[CONTRIBUTION FROM THE PROTECTIVE DEVELOPMENT DIVISION, U. S. ARMY CHEMICAL RESEARCH AND DEVELOPMENT LABORATORIES AND THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF DELAWARE]

Observations on the Geometrical Isomerism of Formyl-1-methylpyridinium Iodide Oximes; Carbinolamine Intermediates[1]

EDWARD J. POZIOMEK,[2] DAVID N. KRAMER, BERNARD W. FROMM, AND WILLIAM A. MOSHER

Received March 18, 1960

The synthesis and structure of reported formyl-1-methylpyridinium iodide *syn* and *anti* oximes referred to as A and B series respectively, are examined in detail. The reaction of 2-formyl-1-methylpyridinium iodide with hydrazine and phenylhydrazine is found to give compounds comparable in physical and chemical properties to the A series. It is shown that the A series type compounds are carbinolamine intermediates in the formation of oximes or hydrazones. Previous assignments of geometrical configuration to the A and B series compounds (derived from hydroxylamine) on the basis of a comparison of their physical and chemical properties are thereby invalidated.

Ginsburg and Wilson[3] have reported the preparation of geometrical isomers of 2(3 and 4)-formyl (and keto)-1-methylpyridinium iodide oximes.[4] They compared melting points, acidities and solubilities of an unstable A and a stable B series[5] with pyrrole and benzene oxime derivatives and conjectured the configuration by stating, "It would thus appear by analogy that our A series has the *syn* and our B series the *anti* configuration." The A series was prepared by treating the aldehyde or ketone methiodide with alcoholic hydroxylamine at −5°. The compounds obtained by this method were described as white to pale yellow solids which changed to the B series compounds slowly at room temperature and very rapidly in alkaline aqueous solution. The B series was obtained by oximation of quaternary aldehyde or ketone at room temperature, or by methylation of the corresponding tertiary oxime.

The B series pyridinium oximes such as 2-formyl-1-methylpyridinium iodide oxime[6] (I) have been employed in the treatment of poisoning by the organophosphorus anticholinesterases. A number of bisquaternary pyridine oximes have also been reported[7] and resemble the B series in physical properties and chemotherapeutic activity. Wilson has offered the hypothesis that the chemotherapeutic activity of formylpyridinium oximes is dependent on their ability to associate strongly with inhibited enzyme at the site of phosphorylation, and that in the association complex the reactive oximino function is properly oriented for the displacement of the phosphate or phosphonate grouping from the inhibited enzyme. The *anti* configuration of 1-alkyl-2(and 4)-formylpyridinium halide oximes satisfied geometrical dispositions defined by Wilson[8] for the nucleophilic displacement of the phosphorus moiety and appeared to coincide with enhanced activity of the B series.[8] Only 2-formyl-1-methylpyridinium iodide oxime was claimed sufficiently stable in the A or "*syn* series" for study and was not found active in reactivating inhibited acetylcholinesterase.[8]

Our attempts to repeat the preparation of the A series of "oximes" yielded colorless solids similar to the ones described by Ginsburg and Wilson.[3] These materials were extremely unstable and yielded the B series before characterization could be made through either elemental analyses or spectrophotometry. The inability to characterize adequately the unstable products led us to examine the synthesis and structure of the A series in more detail.

A number of attempts to synthesize the A series compound from 2-formyl-1-methylpyridinium iodide (II) and hydroxylamine produced compounds which varied in stability at room temperature from a few minutes to several days. The variation in the rate of conversion of the A to the B series compound was obviated by use of exactly neutral hydroxylamine solutions. The more stable A series products were easily handled at room temperature and recrystallization from alcohol-ether

(1) Presented in part at the meeting in miniature of the Maryland Section, American Chemical Society, May 1960.

(2) (a) To whom inquiries should be directed at Army Chemical Center, Md. (b) This paper is constructed in part from a dissertation by E. J. Poziomek to be submitted to the faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy. (c) Work done at Army Chemical Center, Md.

