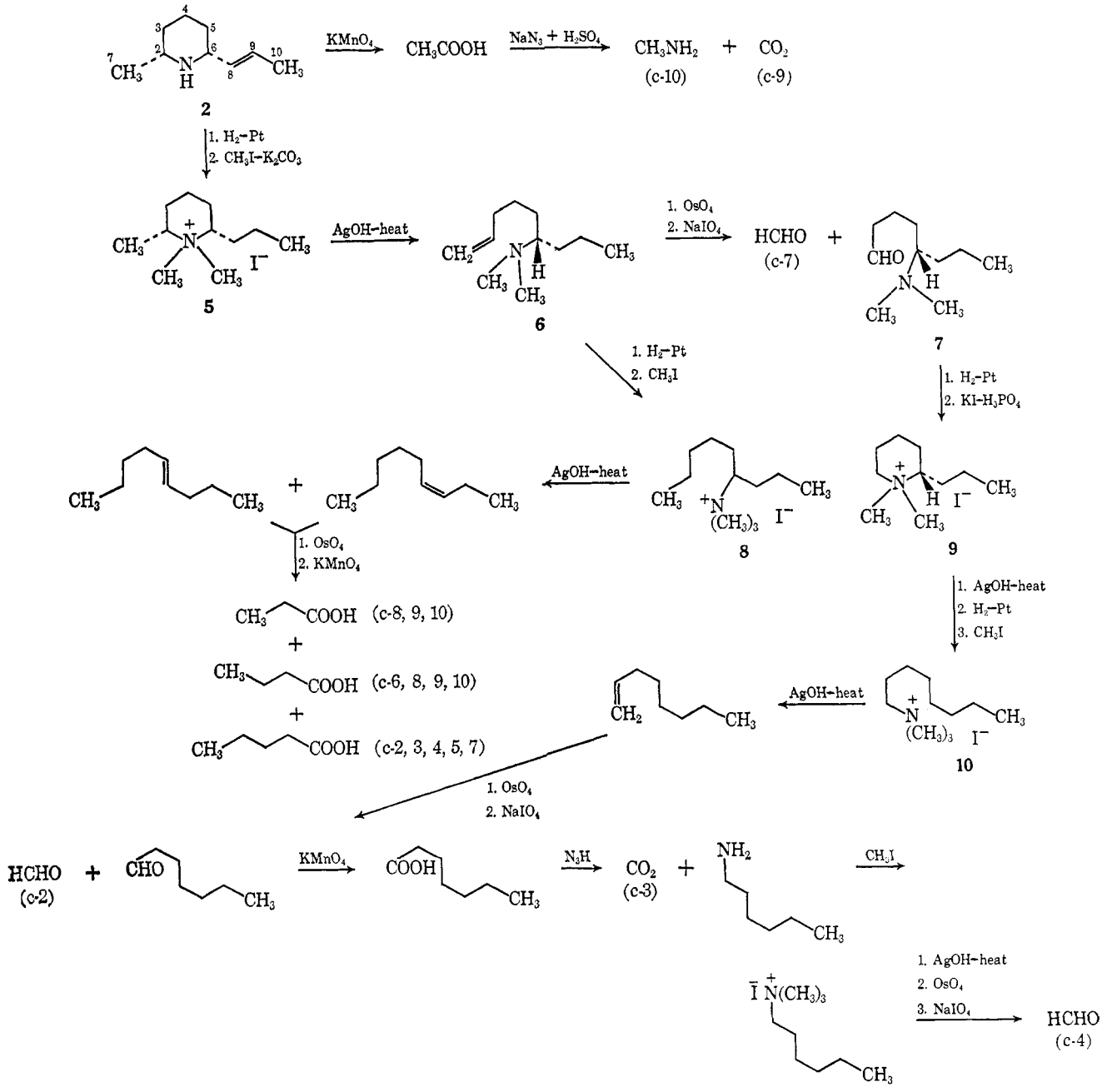
(2) W. H. Tallent, V. L. Stromberg, and E. C. Horning, *J. Am. Chem. Soc.*, 77, 6361 (1955).
(3) W. H. Tallent and E. C. Horning, *ibid.*, 78, 4467 (1956).
(4) R. K. Hill, T. H. Chan, and J. A. Joule, *Tetrahedron*, 21, 147 (1965).
(5) A. J. Haagen-Smit, C. T. Redemann, and N. T. Mirov, *J. Am. Chem. Soc.*, 60, 2014 (1948); *cf.* also W. Karrer, "Konstitution und Vorkommen der organischen Pflanzenstoff," Birkhäuser Verlag, Basel, 1958.
(6) W. Sanderman and W. Schweers, *Ber.*, 93, 2266 (1960).
(7) E. Leete in "Biogenesis of Natural Compounds," P. Bernfeld, Ed., Pergamon Press, Oxford, 1963, Chapter 17, p 753.
(8) E. Leete, *J. Am. Chem. Soc.*, 85, 3523 (1963); *ibid.*, 86, 2509 (1964).

