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Biosynthesis of the Hemlock Alkaloids. The Incorporation of Acetate-1-C^{14} into Coniine and Conhydrine[1]

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The biosynthesis of the alkaloids of *Conium maculatum* (hemlock) has been studied using C^{14}-labeled compounds. The alkaloids obtained from hemlock plants which had been fed DL-lysine-2-C^{14} or cadaverine-1,5-C^{14} had negligible activity. On the other hand, the administration of sodium acetate-1-C^{14} to the plants led to the formation of radioactive coniine and conhydrine. A systematic degradation of the coniine established that almost all the activity was located on the even-numbered carbons and was equally distributed between these four positions. Although the degradation of the conhydrine was not as complete, the activity of the degradation products obtained was consistent with a similar pattern of labeling in this alkaloid. These results favor the hypothesis that these piperidine alkaloids are formed by the cyclization of an eight-carbon chain derived from four acetate units.

Coniine (III), one of the major alkaloids of the common hemlock plant (*Conium maculatum*), was the first alkaloid to be synthesized.[3] The poisonous nature of hemlock was known to the ancient Greeks who used an extract of the plant for the elimination of undesirables.[4] We have been studying the biosynthesis of the hemlock alkaloids for several years using radioactive tracers. The direction our initial experimentals took was influenced by the ideas of Robinson on the biosynthesis of these alkaloids[5] (*cf.* Fig. 1). He suggested that lysine (I) is a precursor of the piperidine ring of coniine. Oxidative deamination and decarboxylation of this amino acid could lead to Δ^1-piperideine (II). Condensation of this imine with acetoacetic acid would afford the intermediate IV which on decarboxylation and reduction yields coniine. Condensations of this type involving Δ^1-piperideine have actually been achieved *in vitro*.[6]

In 1955, DL-lysine-2-C^{14} was fed to 3-month-old hemlock plants which were cultivated in a hydroponic solution[7] by addition to the nutrient solution. The radioactive lysine was absorbed by the roots and radioactivity was detected in the plant tissues. However, the crude alkaloid fraction had negligible activity. We had shown that the piperidine ring of anabasine (V) is derived from lysine,[7] but we also found that cadaverine was a much more efficient precursor of the piperidine ring of this alkaloid.[8] Therefore cadaverine-1,5-C^{14} hydrochloride was administered to hemlock plants, but again alkaloids of very low activity were obtained. Negative results in tracer experiments involving plants must be interpreted with caution since there are several factors which can prevent the incorporation of activity into an alkaloid. The labeled compound may not reach the site of alkaloid synthesis, or it may be metabolized to other compounds before it does. The method of feeding tracers to plants can have a pronounced effect on the degree of incorporation of tracer. Thus it was found that the administration of tryptophan-2-C^{14} to *Rauwolfia serpentina* plants yielded radioactive alkaloids when the tracer was fed by means of a cotton wick inserted into the stems of the plants, while alkaloids with negligible activity were obtained when the tracer was fed to the roots in a hydroponic solution.[9] We therefore repeated the feeding with DL-lysine-2-C^{14} using the cotton wick method. However, we again failed to obtain any significant activity in the alkaloids. In the meantime Schiedt and Höss[10] reported that the administration of uniformly labeled L-lysine-C^{14} to hemlock plants afforded radioactive coniine. However, the alkaloid was not degraded to determine whether radioactivity was confined to the piperidine ring.

In view of our repeated failure to obtain radioactive alkaloids when we fed radioactive lysine or cadaverine to hemlock plants, we considered an alternate hypothesis for the biosynthesis of coniine and related piperidine alkaloids. It was proposed[11] that the carbon skeleton of these alkaloids is derived from acetic acid units which had combined together to form a polyacetyl chain.[12] Thus coniine could be formed by the cyclization of the hypothetical poly-β-keto acid VI (Fig. 2) with ammonia to yield the intermediate VII which on reduction and dehydration could afford γ-coniciene (VIII),[13] also found in hemlock. Further reduction would then yield coniine. The minor hemlock alkaloid, conhydrine (IX), could be plausibly formed *via* the alcohol X, produced by the allylic oxidation of γ-coniceine. Recent investigations[14] on the alkaloid content of hemlock plants at various stages of growth strongly suggest that γ-coniceine plays a central role in the biosynthesis of the other alkaloids.

