Biosynthesis of the Nicotiana Alkaloids. XIV. The Incorporation of Δ^1-Piperideine-6-^{14}C into the Piperidine Ring of Anabasine[1]

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Abstract: Reaction of Δ^1-piperideine-6-^{14}C with acetoacetic acid yielded isopelletierine which was labeled solely at C-6. This result indicates that the 1,2 double bond in Δ^1-piperideine is not capable of a tautomeric shift to the 1,6 position. The administration of Δ^1-piperideine-6-^{14}C to intact *Nicotiana glauca* plants afforded radioactive anabasine (1.2% incorporation) which had all its activity located at C-6′ of the piperidine ring.

The piperidine ring of anabasine (**7**), the major alkaloid in *Nicotiana glauca*, is derived from lysine. The probable route from lysine to the piperidine ring is illustrated in Scheme I and is strongly supported by feeding experiments with labeled precursors. The administration of lysine-2-^{14}C to *N. glauca* afforded radioactive anabasine which was labeled solely at C-2′ of the piperidine ring.[2] By the use of ^{15}N-labeled lysine, it was established that the nitrogen of the ε-amino group, but not the α-amino group, was incorporated into the piperidine ring.[3] It was thus suggested[4] that lysine (**1**) undergoes α-transamination to yield α-keto-ε-aminocaproic acid (**2**) which cyclizes to Δ^1-piperideine-2-carboxylic acid (**3**). Decarboxylation then yields Δ^1-piperideine (**6**) which is considered to be the immediate precursor of the piperidine ring, condensing at its 2 position with some derivative of nicotinic acid,[5] an established precursor of the pyridine ring of anabasine.[6] Cadaverine-1,5-^{14}C (**4**) was also found to be a precursor of the piperidine ring of anabasine,[7] the alkaloid having half of its activity located at C-2′, the rest presumably being at C-6′. Cadaverine could be converted to Δ^1-piperideine *via* 5-aminopentanal (**5**), a transformation which has been accomplished *in vitro*.[8] Cadaverine is formed by the decarboxylation of lysine in other species.[9] However free cadaverine cannot be an intermediate in the normal biosynthesis of anabasine, since the production of this symmetrical compound from lysine-2-^{14}C would ultimately yield anabasine labeled equally at C-2′ and C-6′, whereas all the activity is found at C-2′. At this time we consider that the utilization of cadaverine in the *N. glauca* plant is an "aberrant reaction."[10]

In order to further substantiate the biosynthetic scheme in Scheme I, it was considered desirable to prepare radioactive Δ^1-piperideine and test it as a precursor of the piperidine ring of anabasine. Shibata and Sankawa[11] reported the use of Δ^1-piperideine-6-^{14}C in a study of the biosynthesis of matrine, but gave no details of its synthesis. We obtained this compound by the following route. The diethyl acetal of 4-chlorobutanal[12] was refluxed in ethanol with potassium cyanide-^{14}C and potassium iodide to afford the diethyl acetal of 4-cyanobutanal. The nitrile was reduced with lithium aluminum hydride yielding the diethyl acetal of 5-aminopentanal. Hydrolysis with 2 *N* hydrochloric acid yielded 5-aminopentanal which cyclized to Δ^1-piperideine-6-^{14}C in the acidic solution. The location of the carbon-14 in the radioactive Δ^1-piperideine was established by treating it with acetoacetic acid to yield isopelletierine (**9**),[13] which was degraded as illustrated in Scheme II. The hydrazone of isopelletierine was heated with sodium ethoxide yielding coniine (**10**) which was degraded as previously described.[14] The mixture of alkenes obtained by a Hofmann reaction on N-methylconiine methiodide (**11**) was hydrogenated over platinum and treated with methyl iodide to afford 1-dimethylaminooctane methiodide (**13**) and 4-dimethylaminooctane methiodide (**14**). The methiodide **13** was subjected to a second Hofmann degradation yielding 1-octene (**12**) which was cleaved with osmium tetroxide and sodium metaperiodate. The resultant formaldehyde was collected as its dimedone derivative and heptanal as its oxime. The activities of isopelletierine and its degradation products are recorded in Table I, and it is apparent that all the activity was located at C-6, indicating that the Δ^1-piperideine was also labeled at C-6. It is also clear that there is no tendency for the double bond in Δ^1-piperideine to tautomerize to the 1,6 position.