(3) S. Ginsburg and I. B. Wilson, *J. Am. Chem. Soc.*, 79, 481 (1957).

(4) The naming of compounds throughout this paper is in accordance with the principles of the system of nomenclature currently used by *Chemical Abstracts*.

(5) The terms A and B series as used throughout this paper refer to colorless solids melting below 150° and yellow solids melting above 150°, respectively.

(6) This compound has been commonly referred to in the pharmacological and biochemical literature as 2-pyridinealdoxime methiodide or 2-PAM. For historical references see I. B. Wilson, *Biochim. et Biophys. Acta*, 27, 196 (1958).

(7) (a) E. J. Poziomek, B. E. Hackley, Jr., and G. M. Steinberg, *J. Org. Chem.*, 23, 714 (1958). (b) F. Hobbiger, D. G. O'Sullivan, and P. W. Sodler, *Nature*, 182, 1498 (1958). (c) E. Bay, S. Krop, and L. F. Yates, *Proc. Soc. Exp. Biol.*, 98, 107 (1958). (d) W. K. Berry, D. R. Davies, and A. L. Green, *Brit. J. Pharmacol.*, 14, 186 (1959). (e) F. Hobbiger and P. W. Sodler, *Brit. J. Pharmacol.*, 14, 192 (1959).

(8) I. B. Wilson, *Federation Proc.*, 18, 752 (1959).

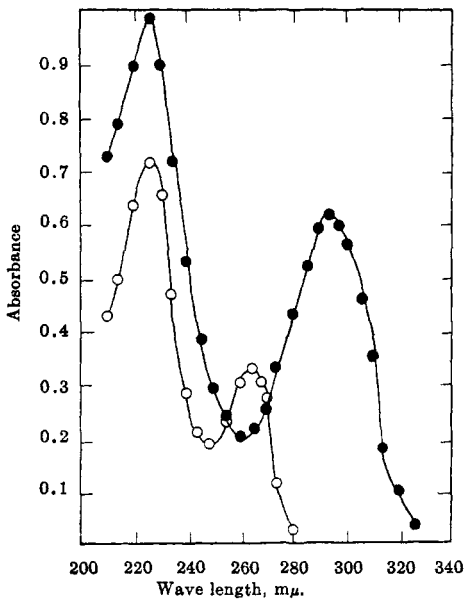


Fig. 1. Absorption spectra of A series ($5.0 \times 10^{-5}M$, open circles) and B series ($5.0 \times 10^{-5}M$, filled circles) products from the reaction of 2-formyl-1-methylpyridinium iodide (II) with hydroxylamine. Both are dissolved in water, *p*H 6.5

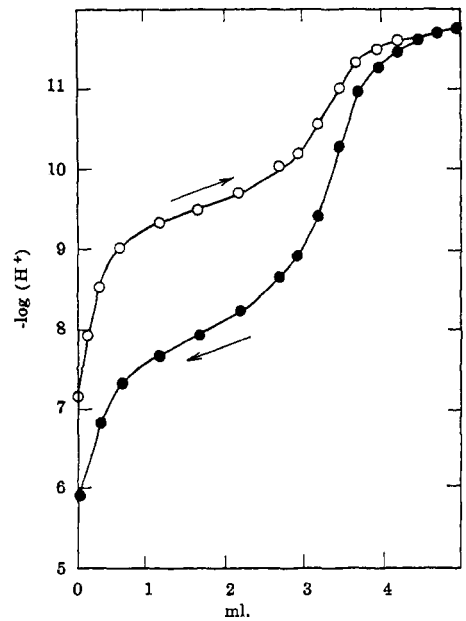


Fig. 2. Potentiometric titration of the A series product (from 2-formyl-1-methylpyridinium iodide and hydroxylamine) with 0.1*N* sodium hydroxide (open circles) followed by a back titration with 0.1*N* hydrochloric acid (filled circles); ml., volume of acid or base used