We have now tested this hypothesis by feeding sodium acetate-1-C^{14} to 2-year-old hemlock plants by the cotton wick method. The crude alkaloids isolated from the plants 8 days after the feeding had appreciable activity. Coniine and conhydrine isolated from the alkaloid mixture had high specific activities and were subjected to the following reactions to determine the distribution of radioactivity in their molecules.

(1) This investigation was supported by a research grant GB-363 from the National Science Foundation. A preliminary account of part of this work has been reported: E. Leete, *J. Am. Chem. Soc.*, **85**, 3523 (1963).

(2) Alfred P. Sloan Fellow, 1962–1965.

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

(4) *Cf.* Plato's "Phaedo" (387 B.C.) for a description of the death of Socrates in 399 B.C. (translated by R. Hackforth, Cambridge University Press, 1955, p. 187).

(5) R. Robinson, *J. Chem. Soc.*, **111**, 876 (1917); also in "The Structural Relations of Natural Products," Clarendon Press, Oxford, 1955.

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

(7) E. Leete, *J. Am. Chem. Soc.*, **78**, 3520 (1956).

(8) E. Leete, *ibid.*, **80**, 4393 (1958).

(9) E. Leete, *ibid.*, **82**, 6338 (1960).

(10) U. Schiedt and H. G. Höss, *Z. Naturforsch.*, **13b**, 691 (1958); *Z. physiol. Chem.*, **330**, 74 (1962).

(11) E. Leete, "Biogenesis of Natural Compounds," P. Bernfeld, Ed., Pergamon Press, Oxford, 1963, Chapter 17, p. 751.

(12) K. Biemann, G. Büchi, and B. H. Walker, *J. Am. Chem. Soc.*, **79**, 5558 (1957), suggested a similar scheme for the biosynthesis of muscopyridine. A. R. Battersby, *Quart. Rev.* (London), **15**, 259 (1961), independently proposed this new biogenetic scheme for coniine.

(13) H. C. Beyerman, M. van Leeuvan, J. Smidt, and A. van Veen, *Rec. trav. chim.*, **80**, 513 (1961).

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

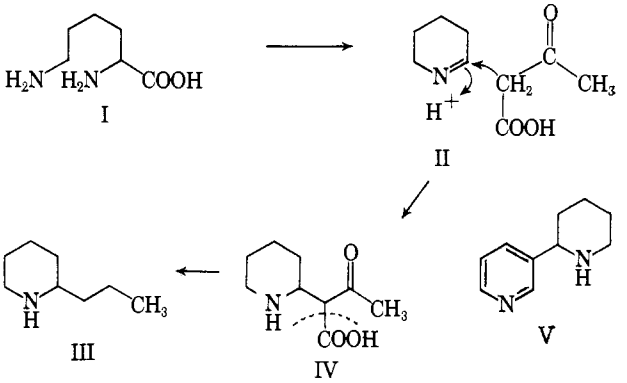

Fig. 1.—Robinson's hypothesis for the biosynthesis of coniine.

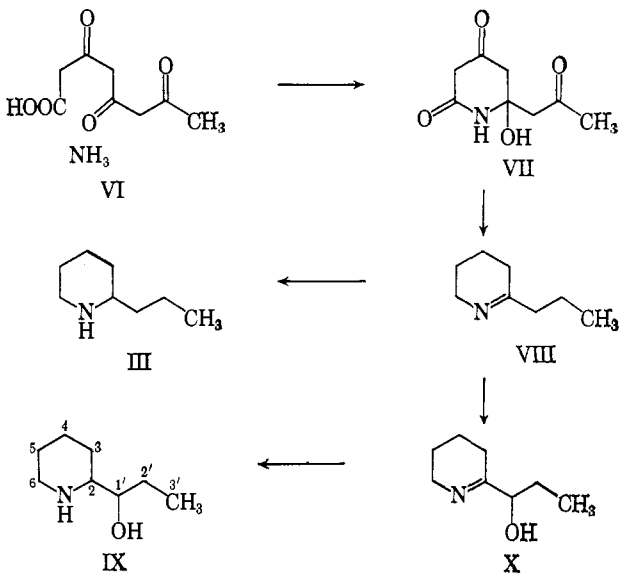

Fig. 2.—New hypothesis for the biosynthesis of the hemlock alkaloids.