Scheme II. Degradation of the Radioactive Pinidine



aldehyde, presumably formed in this oxidation, were not successful. Oxidation of the acetaldehyde with chromic acid yielded acetic acid, assayed as its α-naphthylamide.[9] A Schmidt reaction on the acetic acid yielded carbon dioxide collected as barium carbonate (C-9) and methylamine assayed as N-methylbenzamide (C-10). Dihydropinidine, obtained by catalytic reduction of pinidine, was refluxed with methyl iodide in the presence of potassium carbonate in ethanol yielding N-methyldihydropinidine methiodide (**5**). A Hofmann degradation yielded 6-dimethylamino-1-nonene (**6**), some of which was catalytically reduced and treated with methyl iodide to afford 4-dimethylaminononane methiodide (**8**). On carrying through this series of reactions with *dl*-dihydropinidine,[3] *dl*-4-dimethylaminononane methiodide was obtained, identical with material obtained from 4-nonanone by oximation, reduction, and methylation. A Hofmann degradation on the methiodide **8** yielded a mixture of 3- and 4-nonene which was treated with osmium tetroxide. The resultant diols were oxidized with potassium permanganate yielding a mixture of propionic, butyric, valeric, and caproic acids, which were converted to their α-naphthylamides and separated by thin layer chromatography. By comparison of the specific activity of these amides with the activity of the other degradation products of pinidine (see Table II) it was possible to deduce the activity at C-5, 6, and 8. The 6-dimethylamino-1-nonene was converted to the 1,2-diol with osmium tetroxide and cleaved with sodium metaperiodate yielding formaldehyde (C-7) and 4-dimethylaminooctanal (**7**), which was reduced catalytically affording 4-dimethylamino-1-octanol. Treatment of this alcohol with potassium iodide in 95% phosphoric acid, followed by chloroform extraction of the basified reaction mixture yielded N-methylconiine methiodide (**9**), identical with material prepared from

(9) E. Leete, H. Gregory, and E. G. Gros, *J. Am. Chem. Soc.*, **87**, 3475 (1965).

Table I

Expt no.	Precursor fed (wt activity)	Duration of feeding	Pinidine hydrochloride		
			Wt, (mg)	Activity, dpm/mmole	% incorporation
1	Sodium acetate-1-^{14}C[a] (1 mg, 0.5 mCi)	2 weeks (Dec 1965)	188	$<4 \times 10^2$	
2	Sodium acetate-1-^{14}C (4 mg, 2 mCi)	3 weeks (March 1966)	383	2.8×10^4	0.0014
3	Sodium acetate-1-^{14}C (22 mg, 2 mCi)	4 weeks (April 1968)	88	2.4×10^4	0.0003
4	Sodium acetate-1-^{14}C (11 mg, 1 mCi)	11 weeks (Aug 1968)	19	4.13×10^6	0.022
5	*dl*-Lysine-2-^{14}C hydrochloride[b] (28 mg, 0.2 mCi)	3 weeks (March 1966)	391	6.4×10^3	0.0032

[a] Purchased from Nuclear Research Chemicals, Orlando, Fla. [b] Purchased from Tracerlab, Waltham, Mass.