gave microscopic needles with an analysis corresponding to a monohydrate of the expected oxime. Fig. 1 illustrates the ultraviolet absorption spectra of A and B series products from the reaction of II with hydroxylamine. The marked absorption in the region 225 mμ was assigned to the iodide ion (λmax 2262 Å in water[9]). The wave length of maximum absorption of the A series compound was found to be 264 mμ, corroborating the 263 mμ band reported by Ellin and Kondritzer.[10] Fig. 2 illustrates the titration of the A series compound with aqueous base followed in five minutes by a back titration with acid. The back titration curve delineates neutralization of the conjugate base of the oxime. If the titration of the A series compound was not performed rapidly a pK_a value equivalent to that of the B series oxime was found. The initial titration depicted in Fig. 2 (approximate pK_a, 9.5) was completed within five minutes but even then the reported pK_a value of 9.9 was not substantiated.

Other nitrogen nucleophiles, namely phenylhydrazine and hydrazine, were treated with II using the methods previously described for hydroxylamine. Phenylhydrazine gave two products corresponding to the A and B series while hydrazine gave only the "isomer" resembling the A series. Though *syn* and *anti* tertiary pyridine carboxaldehyde phenylhydrazones are known[11] only one quaternary isomer has been reported.[12] The colorless A product from phenylhydrazine had an analysis corresponding to a monohydrate and was converted to the yellow-orange B form very slowly at room temperature and more rapidly at elevated temperatures. Dilute acid catalyzed the conversion while dilute base yielded a dark red precipitate, presumably the conjugate base of the quaternary hydrazone.[13] Neutralization of the conjugate base with acid also yielded the B isomer; back titration with sodium hydroxide reprecipitated the red compound. The B isomer was also obtained by alkylating *syn*-picolinaldehyde phenylhydrazone with methyl iodide. The reactions using phenylhydrazine analogs are summarized in Scheme I. The compound obtained from hydrazine was colorless, decomposed at approximately 120°, and had an analysis corresponding to a monohydrate. In contrast to the A series derived from hydroxylamine or phenylhydrazine, it could be stored at room temperature without appreciable change. Reaction of the colorless hydrate with dilute acid or base at room temperature had no visible effect.

In an endeavor to characterize more thoroughly the reaction products of II with nitrogen nucleophiles, ultraviolet absorption was measured (Table I). The ultraviolet absorption data found by

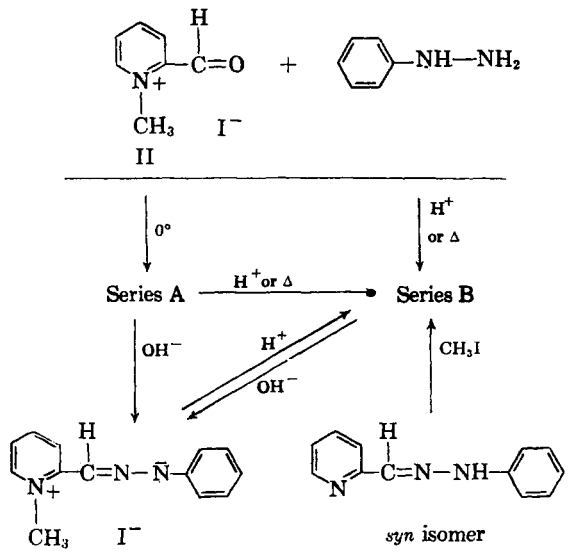
(9) E. M. Kosower, R. L. Martin, and V. M. Meloche, *J. Chem. Phys.*, **26**, 1353 (1957).

(10) R. I. Ellin and A. A. Kondritzer, *Anal. Chem.*, **31**, 200 (1959).