A Kuhn–Roth oxidation of coniine with chromium trioxide in 2 *N* sulfuric acid yielded solely acetic acid. A Schmidt reaction on this acetic acid afforded carbon dioxide collected as barium carbonate [C-2′][15] and methylamine collected as N-methylbenzamide [C-3′]. Coniine was refluxed in ethanol with methyl iodide in the presence of sodium bicarbonate affording N-methylconiine methiodide (XI, Fig. 3). This methiodide was subjected to the Hofmann elimination yielding a mixture of 1-dimethylamino-4- (and -5-) octene (XII) and 5-dimethylamino-1-octene (XIII). This mixture was hydrogenated in the presence of platinum and the resultant saturated amines were treated with methyl iodide yielding 1-dimethylaminooctane methiodide (XIV) and 4-dimethylaminooctane methiodide (XV). Mugdan first obtained these methiodides from (+)-coniine in 1897 and separated them by fractional crystallization.[16] We obtained a more complete separation by column chromatography on alumina. The ratio of the yields of the 1-isomer to the 4-isomer was 3:1. We were carrying out the degradation with *dl*-coniine and a

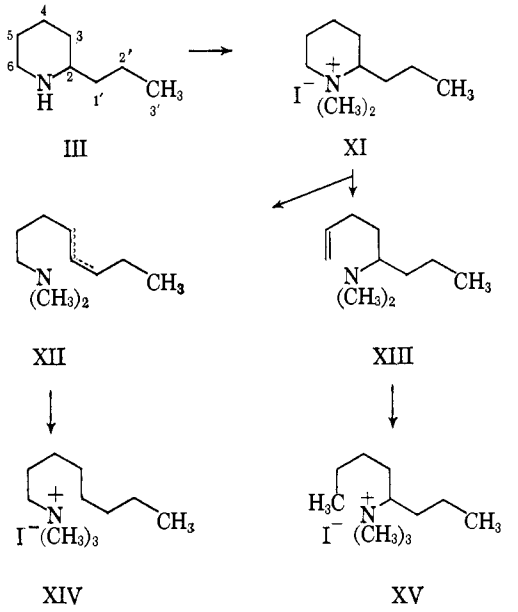

Fig. 3.—Preliminary degradation of coniine.

sample of *dl*-4-dimethylaminooctane methiodide was prepared from 4-octanone oxime for comparison. The oxime was reduced with lithium aluminum hydride and the resultant 4-aminooctane methylated with excess methyl iodide in the presence of sodium bicarbonate. A Hofmann elimination on the methiodide XIV afforded 1-octene which was converted to octane-1,2-diol by treatment with osmium tetroxide. Cleavage of this diol with sodium metaperiodate yielded formaldehyde [C-6] collected as its dimedone derivative and heptanal which was oxidized with potassium permanganate to heptanoic acid. A Schmidt reaction on this acid yielded carbon dioxide collected as barium carbonate [C-5] and hexylamine which was refluxed with methyl iodide in ethanol in the presence of sodium bicarbonate to yield 1-dimethylaminohexane methiodide. This compound was degraded in the same way as the methiodide XIV affording C-4 as formaldehyde and C-3 as barium carbonate. Further degradation by this route was not possible because of lack of material. A Hofmann elimination on 4-dimethylaminooctane methiodide afforded a mixture of 3- and 4-octene, which was oxidized with osmium tetroxide and then potassium permanganate yielding a mixture of valeric, butyric, and propionic acids. These were separated by partition chromatography on silicic acid.[17] Insufficient pure valeric acid was obtained for further degradation. However butyric acid resulting from the oxidation of the 4-octene was obtained in good yield and a Schmidt reaction on this acid yielded carbon dioxide originating from C-2 and C-3. Since activity at C-3 had been previously obtained directly, activity at C-2 could be determined by difference. A Schmidt reaction on the propionic acid afforded carbon dioxide [C-1′].

The radioactive conhydrine was converted to *erythro*-octane-3,4-diol using the procedure of Späth and Adler[18] utilizing some of the modifications described by Hill.[19]

(15) This indicates that the activity of the barium carbonate represented the activity at C-2′ in the alkaloid. Other degradation products are similarly identified.

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

(17) H. F. Mueller, T. E. Larson, and W. J. Lennarz, *Anal. Chem.*, **30**, 41 (1958).

(18) E. Späth and E. Adler, *Monatsh.*, **68**, 127 (1933).

(19) R. K. Hill, *J. Am. Chem. Soc.*, **80**, 1609 (1958).