Table II. Pinidine and Its Degradation Products

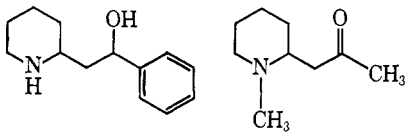
No.		Sp act., dpm/mmol $\times 10^{-6}$	Labeled atoms	Distribn of act., %
	Pinidine Derived from Sodium Acetate-1-^{14}C (Expt 4)			
1	Pinidine hydrochloride	4.13	All	100
2	Dihydropinidine hydrochloride	4.15	All	
3	N-Methyldihydropinidine methiodide	4.06	All	
4	Formaldehyde dimedone[a]	0.04	7	<1
5	N-Methylconiine methiodide	4.15	All except 7	100
6	1-Dimethylaminooctane methiodide	4.12	All except 7	100
7	4-Dimethylaminooctane methiodide	4.12	All except 7	100
8	Formaldehyde dimedone[b]	1.05	2	25
9	Acetyl-α-naphthylamide[c]	0.95	9,10	23
10	Barium carbonate[d]	0.89	9	22
11	N-Methylbenzamide[d]	0.01	10	<1
12	Barium carbonate[e]	0.06	3	1
13	Formaldehyde dimedone[f]	0.92	4	22
14	4-Dimethylaminononane methiodide	4.21	All	
15	Propionyl-α-naphthylamide	1.00	8, 9, 10	24
	By difference (15 − 9)	0.05	8	1
16	Butyryl-α-naphthylamide	2.02	6, 8, 9, 10	49
	By difference (16 − 15)	1.02	6	25
17	Valeryl-α-naphthylamide	2.06	2, 3, 4, 5, 7	50
	By difference (17 − (4 + 8 + 12 + 13))	0	5	<1
	Pinidine Derived from Lysine-2-^{14}C (Expt 5)			
		Sp act., dpm/mmol $\times 10^{-3}$		
18	Pinidine hydrochloride	6.4	All	100
19	Dihydropinidine hydrochloride	6.4	All	
20	N-Methyldihydropinidine methiodide	6.5	All	
21	Formaldehyde dimedone[a]	0.24	7	4
22	Acetyl-α-naphthylamide[c]	1.55	9, 10	24
23	Barium carbonate[d]	1.39	9	22
24	N-Methylbenzamide[d]	0.22	10	3

[a] Obtained from the oxidation of 6-dimethylamino-1-nonene. [b] Obtained by the oxidation of 1-octene. [c] Derivative of the acetic acid produced by the oxidation of pinidine. [d] Obtained by a Schmidt reaction on the previously mentioned acetic acid. [e] Obtained by a Schmidt reaction on heptanoic acid. [f] Obtained by the oxidation of 1-hexene.

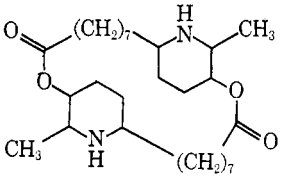
(+)-coniine, and having the illustrated stereochemistry.[10] This series of reactions thus confirms the stereochemistry at C-6 of pinidine previously established by Hill.[4] The N-methylconiine methiodide was then degraded as previously described.[8] The products obtained from a Hofmann degradation on the methiodide **9** were hydrogenated, and methylated yielding a mixture of 1-dimethylaminooctane methiodide (**10**) and 4-dimethylaminooctane methiodide. The 1-octene obtained by a Hofmann degradation on the former methiodide was cleaved with osmium tetroxide and sodium metaperiodate yielding formaldehyde (C-2) and heptanal, which was oxidized with permanganate to heptanoic acid. A Schmidt reaction on this acid yielded carbon dioxide (C-3) and 1-aminohexane which was converted to 1-dimethylaminohexane methiodide and subjected to another Hofmann degradation. The resultant 1-hexene was oxidized as before yielding formaldehyde (C-4). The activities of these degradation products are recorded in Table II, and it is clear that the data substantiate the hypothesis illustrated in Scheme I, essentially all the radioactivity being located at the four expected positions. The results obtained do not enable us to distinguish between the cyclization illustrated in Scheme I where C-10 is derived from the "methyl end"