(11) D. Schulte-Frohlinde, R. Kuhn, W. Münzing, and W. Otting, *Ann.*, **622**, 43 (1959).

(12) G. H. Lenart, *Ann.*, **410**, 104 (1915).

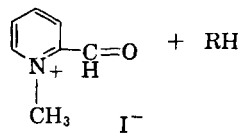
(13) The strong electron withdrawing effect of the pyridinium ring was illustrated by the pK_a of the quaternary hydrazone found to be in the range of 10 to 10.5 in a 1:1 methanol-water solution. The pK_a value of the corresponding oxime also determined in a 1:1 methanol-water solution using the same molar concentrations was found to be 8.2.



SCHEME I

TABLE I

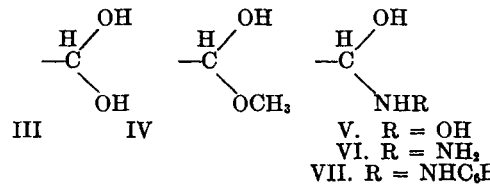
ULTRAVIOLET ABSORPTION OF PRODUCTS FROM 2-FORMYL-1-METHYLPYRIDINIUM IODIDE REACTIONS



RH Reactant	Compound	Series	λmax, mμ (log ε)	Ref.
H_2O	III	..	263(3.79)	10
CH_3OH	IV	..	264(3.82)	14
NH_2OH	V	A	263(3.79)	10
NH_2OH	V	A	264(3.82)[a]	..
NH_2NH_2	VI	A	264(3.82)[a]	..
$C_6H_5NHNH_2$	VII	A	264(3.92)[a]	..
NH_2OH	I	B	292(4.09)	10
NH_2OH	I	B	293(4.09)[a]	..
$C_6H_5NHNH_2$	VIII	B	408(4.49)[a]	..

[a] Water, *p*H 6.5.

Ellin and Kondritzer[10] for the A and B series compounds derived from hydroxylamine and II are also included in Table I. These workers in a "quantitative estimation of the *syn* and *anti* configurations" reported without comment that the ultraviolet absorption spectrum of the A "isomer" was identical with the parent quaternary pyridine carboxaldehyde. Neither elemental analyses, nor any other characterization of the A series compound were presented by Ellin and Kondritzer.[10] In a cognate spectral study of the isolated reaction products of quaternary pyridine carboxaldehydes with alkanols it was shown that hemiacetals such as IV rather than molecular complexes were formed.[14] It was also observed that the ultraviolet absorption of quaternary pyridine carboxaldehyde dissolved in water was not that of a true carbonyl function but that of a *gem*-glycol (III). In fact, ultraviolet absorption spectra do not differentiate the *gem*-glycol, hemiacetal, and A series of compounds. It would be expected on comparing the vastly different spectra of *syn*-picolinaldehyde oxime[15] and phenylhydrazone[11] that the absorption spectra between the various A series compounds would be quite different. Furthermore, the small differences between maximum absorption bands of isomeric furfuraldehyde oximes (Δ5 mμ, water),[16] benzaldehyde oximes (Δ6 mμ, ethanol)[17] and picolinaldehyde phenylhydrazones (Δ15 mμ, ethanol)[11] would not predict the large differences in absorption found between the A and the B series derived from hydroxylamine (Δ30 mμ, Fig. 1) or phenylhydrazine (Δ 144 mμ). In view of the spectral data carbinolamine structures (V–VII) are proposed for the A series[18]; elemental



analyses of the reaction products of hydroxylamine, hydrazine, and phenylhydrazine with II agree closely with calculated values for hydroxaminohydroxymethyl (V), hydrazinohydroxymethyl (VI), and hydroxyphenylhydrazomethyl (VII) substituted pyridinium salts, respectively. The B series compounds (assigned an oxime or hydrazone structure) were obtained by direct quaternization of the *syn*-picolinaldehyde oxime or phenylhydrazone. A summary of the reactions discussed using hydroxylamine analogs is presented in Scheme II.