Oxidation of this diol with potassium permanganate yielded a mixture of valeric and propionic acids. A Schmidt reaction on the propionic acid yielded carbon dioxide [C-1′]. A Schmidt reaction on some of the valeric acid yielded carbon dioxide [C-2], while another portion of the acid was oxidized with chromium trioxide to acetic acid [C-5 + C-6] which yielded carbon dioxide [C-5] and methylamine [C-6] by a Schmidt reaction. The acetic acid resulting from a Kuhn–Roth oxidation of conhydrine was subjected to the same degradation affording activity at C-2′ and C-3′ as carbon dioxide and methylamine, respectively. Lack of material prevented determination of activity at C-3 and C-4.

Table I records the activities of the degradation products of coniine and conhydrine derived from acetate-1-C^{14}. Most of the activity of the coniine was located on the even numbered carbons and, within experimental error, equally distributed between these four positions. Degradation of the conhydrine was not as complete; however, the pattern of labeling in the six atoms whose activity was determined was essentially the same as in coniine.

Our results strongly support the new hypothesis that the hemlock alkaloids are derived from four acetate units. Recently it has been established that the formation of fatty acids from acetic acid proceeds *via* malonyl coenzyme A, only the two carbons at the methyl end of the chain being formed directly from acetyl coenzyme A.[20] If the eight carbon chain of coniine is produced in an analogous manner we would expect only C-2′ and C-3′ to be formed directly from acetyl coenzyme A, the other six carbons being derived from malonyl coeznyme A.

The results of Schiedt and Höss[10] involving uniformly labeled lysine-C^{14} may be rationalized by postulating that the lysine underwent metabolism in the plant ultimately yielding radioactive acetate which was then incorporated into coniine.

Experimental[21]

Administration of Tracers to *C. maculatum* Plants and Isolation of the Alkaloids.—Sodium acetate-1-C^{14} (41.0 mg., 1.0 mc.)[22] was administered to three 2-year-old hemlock plants by means of a cotton wick inserted into the stems of the plants. The plants were growing out of doors (June, 1963) in soil. Eight days after feeding the tracer, the plants were harvested (wet wt. 2.7 kg.) and macerated in a Waring blendor with a mixture of chloroform (2 l.) and concentrated ammonia solution (200 ml.). After standing for several weeks the mixture was filtered through cloth and the aqueous layer separated (total activity = 2.3×10^8 d.p.m. = 10.5% of the activity fed). The chloroform layer was evaporated *in vacuo* to a volume of 100 ml., diluted with an equal volume of ether, and extracted with 2 *N* sulfuric acid (250 ml.). This acid extract was cooled, made basic with 20% sodium hydroxide, and extracted with chloroform. A saturated solution of hydrogen chloride in ethanol (10 ml.) was added to the dried chloroform extract which was then evaporated to yield the crude alkaloids as their hydrochlorides (total activity = 7.8×10^6 d.p.m.). This semicrystalline residue was dissolved in water, made basic with sodium hydroxide, and extracted with chloroform. The dried chloroform extract was evaporated and the residue distilled (110°, 0.1 mm.) in a hot air bath, the volatile coniine and γ-coniceine being collected in a U-tube cooled in liquid nitrogen. The less volatile conhydrine accumulated as a white solid near the exit from the hot air bath. This solid (87 mg.) was washed out with diethyl ether. The residue obtained on evaporation of

TABLE I

	Spec. activity d.p.m./m*M* × 10^{-5}	Labeled atom	Distribution of activity, %
Coniine and its degradation products			
Coniine hydrochloride	3.8	All	100
N-Methylconiine methiodide	3.8		
1-Dimethylaminooctane methiodide	3.7		
4-Dimethylaminooctane methiodide	3.7		
Formaldehyde dimedone[a]	0.90	6	24
Barium carbonate[b]	0.04	5	1
1-Dimethylaminohexane methiodide	2.7		
Formaldehyde dimedone[c]	0.82	4	22
Barium carbonate[d]	0.06	3	1.6
Sodium butyrate[e]	1.8		
Barium carbonate[f]	0.52	(2 + 3)/2	
(by difference)		2	26
Sodium propionate	1.1		
Barium carbonate[g]	0.05	1′	1.3
N-Ethylbenzamide[g]	0.9		
Sodium acetate[h]	1.0		
Barium carbonate[i]	0.85	2′	22
N-Methylbenzamide[i]	0.04	3′	1
Conhydrine and its degradation products			
Conhydrine	1.16[p]	All	100
N-Methylconhydrine methiodide	1.10		
1-Dimethylamino-5,6-epoxyoctane methiodide	1.18		
erythro-Octane-3,4-diol	1.16		
Sodium valerate	0.90		
Barium carbonate[j]	0.27	2	23
Sodium acetate[k]	.35		
Barium carbonate[l]	.037	5	3
N-Methylbenzamide[l]	.26	6	22
Sodium propionate	.32		
Barium carbonate[m]	.046	1′	4
Sodium acetate[n]	.29		
Barium carbonate[o]	.25	2′	21
N-Methylbenzamide[o]	.05	3′	4