(10) K. Löffler and G. Friedrich, *Ber.*, **42**, 107 (1909).

of the hypothetical ten-carbon poly-β-keto acid, and an alternate scheme in which C-7 would be the "methyl end" of the ten-carbon precursor. Recently it has been established that the piperidine rings of sedamine (**11**)[11] and N-methylisopelletierine (**12**)[12,13] can be derived from lysine and not from acetic acid as had previously been suggested.[7] In preliminary work we fed *dl*-lysine-2-^{14}C to *P. jeffreyi* plants and obtained a low incorporation of activity into pinidine. The activity was high enough to carry out a partial degradation and the following distribution of activity was found at C-7 (4%), C-9 (22%), and C-10 (3%). These results indicate that



11 12



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there was not a specific incorporation of the lysine into the piperidine ring of pinidine. The results can be rationalized by postulating that the lysine is catabolized in higher plants by a route similar to that operating in animals and microorganisms.[14] By this route, lysine-2-^{14}C affords acetate-1-^{14}C, which could then be incorporated into pinidine, labeling alternate carbon atoms.

Another piperidine alkaloid which may be derived from a poly-β-keto acid is carpaine (**13**) and preliminary feeding experiments with acetate[15] are consistent with this hypothesis.

Experimental Section

General Methods. Melting points are corrected. Radioactivity measurements were carried out in a Nuclear Chicago Model 724 liquid scintillation system using as solvents either toluene or dioxane–water with the usual scintillators.[16] Microanalyses were carried out by Clark Microanalytical Laboratories Urbana, Ill. Mass spectra were determined by Adrian Swanson on an Hitachi Perkin-Elmer RMU-6D mass spectrometer.

Administration of Tracers to *Pinus jefferyi* and Isolation of the Pinidine. The amounts of the precursors which were fed to the plants, growing in soil in a greenhouse, are recorded in Table I. The plants[17] were 2–3 years old, and 30–40 cm tall, when fed. In expt 1, the pine needles were cut off by means of a razor blade and the cut ends were placed in a solution of sodium acetate-1-^{14}C in water. After 2 weeks, only 0.62% of the radioactivity remained in this solution. In the other experiments cotton wicks were inserted by means of a thin sewing needle about 10 cm below the tops of the stems where the clumps of needles were growing. The following extraction procedure is typical and describes the isolation of pinidine from expt 4. The needles and the tops of the plants (fresh weight 47 g) were homogenized in a Sorval omnimixer with 10% aqueous sodium carbonate (200 ml). The mixture was steam distilled, it being necessary to add 1-octanol to prevent foaming. When 1 l. of distillate had been collected it was extracted with three 200-ml portions of chloroform. This chloroform was then extracted with four 150-ml portions of 2 *N* hydrochloric acid. The acid extract was made strongly basic with potassium hydroxide and extracted with ten 100-ml portions of ether. Hydrogen chloride gas was passed into the ether extract which was then evaporated to dryness yielding crude pinidine hydrochloride (19 mg). Thin layer chromatography on silica gel G (Merck) developing with a mixture of chloroform, methanol, and concentrated ammonia (100:20:1) indicated only one base to be present (R_f 0.7), detected with iodine vapor. 2-Methylpiperidine (R_f 0.4) reported to be present in minor amounts in *P. sabiniana*[2] was not detected in our extracts. Recrystallization of the crude alkaloid from a mixture of ethanol and ethyl acetate yielded colorless needles of pinidine hydrochloride, mp 245–246°, lit.[2] mp 244–246°.