The identification of the A series compounds as carbinolamines is consistent with the generally accepted mechanism for oxime and hydrazone formation. Jencks[20] in a recently published study on the mechanism of oxime and semicarbazone formation presented evidence through ultraviolet and infrared absorption for the reaction of a number of aldehydes and ketones to form carbinol-

(14) G. M. Steinberg, E. J. Poziomek, and B. E. Hackley, Jr., *J. Org. Chem.*, **26**, 368 (1961).

(15) P. Grammatcakis, *Bull. soc. chim. France*, 109 (1956).

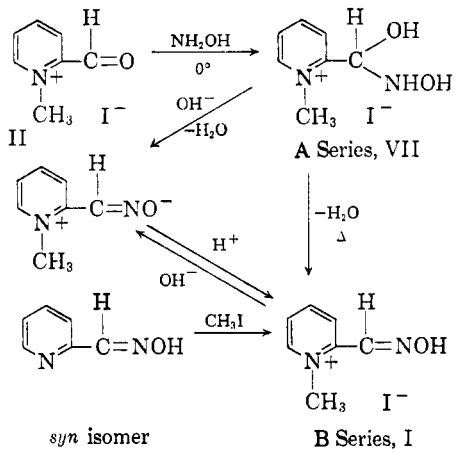
(16) R. F. Raffauf, *J. Am. Chem. Soc.*, **68**, 1765 (1946).

(17)(a) J. Meisenheimer and O. Dorner, *Ann.*, **502**, 156 (1933). (b) R. F. Rekker and J. U. Veenland, *Rec. trav. chim.*, **78**, 739 (1959).

(18) Ginsburg and Wilson[3] characterized the A series as oximes through a qualitative test[19] the first step of which depended on hydrolysis with concentrated HCl to hydroxylamine hydrochloride. V would also be expected to give positive tests through hydrolysis to II and hydroxylamine. Ginsburg and Wilson[3] recorded slightly low but acceptable iodide analyses for the A series. It is conceivable that the samples lost water before analysis through either drying or standing at room temperature.

(19) F. Feigl, *Spot-Tests*, Vol. II, Elsevier Press, New York, 1954, p. 161.

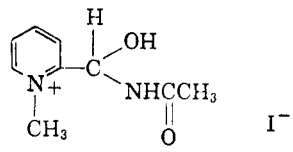
(20) W. P. Jencks, *J. Am. Chem. Soc.*, **81**, 475 (1959).



SCHEME II

amine addition compounds. References were provided to other literature reports suggesting the carbinolamine addition type intermediate. A further analogy may be drawn between the carbinolamine type compounds postulated for the A series and the product formed from chloral hydrate and semicarbazide. It was found that reaction proceeded with the elimination of only one molecule of water.[21] The structure of the product was indicated from infrared analysis by Chang and Ulbricht[21] to be a carbinolamine.

Isolation of the carbinolamine adduct was not limited to the reaction of hydroxylamine, hydrazine, or phenylhydrazine with II, but was also observed using an amide and appeared to occur with a primary amine. Reaction of II with *n*-butylamine in the cold yielded an unstable colorless solid; reaction of II with acetamide yielded a stable, colorless solid whose elemental analyses corresponded to 2-(acetamidohydroxymethyl)-1-methylpyridinium iodide, IX.



IX

The consistent color differences between the colorless A and the yellow B series may be attributed to a variation in strength of a charge transfer complex arising from the "transfer" of an electron of the iodide atom to the π-electron system of the pyridinium ring. The oxime or hydrazone substituent being more effective at electron withdrawal than the carbinolamine function may thus increase electron affinity of the pyridinium ring and its ability to form charge-transfer complexes. Analogy for this phenomenon may be drawn from the studies by Kosower[22] of substituted 1-methylpyridinium iodides.