[a] Obtained from the periodate cleavage of octane-1,2-diol. [b] Obtained from a Schmidt reaction on heptanoic acid. [c] Obtained from the periodate cleavage of hexane-1,2-diol. [d] Obtained from a Schmidt reaction on valeric acid. [e] Obtained from the cleavage of 4-octene. [f] Obtained from a Schmidt reaction on sodium butyrate. [g] Obtained from a Schmidt reaction on the preceding sodium propionate. [h] Obtained by the Kuhn–Roth oxidation of coniine. [i] Obtained by a Schmidt reaction on the preceding sodium acetate. [j] Obtained by a Schmidt reaction on the preceding sodium valerate. [k] Obtained by a Kuhn–Roth oxidation of the sodium valerate. [l] Obtained by a Schmidt reaction on the preceding sodium acetate. [m] Obtained by a Schmidt reaction on the sodium propionate. [n] Obtained by a Kuhn–Roth oxidation of conhydrine. [o] Obtained by a Schmidt reaction on the preceding sodium acetate. [p] This activity is somewhat lower than that reported in our preliminary communication.[1] Repeated crystallization of the alkaloid reduced the activity to this constant level.

(20) S. J. Wakil and J. Ganguly, *J. Am. Chem Soc.*, **81**, 2597 (1959).

(21) Melting points are corrected. We thank Mrs. O. Hamerston and her assistants for the analyses. Radioactivities were determined in a Nuclear Chicago Model 724 liquid scintillation spectrometer using previously described solvent mixtures (E. Leete, *J. Am. Chem. Soc.*, **85**, 3666 (1963)). The dioxane used in some solvent mixtures was reagent grade, purified immediately before use by passage through a column of Woelm alumina (Activity I).

(22) Purchased from New England Nuclear Corp., Boston, Mass.

this ether was crystallized from petroleum ether (b.p. 60–70°) yielding colorless plates of (+)-conhydrine (35 mg.), m.p. 117–118°, having an infrared spectrum identical with that of an authentic specimen. Paper chromatography[14] of the material which condensed in the liquid nitrogen trap indicated the presence of coniine and γ-coniceine. Radioactive assay of the chromatogram indicated that activity was located at positions coincident with these two alkaloids, a larger amount of activity being located in the coniine spot. The material in the liquid nitrogen trap was dissolved in ethanol and treated with an ethanolic solution of hydrogen chloride followed by ether when crude coniine hydrochloride separated. After several crystallizations from a mixture of ethanol and acetone, coniine hydrochloride (68 mg.), m.p. 221–222°, was obtained, having an infrared spectrum identical with that of an authentic specimen. Attempts to isolate γ-coniceine from the mother liquors were not successful.

Degradation of the Coniine.—The radioactive coniine hydrochloride was diluted ten times with inactive *dl*-coniine hydrochloride prior to the following degradation. Specific activities reported in Table I are for undiluted material.

***dl*-N-Methylconiine Methiodide.**—A solution of coniine hydrochloride (300 mg.) in absolute ethanol (20 ml.) and methyl iodide (5 ml.) was refluxed in the presence of sodium bicarbonate (0.8 g.) for 20 hr. The solution was then evaporated to dryness and the residue extracted with boiling chloroform. The filtrated chloroform extract was taken to dryness and the residue crystallized from a mixture of ethanol and ethyl acetate yielding colorless needles of *dl*-N-methylconiine methiodide (482 mg., 93%), m.p. 154–155°. (The corresponding methiodide prepared from (+)-coniine[16] had m.p. 186–188°.)

Anal. Calcd. for $C_{10}H_{22}NI$: C, 42.41; H, 7.83; N, 4.95. Found: C, 42.27; H, 7.78; N, 4.57.