The Δ^1-piperideine-6-^{14}C was fed to *Nicotiana glauca*

(1) This investigation was supported by a research grant GM-13246 from the U. S. Public Health Service. Part XIII in this series on the Biosynthesis of the Nicotiana alkaloids is: E. Leete, *Tetrahedron Letters*, 4433 (1968).

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

(3) E. Leete, E. G. Gros, and T. J. Gilbertson, *ibid.*, **86**, 3907 (1964).

(4) E. Leete in "Biogenesis of Natural Compounds," 2nd ed, P. Bernfeld, Ed., Pergamon Press, New York, N. Y., 1967, p 964.

(5) E. Wenkert (*Accounts Chem. Res.*, 1, 78 (1968)) has proposed that the isomeric alkaloid nicotine is formed by a condensation between an N-glycoside of nicotinic acid and the N-methyl-Δ^1-pyrrolinium cation.

(6) M. L. Solt, R. F. Dawson, and D. R. Christman, *Plant Physiol.*, **35**, 887 (1960).

(7) E. Leete, *J. Am. Chem. Soc.*, **80**, 4393 (1958).

(8) K. Hasse and G. Schmid, *Biochem. Z.*, **337**, 69 (1963); K. Hasse and H. Maisack, *ibid.*, **327**, 296 (1955); P. G. J. Mann and W. R. Smithies, *Biochem. J.*, **61**, 89 (1955); H. R. Schütte, D. Knöfel, and O. Heyer, *Z. Pflanzenphysiol.*, **55**, 110 (1966).

(9) K. Hasse, O. T. Ratych, and J. Salnikow, *Z. Physiol. Chem.*, **348**, 843 (1967).

(10) We are only just beginning to realize the potentiality of higher plants to carry out syntheses and degradations which are not part of their normal metabolism. Such reactions are conveniently referred to as aberrant reactions.

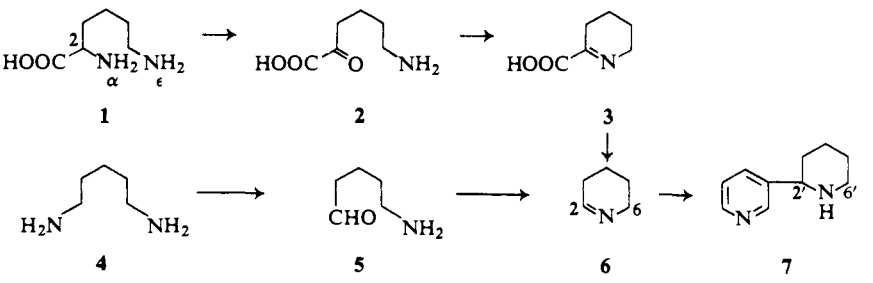
(11) S. Shibata and U. Sankawa, *Chem. Ind.* (London), 1161 (1963).

(12) R. B. Loftfield, *J. Am. Chem. Soc.*, **73**, 1365 (1951).

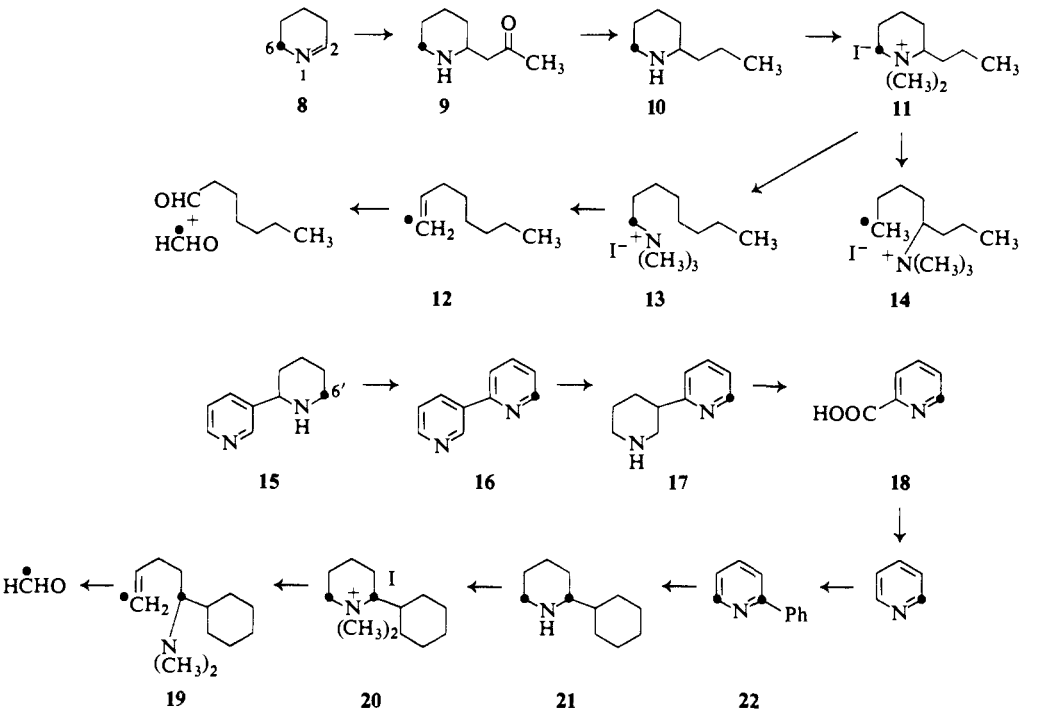
(13) C. Schöpf, F. Braun, K. Burkhardt, G. Dummer, and H. Müller, *Ann.*, **626**, 123 (1959).