Degradation of the Radioactive Pinidine. Pinidine hydrochloride obtained from expt 4 was subjected to the following degradation. Duplicate assays were carried out on 2–6 mg of each degradation product, and the results reported in Table II are average values, the probable error being ±5%. Dilutions with inactive materials were carried out as required; however, the activities reported in Table II are calculated for undiluted material. Partial degradations were carried out on pinidine obtained in the earlier experiments and the same pattern of labeling was found. The pinidine derived from lysine-2-^{14}C was not diluted prior to degradation.

Dihydropinidine. Pinidine hydrochloride (217 mg) was dissolved in ethanol (30 ml) and hydrogenated in the presence of Adams catalyst (0.1 g) for 3 hr at 3 atm pressure. The filtered reaction mixture was evaporated and the residue crystallized from ethanol and ethyl acetate affording colorless crystals of dihydropinidine hydrochloride (206 mg), mp 248–249° (lit.[2] mp 247–248°).

N-Methyldihydropinidine Methiodide. Dihydropinidine hydrochloride (203 mg) was refluxed overnight with a mixture of potassium carbonate (1.0 g), methyl iodide (10 ml), and ethanol (20 ml). The mixture was evaporated to dryness and the residue extracted with chloroform. Addition of ethyl acetate to the residue obtained on evaporation of the chloroform afforded colorless prismatic needles of N-methyldihydropinidine methiodide (284 mg), mp 101–102°.

Anal. Calcd for $C_{11}H_{24}NI$: C, 44.45; H, 8.14; N, 4.71. Found: C, 43.72; H, 8.10; N, 4.63.

The racemic methiodide prepared from *dl*-dihydropinidine[3] had mp 122–123°.

4-Dimethylaminononane Methiodide. (a) From 4-Nonanone. 4-Nonanone (1.0 g), hydroxylamine hydrochloride (1.0 g), and potassium hydroxide (4 g) were refluxed for 2 hr in 95% ethanol (20 ml). The reaction mixture was then added to water (100 ml), neutralized with hydrochloric acid, and extracted with ether. Evaporation of the dried ($MgSO_4$) ether extract yielded 4-nonanone oxime (674 mg) as a colorless oil. The oxime was dissolved in ether (25 ml) and a solution of lithium aluminum hydride (0.36 g) in ether (10 ml) was slowly added and the mixture then refluxed for 5 hr. Water was added to the reaction mixture, followed by dilute hydrochloric acid. The mixture was extracted with ether, and the aqueous solution made basic with potassium hydroxide and then extracted with ether. Evaporation of the dried ether extract yielded 4-aminononane (288 mg) which was dissolved in ethanol (20 ml) and refluxed with methyl iodide (5 ml) in the presence of potassium carbonate (1.0 g) for 21 hr. Evaporation of the reaction mixture, followed by extraction with chloroform, yielded *dl*-4-dimethylaminononane methiodide (320 mg), obtained as colorless plates from a mixture of ethanol and ethyl acetate, mp 215–216°.

Anal. Calcd for $C_{12}H_{28}NI$: C, 46.01; H, 9.01. Found: C, 45.66; H, 8.99.

(b) From N-Methyldihydropinidine Methiodide. *dl*-N-Methyldihydropinidine methiodide (300 mg) was dissolved in water (10 ml) and shaken with silver hydroxide (from 0.5 g of silver nitrate). The filtered mixture was evaporated to dryness and the residue distilled (180°, 0.01 mm). The distillate which was collected in a U tube cooled in liquid nitrogen, was dissolved in ethanol (20 ml), and hydrogenated at 3 atm pressure in the presence of Adams catalyst (0.2 g) for 15 min. The catalyst was filtered off and methyl iodide (5 ml) was added to the filtrate which was then refluxed for 1 hr. Evaporation yielded *dl*-4-dimethylaminononane methiodide (243 mg, 78%), mp 215–216°, not depressed on admixture with material obtained from 4-nonanone. Material obtained from natural pinidine had mp 216–218°, and had an infrared spectrum identical with the *dl*-methiodide.