1- and 4-Dimethylaminooctane Methiodides.—*dl*-N-Methylconiine methiodide (455 mg.) was dissolved in water (20 ml.) and stirred with freshly prepared silver hydroxide (from 0.8 g. of silver nitrate) for 10 min. The mixture was then filtered and added to a solution of potassium hydroxide (20 g.) in water (20 ml.) and distilled in a metal bath at 200°. The aqueous distillate was extracted with ether which was then dried and evaporated under reduced pressure. The residue was dissolved in ethanol (20 ml.) and hydrogenated in the presence of platinum oxide (100 mg.) at 25 p.s.i. for 6 hr. The platinum was filtered off and methyl iodide (3 ml.) added to the filtrate which was allowed to stand at room temperature for 16 hr. Evaporation then yielded a mixture of 1- and 4-dimethylaminooctane methiodides (344 mg.). These methiodides were dissolved in a 1:1 mixture of chloroform and benzene and chromatographed on Woelm alumina (70 g. of Activity III) in a column 2 cm. in diameter. The column was first eluted with 1:1 chloroform–benzene (200 ml.), then with chloroform (200 ml.). *dl*-4-Dimethylaminooctane methiodide (70 mg.) was eluted with a 3% solution of ethanol in chloroform. The 1-isomer (204 mg.) was eluted with absolute ethanol. Recrystallization of 1-dimethylaminooctane methiodide from ethyl acetate yielded colorless needles, m.p. 140–141° (lit.[23] 138°). The *dl*-4-dimethylaminooctane methiodide crystallized from ethyl acetate in colorless plates, m.p. 225–226°, identical (mixture m.p., infrared spectrum) with material prepared as described below. The optically active methiodide obtained from (+)-coniine by Mugdan[16] had m.p. 190°.

dl-4-Dimethylaminooctane Methiodide.—4 Octanone[24] was converted to its oxime and then reduced in ether solution with lithium aluminum hydride for 18 hr. The resultant 4-aminooctane was refluxed in ethanol with methyl iodide in the presence of sodium bicarbonate yielding *dl*-4-dimethylaminooctane methiodide, m.p. 225–226°.

Anal. Calcd. for $C_{11}H_{26}NI$: C, 44.15; H, 8.75; N, 4.68. Found: C, 44.16; H, 8.73; N, 4.72.

Degradation of the 1-Dimethylaminooctane Methiodide.—The methiodide (300 mg.) was dissolved in water (10 ml.) and shaken with silver hydroxide (from 0.4 g. of silver nitrate) for 10 min. The filtered solution was evaporated to dryness *in vacuo* and then distilled (180°, 1 mm.), the volatile products being collected in a liquid nitrogen trap. The contents of the trap were dissolved in ether, osmium tetroxide (310 mg.) and a drop of pyridine added, and the solution was allowed to stand at room temperature for 16 hr. The ether was then evaporated and the black residue dissolved in methanol (20 ml.). A solution of sodium sulfite (1 g.) in water (10 ml.) was added and the mixture refluxed for 1 hr. The hot mixture was then filtered and the filtrate evaporated until all the methanol was removed. The aqueous solution was extracted with ether which was then evaporated. The residue was shaken for 1 hr. with a solution of sodium metaperiodate (400 mg.) in water (10 ml.). The solution was then extracted several times to remove heptanal. The residual aqueous solution was distilled into an aqueous solution of dimedone (300 mg.), which on standing overnight afforded crystals of formaldehyde dimedone (170 mg., 58%). The ether solution of heptanal was dried, evaporated, and the residue dissolved in acetone (10 ml.) containing potassium permanganate (120 mg.). After standing overnight at room temperature the mixture was diluted with water (20 ml.) and the acetone removed by evaporation under reduced pressure. Sulfur dioxide was passed into the residual aqueous solution which was then extracted with ether. The dried ether solution was evaporated and the residual heptanoic acid dissolved in benzene (3 ml.). Concentrated sulfuric acid (0.3 ml.) was added and the mixture cooled to 0° with stirring. A chloroform solution of hydrazoic acid (1.5 ml. of a 5.8% solution) was added and the temperature raised to 40° during 2 hr. The evolved carbon dioxide was collected in 0.2 *N* barium hydroxide solution yielding barium carbonate (64 mg., 32%). The residue in the reaction flask was added to water, made basic with sodium hydroxide, and extracted with ether. The ether extract was acidified with a few drops of ethanolic hydrogen chloride and taken to dryness. The residual hexylamine hydrochloride was dissolved in ethanol (10 ml.) and refluxed with methyl iodide (1 ml.) in the presence of sodium bicarbonate (0.5 g.) for 16 hr. The mixture was then evaporated to dryness and the residue extracted with chloroform. The residue obtained after evaporation of the chloroform was crystallized from a mixture of methanol and ethyl acetate yielding 1-dimethylaminohexane methiodide (42.5 mg.), m.p. 165–166° (lit.[23] 167°), having an infrared spectrum identical with that of an authentic specimen. This methiodide was further degraded to valeric acid using essentially the same reaction conditions as those described for the conversion of 1-dimethylaminooctane methiodide to heptanoic acid.