(14) E. Leete, *J. Am. Chem. Soc.*, **86**, 2509 (1964).

Scheme I. Hypothetical Biosynthesis of the Piperidine Ring of Anabasine



Scheme II. Degradation of the Isopelletierine and Anabasine Derived from Δ^1-Piperideine-6-^{14}C



plants which were growing in hydroponics, the tracer being added to the inorganic nutrient solution. After 7 days the plants were harvested yielding anabasine as the main alkaloid, with smaller amounts of nicotine and nornicotine. The two latter alkaloids were nonradioactive, but the anabasine had an activity indicating an incorporation of 1.2%. This incorporation is significantly higher than that obtained in anabasine after feeding cadaverine-1,5-^{14}C (0.33%) or lysine-2-^{14}C (0.046%) to *N. glauca* plants growing in hydroponics.[7] If the Δ^1-piperideine-6-^{14}C is incorporated into anabasine by reaction with a nicotinic acid derivative at C-2, all the activity should be located at C-6′ of the piperidine ring. The degradation used to determine the activity at C-6′ is illustrated in Scheme II. Oxidation of anabasine with nitric acid yielded nicotinic acid having negligible activity, indicating that C-2′ of the piperidine ring was inactive. Dehydrogenation of anabasine with silver acetate afforded α,β-dipyridyl (**16**). Smith[15] reported that the reduction of this compound with tin and hydrochloric acid yielded isoneonicotine (2,3′-piperidylpyridine, **17**). We confirmed this remarkably selective reduction, and oxidation of **17** with potassium permanganate yielded α-picolinic acid (**18**). Heating with copper chromite afforded pyridine which was phenylated at the α position with phenyllithium in boiling toluene. It was then our intention to reduce the 2-phenylpyridine (**22**) to 2-phenylpiperidine which could then be oxidized to benzoic acid, the activity of which would represent the activity at the α position of the pyridine. However, hydrogenation of **22** in the presence of Adams catalyst in various solvents yielded only 2-cyclohexylpiperidine (**21**). This was converted to the methiodide **20**, and subjected to a Hofmann degradation. The reaction product was treated with osmium tetroxide and sodium metaperiodate yielding formaldehyde, which could only have originated from the terminal carbon of the alkene **19**. The activity of the formaldehyde represents the average activity of the two α positions of the piperidine ring of anabasine. As indicated in Table I, the specific activity of the formaldehyde dimedone was half that of the anabasine. Since no activity was present at C-2′, all the activity of the anabasine must have been

(15) C. R. Smith, *J. Am. Chem. Soc.*, **53**, 277 (1931).

Table I. Activities of Isopelletierine and Anabasine and Their Degradation Products

Compound	Spec act., dpm/mM × 10^{-4}
Isopelletierine oxime	1.40
Isopelletierine hydrobromide	1.36
Coniine hydrochloride	1.30
N-Methylconiine methiodide	1.32
1-Dimethylaminooctane methiodide	1.37
4-Dimethylaminooctane methiodide	1.32
Formaldehyde dimedone	1.34
Heptanal oxime	<0.01
Anabasine diperchlorate	355
Nicotinic acid	<1
α,β-Dipyridyl diperchlorate	357
α-Picolinic acid	344
Pyridine oxalate	340
2-Cyclohexylpiperidine hydrochloride	342
2-Cyclohexyl-N-methylpiperidine methiodide	340
Formaldehyde dimedone	175

at C-6′. Therefore our results further substantiate the biosynthetic scheme illustrated in Scheme I.