(11) R. N. Gupta and I. D. Spenser, *Chem. Commun.*, 893 (1966); *Can. J. Chem.*, **45**, 1275 (1967).
(12) D. G. O'Donovan and M. F. Keogh, *Tetrahedron Letters*, 265 (1968).
(13) R. N. Gupta and I. D. Spenser, *Chem. Commun.*, 85 (1968).
(14) *Cf.* I. D. Spenser in "Comprehensive Biochemistry, Vol. 20, Metabolism of Cyclic Compounds," M. Florkin and E. H. Stotz, Ed., Elsevier Publishing Co., New York, N. Y., 1968, Chapter VI, p 256.
(15) C. W. L. Bevan and A. U. Ogan, *Phytochemistry*, **3**, 591 (1964).
(16) A. R. Friedman and E. Leete, *J. Am. Chem. Soc.*, **85**, 2141 (1963).
(17) Obtained from Sherwood Nursery, Corbett, Oregon.

Hofmann Degradation on 4-Dimethylaminononane Methiodide and Oxidation of the Resultant Nonenes. 4-Dimethylaminononane methiodide (220 mg) was dissolved in water (10 ml) and shaken with silver hydroxide (from 0.5 g of silver nitrate). The residue obtained on evaporation of the filtered mixture was distilled (180°, 0.01 mm), and the distillate dissolved in ether (100 ml) containing 1 drop of pyridine and osmium tetroxide (200 mg). After standing overnight at room temperature the black solution was evaporated, and the residue refluxed with a mixture of methanol (15 ml), water (15 ml), and sodium sulfite (1.0 g) for 1 hr. The mixture was filtered and the filtrate evaporated to dryness, and then extracted with ether. The residue obtained on evaporation of the ether was suspended in water (30 ml) and potassium permanganate (300 mg) added. After heating on a steam bath for 3 hr, 2 *N* sulfuric acid (5 ml) was added and the mixture distilled, water being added to maintain the volume in the distillation flask. When 50 ml of distillate had been collected it was titrated with 0.1 *N* sodium hydroxide (9.2 ml required). The neutralized solution was evaporated to dryness and the residue dissolved in water (3 ml) to which was then added α-naphthylamine hydrochloride (200 mg) and 1-ethyl-3-(3-dimethylaminopropyl)carbodimide hydrochloride (1.0 g). After standing for 30 min, the reaction mixture was extracted with chloroform, which was then washed with dilute hydrochloric acid and 10% sodium carbonate. Evaporation of the dried ($MgSO_4$) chloroform extract yielded a mixture of amides which were separated on preparative plates of silica gel F_{254} (Merck), developing three times with a mixture of chloroform, ethyl acetate, and ethanol (100:10:5). The zones, visible as dark bands in ultraviolet light, were removed and extracted with boiling ethanol. The filtered ethanol extracts were evaporated, and the residues sublimed (150°, 0.001 mm). Crystallization of the sublimates from hexane yielded the following amides: acetyl-α-naphthylamide (6 mg), mp 159–160°; propionyl-α-naphthylamide (17 mg), mp 118–119°; butyryl-α-naphthylamide (17 mg), mp 120°; valeryl-α-naphthylamide (11 mg), mp 118°; and caproyl-α-naphthylamide (7 mg), mp 112–113°. The valeryl- and caproyl-α-naphthylamides had to be rechromatographed on silica gel plates, with chloroform as the developing solvent, to achieve complete separation from each other. The acetic acid obtained in this reaction probably resulted from oxidation of some of the propionic acid. The acetyl-α-napthylamide had the same specific activity (0.97×10^6 dpm/mmole) as material obtained by the direct oxidation of pinidine.