Degradation of the 4-Dimethylaminooctane Methiodide.—The methiodide (250 mg.) was subjected to the Hofmann elimination as described for the 1-isomer, and the resultant mixture of octenes was hydroxylated with osmium tetroxide as previously described for 1-octene. The mixture of diols was dissolved in water (20 ml.) containing potassium permanganate (316 mg.). After heating for 1 hr. on a steam bath the mixture was acidified with sulfuric acid and distilled, water being added to the distillation flask during the distillation. When about 100 ml. had distilled it was titrated with 0.1 *N* sodium hydroxide and then evaporated. A small sample of the residue was chromatographed on Whatman No. 4 paper developing with a solution obtained by mixing 100 ml. of 95% ethanol with 10 ml. of 10% sodium hydroxide. With this system the R_f values for sodium acetate, propionate, butyrate, and valerate were 0.34, 0.44, 0.53, and 0.60, respectively. The salts showed up as blue spots on a yellow background by spraying with a solution of bromocresol blue (50 mg.), and citric acid (200 mg.) in water (100 ml.).[25] Only the last three named acids were detected in the oxidation mixture from the diols. They were separated on a silicic acid column as previously described.[17] Initial fractions from the column consisted of a mixture of valeric and butyric acids. The later fractions contained pure butyric acid and then propionic acid. These were isolated as their sodium salts which were then subjected to Schmidt reactions with sodium azide and concentrated sulfuric acid.[26]

Kuhn–Roth Oxidation of Coniine.—Coniine hydrochloride (160 mg.) was dissolved in 2 ml. of 2 *N* sodium hydroxide and then extracted with chloroform. The chloroform extract was evaporated in the presence of 1 ml. of 2 *N* sulfuric acid. The residual aqueous solution was washed with another 1 ml. of 2 *N* sulfuric acid into a refluxing solution of chromium trioxide (5 g.) in 2 *N* sulfuric acid (10 ml.). The reaction mixture was then distilled, water being added to the distillation flask to maintain the volume at about 10 ml. Distillation was continued until the distillate was no longer acidic (about 50 ml.). The distillate was titrated with 0.1 *N* sodium hydroxide (6.6 ml.) and evaporated to dryness. The residue was crystallized from absolute ethanol affording sodium acetate (15 mg.). On addition of ether to the

(23) J. von Braun, *Ann.*, **382**, 1 (1911).
(24) M. S. Newman and A. S. Smith, *J. Org. Chem.*, **13**, 592 (1948).
(25) E. P. Kennedy and H. A. Barker, *Anal. Chem.*, **23**, 1033 (1951).
(26) A. R. Friedman and E. Leete, *J. Am. Chem. Soc.*, **85**, 2141 (1963).

mother liquor an additional 20 mg. was obtained. Paper chromatography of the sodium acetate failed to show any propionate or butyrate.

Degradation of the Conhydrine.—The radioactive conhydrine from the plant was diluted with natural (+)-conhydrine.