Experimental Section

Melting points are corrected. Elementary analyses were carried out by the Clark microanalytical laboratory, Urbana, Ill. Radioactivity measurements were carried out in a Nuclear Chicago liquid scintillation spectrometer, Model 724, using as solvents either toluene or dioxane–water with the usual scintillators.[16]

Δ^1-Piperideine-6-^{14}C Hydrochloride. The diethyl acetal of 4-chlorobutanal (0.90 g)[12] was refluxed in absolute ethanol (10 ml) with potassium cyanide-^{14}C (0.325 g, 0.5 mCi)[17] and potassium iodide (0.1 g) for 18 hr. The solution was then concentrated to about 5 ml, diluted with water, and extracted with chloroform. The dried (Na_2SO_4) extract was evaporated affording the diethyl acetal of 4-cyanobutanal (total activity = 7.0×10^8 dpm, 65% radiochemical yield). This nitrile was dissolved in ether (40 ml) and 20 ml of 0.5 M lithium aluminum hydride in ether added. The mixture was refluxed overnight and then the excess hydride decomposed with a little water. After refluxing for 1 hr, the mixture was filtered and the filtrate extracted with 2 N hydrochloric acid (three 30-ml portions). Evaporation of this acid extract at 30°, under reduced pressure, afforded Δ^1-piperideine hydrochloride as a pale brown crystalline solid (145 mg, with an activity of 1.80×10^6 dpm/mg = 23% radiochemical yield).

Isopelletierine from Δ^1-Piperideine-6-^{14}C. Δ^1-Piperideine-6-^{14}C hydrochloride (3.0 mg, 5.4×10^6 dpm) was added along with N-chloropiperidine[18] (31 g) to a solution of potassium hydroxide (16 g) in 95% ethanol (160 ml). The mixture was boiled for 10 min and then cooled and filtered. The clear yellow filtrate was evaporated *in vacuo* and the residue was shaken with 50% potassium hydroxide solution and extracted with ether. The dried ($MgSO_4$) ether extract was evaporated and the viscous oil which remained heated on a steam bath for 1 hr. The reaction product was dissolved in hot acetone (60 ml) and on prolonged cooling at 0° the symmetrical trimer of Δ^1-piperideine, α-tripiperideine (11 g), mp 61–62°, separated as colorless plates. After crystallization from acetone the α-tripiperideine had an activity of 158 dpm/mg.

The α-tripiperideine was converted to isopelletierine by reaction with sodium acetoacetate in aqueous solution at pH 11.5.[13] The freshly distilled isopelletierine (bp 93–94° (15 mm)) was converted to its hydrobromide, mp 137–138° (lit.[13] 136–138°), and oxime, mp 99–100° (lit.[13] 95–97°), for radioactive assay.

Degradation of Isopelletierine-6-^{14}C. Isopelletierine (1.118 g) and 95% hydrazine (4 ml) were refluxed for 4 hr. The solution was then cooled, diluted with 50% potassium hydroxide, and extracted with ether. Evaporation of the dried ($MgSO_4$) ether yielded a colorless oil which was dissolved in ethanol (20 ml) in which sodium (1 g) had been previously dissolved. The solution was heated in a sealed tube at 180–200° for 12 hr. The contents of the tube were neutralized with concentrated hydrochloric acid and evaporated to dryness. The residue was made basic with 50% potassium hydroxide and extracted with ether. The ether was extracted with 2 N hydrochloric acid (three 30-ml portions). Evaporation of the acid extract yielded a colorless residue which was crystallized from a mixture of acetone and ether yielding DL-coniine hydrochloride (820 mg, 64%), mp 216–217°, not depressed on admixture with an authentic specimen.

The coniine hydrochloride was degraded as previously described,[14] and the activities of these degradation products are recorded in Table I.