The A and B series of "oximes" have been used for molecular complementarity studies in alkylphosphate poisoning,[8] and in quantitative ultraviolet analysis,[10] as possessing *syn* and *anti* configurations, respectively. These configurations were based on a comparison of physical and chemical properties of the two series with oximes of the benzene and pyrrole systems; since it has been found that the A series is a carbinolamine, reference to the configuration of the B series on this basis is invalidated.

Work is in progress to synthesize geometrically isomeric oximes in the pyridine series and will be the subject of a future communication.

EXPERIMENTAL[23]

2-(Hydroxaminohydroxymethyl)-1-methylpyridinium iodide. (A series, 2-formyl-1-methylpyridinium iodide oxime[2]) (V). The method of Ginsburg and Wilson[3] for A series formyl (and keto)-1-methylpyridinium iodide oximes was exactly followed in that, "A methanolic solution of hydroxylamine was prepared by dissolving hydroxylamine hydrochloride (30% excess) in a minimum amount of warm methanol and neutralized with methanolic potassium hydroxide. After cooling the potassium chloride was filtered off, the solution was cooled to −5° with an ice–sodium chloride mixture and the pyridine aldehyde (or ketone) methiodide was added in small portions under vigorous stirring and cooling. The orange-yellow salt gradually dissolved and white precipitate appeared. The mixture was kept at −10 to −12° for 20 min., then filtered on a well cooled funnel, and washed quickly with cold absolute ether. Where the compound was too soluble in cold methanol, cold absolute ether was added to precipitate the product."

A near colorless solid from 2-formyl-1-methylpyridinium iodide[3,10,14] and hydroxylamine was obtained as described by the previous authors.[3] This material decomposed within minutes at room temperature to a yellow solid. The A series compound melted at approximately 100°, (exact melting point varied within a 10° range depending on the rate of heating; 105–106° reported[3]) resolidified to a yellow solid and finally decomposed between 200 and 220° (224–225° reported for B series 2-formyl-1-methylpyridinium iodide oxime,[3] I). The rapid decomposition of the A series compound at room temperature prevented accurate elemental analysis or pK_a determination.

In view of the greater stability of the A series compound[10] in acid solution the parent quaternary carboxaldehyde was allowed to react with an excess of partially neutralized hydroxylamine hydrochloride in lieu of neutral hydroxylamine. This, however, did not tend to increase stability of the product but only complicated product recovery by a coprecipitation of hydroxylamine hydrochloride. For this

(21) P. K. Chang and T. L. Ulbricht, *J. Am. Chem. Soc.*, 80, 976 (1958).

(22) (a) E. M. Kosower, *J. Am. Chem. Soc.*, 77, 3883 (1955). (b) E. M. Kosower and P. E. Klinedinst, Jr., *J. Am. Chem. Soc.*, 78, 3493 (1956). (c) E. M. Kosower, *J. Am. Chem. Soc.*, 78, 3497 (1956).

(23) All melting points were determined using a Fisher-Johns melting point apparatus and are uncorrected. Ultraviolet absorption spectra were determined with the aid of a Beckman quartz spectrophotometer, model DU. All pK_a values were determined at room temperature (25–27°), from potentiometric titration data, assuming pK_a to be the pH of half neutralization. In each case approximately 100 mg. of sample dissolved in 10 ml. of water (or other solvent as indicated) was titrated with 0.1*N* sodium hydroxide.

reason and because of the catalytic effect of base, the use of an exactly neutral solution of hydroxylamine is recommended. After a number of syntheses it was also found that if the addition of ether was avoided a purer product was obtained. It was found convenient to concentrate the methanolic solution of hydroxylamine below room temperature using a rotating type evaporator before adding the carboxaldehyde. The purer carbinolamine sample could be handled easily at room temperature and was recrystallized from methanol-ether to give (25%) colorless microscopic needles, m.p. 107–109° dec.