erythro-**Octane-3,4-diol.**—A solution of conhydrine (200 mg.) in a mixture of ethanol (10 ml.) and methyl iodide (3 ml.) was refluxed in the presence of sodium bicarbonate (0.4 g.) for 24 hr. The filtered reaction mixture was evaporated to small bulk when N-methylconhydrine methiodide separated (390 mg.), m.p. 221–223° (lit.[18] 221°). This methiodide (380 mg.) was dissolved in warm water (20 ml.) and stirred with silver hydroxide (from 0.4 g. of silver nitrate) for 10 min. The filtered solution was evaporated and the residue distilled (200°, 0.01 mm.). The distillate was dissolved in ethanol (20 ml.) and methyl iodide (3 ml.) was added. After standing overnight at room temperature the solution was evaporated and the residue crystallized from a mixture of ethanol and ethyl acetate affording colorless needles of 1-dimethylamino-5,6-epoxyoctane methiodide (331 mg.), m.p. 134–135° (lit.[18] 134–135°). This methiodide (300 mg.) was subjected to a Hofmann elimination using the same conditions as those described for the N-methylconhydrine methiodide. The distillate from the elimination was washed out with methanol (10 ml.) and diluted with water (20 ml.). The solution was made acidic with a few drops of perchloric acid and then stirred at room temperature for 3 days. The solution was then extracted with ether which was dried and evaporated. The residue was dissolved in methanol (10 ml.) and hydrogenated in the presence of platinum oxide (50 mg.) at 40 p.s.i. for 45 min. Evaporation of the filtered methanol solution yielded a solid residue which was crystallized from petroleum ether affording colorless plates of *erythro*-octane-3,4-diol (77 mg., 46% yield from conhydrine), m.p. 96–97° (lit.[19] 98°).

Oxidation of *erythro*-**Octane-3,4-diol.**—The diol (73 mg.) was dissolved in water (50 ml.) containing potassium permanganate (158 mg.) and shaken at 35° for 16 hr. The mixture was then filtered and the filtrate acidified with sulfuric acid and distilled. The distillate was titrated with 0.1 *N* sodium hydroxide (9.7 ml. required) and then evaporated to dryness. The residual sodium salts of valeric and propionic acid were separated by chromatography on silicic acid.[17] Schmidt reactions were carried out on the two acids using sodium azide and concentrated sulfuric acid as previously described. A Kuhn–Roth oxidation was carried out on the valeric acid using the conditions described for the oxidation of coniine, except that the mixture was refluxed for 3 hr. before distillation was carried out. A Kuhn–Roth oxidation of conhydrine using these conditions also afforded only acetic acid.

COMMUNICATIONS TO THE EDITOR

The Zeeman Effect of Nuclear Quadrupole Resonance in Single Crystals of Sodium Bromate and *p*-Dibromobenzene

Sir:

In a preceding paper,[1] a spectrometer for the study of the Zeeman effect of the n.q.r. in the range 100–400 Mc.p.s. was described.

This spectrometer was first tested by measurements on a single crystal of $NaBrO_3$, then *p*-dibromobenzene was studied.

$NaBrO_3$.—This substance has a cubic cell[2] with four pyramidal BrO_3^- groups, each having a ternary symmetry axis through the Br atom, arranged parallel to the four body diagonals of the cube. The Na^+ ions lie also on these axes on the Br side. A single crystal of $NaBrO_3$ should then show, by Zeeman effect, four electric field gradients, all with the same value of q_{zz} and with $\eta = 0$, but with distinct orientations making tetrahedral angles. The single crystals of $NaBrO_3$ were prepared by slowly cooling (0.5° per day) an aqueous saturated solution. The measurements were carried out, at 23°, by a technique already described[3,4] and have fully confirmed the provision, giving, for the unique resonance frequency (~178.9 Mc.p.s.) of ^{79}Br, four directions of *Z*-axis of the electric field gradient all making experimental angles of 109° 28′ ± 9′ and a value of $\eta = 0.001 \pm 0.001$, *i.e.*, zero. These results show the absence of any disturbance in the direction and homogeneity of the external magnetic field which might arise from the heavy brass shielding which contains the sample and the radiofrequency coil.

p-**Dibromobenzene.**—Two values of the asymmetry parameter η are found in the literature: the first, 0.12 ± 0.01, reported by Kojima[5] and the second, 0.05 ± 0.01, given by Shimomura.[6] The crystal structure was determined by Croatto and Bezzi[7] in 1949 by X-ray diffraction. They concluded that *p*-dibromobenzene in the stable phase at room temperature is isomorphous with *p*-dichlorobenzene in the α-phase. It is then monoclinic with two molecules per unit cell. The large discrepancy between the two reported values of η induced us to study again this substance. A single crystal of *p*-dibromobenzene was easily obtained by sealing the melted substance in a glass tube (diameter 12 mm.) terminating with a 5–10 cm. long capillary, as generally used, and letting it

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(6) K. Shimomura, *ibid.*, **14**, 235 (1959).
(7) U. Croatto and S. Bezzi, *Gazz. chim. ital.*, **79**, 240 (1949).