Administration of Δ^1-Piperideine-6-^{14}C to *N. glauca* Plants and Isolation of the Alkaloids. The *N. glauca* plants were grown from seed[19] in soil until they were about 4 months old. They were then transferred to an aerated hydroponic nutrient solution.[2] After 2 weeks many new roots were formed and Δ^1-piperideine-6-^{14}C hydrochloride (50 mg, 9.0×10^7 dpm) was divided equally between four plants. After 7 days, only 2% of the initial activity remained in the nutrient solutions and the plants were harvested. The fresh plants (260 g) were macerated in a Waring Blendor with chloroform (2 l.) and concentrated ammonia (100 ml). The chloroform layer obtained after filtration was evaporated on a rotary evaporator in the presence of 2 N hydrochloric acid (200 ml). The aqueous layer was decanted from tarry material and made basic with ammonia. The mixture was extracted with chloroform. The crude alkaloids obtained on evaporation of the dried ($MgSO_4$) extract were subjected to thin layer chromatography on silica gel G (Merck). Development with a mixture of chloroform, methanol, and concentrated ammonia (60:20:1) indicated the presence of the following alkaloids with the indicated R_f values: nicotine (0.78), anabasine (0.58), and nornicotine (0.38). The alkaloids were detected by spraying with an ethanolic solution of *p*-aminobenzoic acid, followed by exposure to cyanogen bromide vapor. In addition to the above known alkaloids which gave reddish brown spots, a red spot was detected at an R_f of 0.63, and a yellow spot at 0.17. The total crude alkaloids were chromatographed on 2 mm thick plates of silica gel PF-54 (Merck) using the same developing solvent. The main zone containing anabasine was extracted with boiling ethanol and evaporated in the presence of 0.2 ml of 70% perchloric acid. The residue was crystallized from a mixture of ethanol and ethyl acetate yielding anabasine diperchlorate (116 mg with an activity of 9800 dpm/mg). The zone containing nicotine was also extracted with ethanol and evaporated in the presence of picric acid (30 mg). The residue was crystallized from ethanol yielding nicotine dipicrate (6.82 mg). This was diluted with inactive nicotine dipicrate. After several crystallizations the picrate was decomposed with sodium hydroxide and the liberated nicotine extracted with ether. The nicotine was distilled and converted to its diperchlorate. The nicotine diperchlorate had negligible activity. Nornicotine was isolated directly as its diperchlorate (12 mg) and was also inactive.

Degradation of the Radioactive Anabasine. The anabasine was diluted with inactive material prior to the following reactions. Dilutions were also carried out in the course of the following degradation. The activities reported in Table I are calculated for undiluted material.

Nicotinic Acid. This was obtained from anabasine by oxidation with concentrated nitric acid as previously described.[2]

α,β-Dipyridyl. Anabasine diperchlorate (124 mg), sodium acetate dihydrate (400 mg), silver acetate (2.0 g), and 4 ml of 10% acetic acid were heated in a sealed tube at 225° for 15 hr. The contents of the tube were made basic with sodium hydroxide and extracted with ether in a continuous extractor for 22 hr. The residue obtained on evaporation of the ether was distilled (140° (0.1 mm)) affording a colorless oil (35 mg) which was dissolved in ethyl acetate (1 ml). Addition of 0.04 ml of 70% perchloric acid yielded a crystalline precipitate of α,β-dipyridyl diperchlorate (70 mg). Crystallization from ethanol yielded colorless plates, mp 215–216°.

(16) A. R. Friedman and E. Leete, *J. Am. Chem. Soc.*, **85**, 2141 (1963).

(17) Purchased from International Chemical and Nuclear Corp., City of Industry, Calif.

(18) C. Schöpf, A. Komzak, F. Braun, and E. Jacobi, *Ann.*, **559**, 1 (1948).

(19) The author thanks Professor T. A. Geissman of the University of California for collecting the seeds in the Santa Monica Mountains.

Anal. Calcd for $C_{10}H_8N_2 \cdot 2HClO_4$: C, 33.63; H, 2.82; N, 7.85. Found: C, 34.13; H, 3.06; N, 7.60.

α-Picolinic Acid. α,β-Dipyridyl diperchlorate (466 mg) was dissolved in water, made basic with potassium hydroxide, and extracted with ether. The residue obtained on evaporation of the ether was dissolved in concentrated hydrochloric acid (5 ml) and granulated tin (1.5 g) was added. The mixture was heated on a steam bath overnight during which time all the tin dissolved. The solution was evaporated to dryness; the residue was made basic with 10% sodium hydroxide. A continuous ether extraction for 12 hr yielded a colorless oil which was free of α,β-dipyridyl (by thin layer chromatography). This oil was refluxed with potassium permanganate (2.0 g) in water (50 ml) for 2 hr. Excess permanganate was destroyed with a little ethanol and the mixture filtered. The filtrate was adjusted to pH 3.2 by the addition of hydrochloric acid and evaporated to dryness. The residue was extracted several times with boiling benzene. Evaporation of the benzene yielded a white residue which was sublimed (140° (0.01 mm)) affording α-picolinic acid (62 mg), mp 132–134°, not depressed on admixture with an authentic specimen.