Conversion of N-Methyldihydropinidine Methiodide to N-Methylconiine Methiodide. N-Methyldihydropinidine methiodide (300 mg) was subjected to a Hofmann degradation as previously described and the resultant 6-dimethylamino-1-nonene dissolved in ether (50 ml) containing 1 drop of pyridine and osmium tetroxide (250 mg). After standing overnight, the osmate ester was decomposed in the usual way with sodium sulfite in aqueous methanol. The diol which was extracted with ether was dissolved in 1% acetic acid (10 ml) and sodium metaperiodate (400 mg) added. After 15 min, barium chloride (1.0 g) in water (10 ml) was added and the precipitated barium iodate and periodate were filtered off. The filtrate was diluted with methanol (100 ml) containing a few drops of concentrated hydrochloric acid, and the mixture hydrogenated in the presence of Adams catalyst (0.2 g) for 12 hr at 3 atm pressure. The filtered solution was evaporated to dryness and the residue made alkaline with sodium hydroxide. Continuous ether extraction for 16 hr yielded on distillation (180°, 0.1 mm) 4-dimethylamino-1-octanol as a colorless oil (75 mg) having an OH absorption in the infrared at 3300 cm^{-1}. The mass spectrum had a molecular ion peak at *m/e* 173 (mol wt of $C_{10}H_{23}NO$, 173). This oil was dissolved in 95% phosphoric acid (0.5 ml) and stirred with potassium iodide (200 mg) in a nitrogen atmosphere for 2 hr at 105°. The reaction mixture was then added to ice, neutralized with potassium carbonate, and extracted with chloroform for 18 hr. Evaporation of the extract yielded a solid residue which was dissolved in ethanol and diluted with ethyl acetate when fine colorless needles of N-methylconiine separated (72 mg), mp 186–187°, not depressed on admixture with N-methyl-(+)-coniine methiodide, mp 188–189°. Mugdan[18] reported mp 186–188° for the methiodide prepared from natural (+)-coniine. The *dl*-N-methylconiine methiodide has mp 154–155°.[8]

This series of reactions was repeated with 70 mg of the N-methyldihydropinidine methiodide up to cleavage of the 1,2-diol in dilute acetic acid with sodium metaperiodate. The reaction mixture was then made basic with sodium carbonate and distilled into a solution of dimedone (100 mg) in water (50 ml). On standing overnight at 0°, the distillate deposited formaldehyde dimedone (37 mg), mp 186–187°. It was crystallized from aqueous methanol prior to radioactive assay.

The N-methyl-(+)-coniine methiodide was degraded as previously described[8] affording 1-dimethylaminooctane methiodide, mp 141–142°, and optically active 4-dimethylaminooctane methiodide, mp 227–228° (the *dl*-methiodide has mp 225–226°[8]).

Oxidation of Pinidine. (a) With Osmium Tetroxide and Sodium Metaperiodate. Pinidine hydrochloride (145 mg) was dissolved in a little water, made alkaline with sodium hydroxide, and extracted with three 30-ml portions of ether. Osmium tetroxide (350 mg) and 2 drops of pyridine were added to the ether extract. After standing overnight, the ether was evaporated and the residue refluxed with 50% aqueous methanol (30 ml) containing sodium sulfite (1.0 g) for 1 hr. The mixture was filtered and evaporated yielding a residue which was dissolved in 0.1 *N* sulfuric acid (10 ml) and treated with sodium metaperiodate (400 mg). After standing for 30 min, the mixture was distilled into a solution of chromium trioxide (3 g) in 2 *N* sulfuric acid (20 ml). This solution was then distilled, water being added to maintain the volume in the distillation flask. The distillate was titrated with 0.1 *N* sodium hydroxide (4.1 ml required). Evaporation yielded sodium acetate (35 mg) crystallized from ethanol and ether.

(b) With Potassium Permanganate. Pinidine hydrochloride (67 mg) was dissolved in water (10 ml) and 1 ml of 1% sodium hydroxide and potassium permanganate (200 mg) were added. After heating on a steam bath for 1 hr, the mixture was acidified with sulfuric acid and distilled. The distillate was neutralized with sodium hydroxide, evaporated to dryness, and the residue extracted with ethanol. Evaporation of the ethanol and addition of ether yielded sodium acetate (17 mg).

(18) M. Mugdan, *Ann.*, **298**, 131 (1887).