2-Phenylpyridine. α-Picolinic acid (200 mg) was mixed with copper chromite (300 mg) and heated at 300° in a current of nitrogen. The volatile products from the reaction were passed into ether, cooled to −80°. The contents of the trap were added to a solution of oxalic acid (200 mg) in ether, when pyridine oxalate (180 mg), mp 150–151°, separated. The pyridine oxalate (160 mg) was dissolved in water (0.2 ml). Powdered potassium hydroxide (1 g) and ether (10 ml) were added. After standing for 1 hr the mixture was filtered into a flask containing a stirred solution of phenyllithium (160 mg) in a mixture of ether (1 ml) and toluene (4 ml). The contents of the flask were distilled until all the ether was removed, and then the reaction mixture refluxed for 16 hr. After cooling, water was added and the mixture was extracted with ether. The ether layer was extracted with 2 *N* hydrochloric acid (two 10-ml portions), which was then made basic with potassium hydroxide, extracted with ether, and dried over sodium sulfate. Evaporation of the ether and distillation of the residue (140° (0.1 mm)) yielded a colorless oil (26 mg) which was dissolved in ethanol (2 ml), and mixed with a solution of picric acid (50 mg) in ethanol (2 ml), when 2-phenylpyridine picrate separated as bright yellow needles (68 mg), mp 177–178° (lit.[20] 175°).

2-Cyclohexylpiperidine. 2-Phenylpyridine picrate (63 mg) was dissolved in 2 *N* hydrochloric acid (10 ml) and the solution was extracted with ether until colorless. The aqueous solution was evaporated to dryness and the residue dissolved in ethanol (20 ml) and was hydrogenated in the presence of Adams catalyst (0.1 g) at 2 atm pressure for 2 hr. The filtered reaction mixture was evaporated to dryness and the residue crystallized from a mixture of ethanol and ethyl acetate, yielding colorless needles of 2-cyclohexylpiperidine hydrochloride (25 mg), mp 256–257° (Salathiel, *et al.*, reported[21] melting points of 197–198 and 250° for isomorphous forms of this compound, obtained by a different method).

Anal. Calcd for $C_{11}H_{22}NCl$: C, 64.84; H, 10.89; N, 6.87. Found: C, 64.64; H, 10.87; N, 6.86.

N-Methyl-2-cyclohexylpiperidine Methiodide. 2-Cyclohexylpiperidine hydrochloride (20 mg), sodium bicarbonate (80 mg), methyl iodide (2 ml), and ethanol (10 ml) were refluxed for 16 hr, and then evaporated to dryness. The residue was extracted with boiling chloroform, which on evaporation yielded the methiodide. Crystallization from a mixture of ethanol and ethyl acetate yielded colorless plates of N-methyl-2-cyclohexylpiperidine methiodide (32 mg), mp 252–253°.

Anal. Calcd for $C_{13}H_{26}NI$: C, 48.30; H, 8.11; N, 4.33. Found: C, 48.27; H, 8.19; N, 4.25.

The methiodide (30 mg) was dissolved in water (5 ml) and shaken with silver hydroxide (from 0.2 g of silver nitrate), filtered, and evaporated. The residue was distilled at 180° (0.1 mm) into a trap cooled to −80°. The trap was washed out with ether (20 ml), and a drop of pyridine and osmium tetroxide (50 mg) was added. After standing overnight at room temperature the dark brown solution was evaporated and the residue was boiled with a solution of sodium sulfite (0.5 g) in methanol (10 ml) and water (10 ml) for 1 hr. The filtered solution was evaporated and the residue was extracted with ether. The evaporated ether extract was shaken with sodium metaperiodate (100 mg) in water (20 ml) for 20 min, and then distilled into a solution of dimedone (50 mg) in water (50 ml). After standing overnight at 0°, the formaldehyde dimedone derivative separated (15 mg), and after crystallization from aqueous methanol had mp 191–193°, not depressed on admixture with an authentic specimen.

(20) K. Ziegler and H. Zeiser, *Ber.*, **63**, 1847 (1930).

(21) R. Salathiel, J. M. Burch, and R. H. Hixon, *J. Am. Chem. Soc.*, **59**, 984 (1937).