Anal. Calcd. for $C_7H_{11}IN_2O_2$: C, 29.8; H, 3.9; O, 11.4; neut. equiv., 282. Found: C, 29.6; H, 3.7; O, 11.2; neut. equiv., 280; pK_a *ca.* 9.5.

On standing 2 days at room temperature in a capped vial or overnight at room temperature under vacuum (25 mm.), the carbinolamine turned pale yellow, but still melted below 110°. If the carbinolamine was refluxed in methanol for 30 min. the B series oxime (m.p. 224–225°) formed.

2-(Hydrazinohydroxymethyl)-1-methylpyridinium iodide (VI). To 2.0 g. (0.008 mole) of 2-formyl-1-methylpyridinium iodide in 20 ml. of methanol was added at 15° 1.0 g. (0.02 mole) of hydrazine hydrate (85% aqueous solution used). The pale yellow mixture was stirred for 30 min., then cooled to 5°, and filtered to give 2.1 g. (93.5% yield) of a colorless solid, m.p. 127–128° dec. The material was red at 110° and purple at 115° before finally decomposing.

Anal. Calcd. for $C_7H_{12}IN_3O$: C, 29.9; H, 4.3; O, 5.7. Found: C, 29.9; H, 4.4; O, 5.7.

Attempted preparation of 2-formyl-1-methylpyridinium iodide hydrazone. In an attempt to prepare the hydrazone, the above reaction of hydrazine hydrate with 2-formyl-1-methylpyridinium iodide was repeated in boiling methanol. A red crystalline material, 1.0 g., separated on cooling, m.p. 158–160° dec.

Anal. Calcd. for $C_7H_{10}IN_3 \cdot 1/2H_2O$; C, 30.9; H, 4.1; O, 2.9. Found: C, 30.2; H, 4.4; O, 2.4. An attempt to dehydrate the isolated 2-(hydrazinohydroxymethyl)-1-methylpyridinium iodide by heating to 100° or acid and base catalysis yielded red products, whose elemental analysis seemed to indicate incomplete dehydration.

2-(Hydroxyphenylhydrazomethyl)-1-methylpyridinium iodide (VII). To 2.5 g. (0.01 mole) of 2-formyl-1-methylpyridinium iodide in 25 ml. methanol was added at 5° 1.2 g. (0.011 mole) of phenylhydrazine. After 15 min. of standing, ether was added and an oil precipitated. On stirring a few minutes the oil solidified and filtration gave 3.0 g. (83.4% yield) of a nearly colorless solid. On standing in the air the product turned pale yellow but this could be avoided by immediately placing product into vials and storing in the refrigerator. Using a Fisher hot stage melting point apparatus and a slow rate of heating no discernible melting point and only a marked color change to orange was observed in the area of 100°. The sample finally melted with decomposition at 240–243° (239–240° reported[12] for 2-formyl-1-methylpyridinium iodide phenylhydrazone).

Anal. Calcd. for $C_{13}H_{16}IN_3O$: C, 43.7; H, 4.5; O, 4.5. Found: C, 44.2; H, 4.7; O, 4.1.

2-Formyl-1-methylpyridinium iodide phenylhydrazone (VIII). To 2.0 g. (0.01 mole) of *syn* 2-pyridinecarboxaldehyde phenylhydrazone[5] in 100 ml. of ethanol was added 4.0 g. (0.03 mole) of methyl iodide. The mixture was refluxed for 3 hr., then cooled and filtered to give 3.0 g. (92% yield) of a bright orange salt, m.p. 245–247° dec. (239–240° reported[10]).

Anal. Calcd. for $C_{13}H_{14}IN_3$: C, 46.0; H, 4.1. Found: C, 46.2; H, 4.3.

The same product as confirmed by ultraviolet analysis and melting point was obtained by reacting 2-formyl-1-methylpyridinium iodide with phenylhydrazine in hot alcohol for 30 min. It was also obtained by heating 2-(hydroxyphenylhydrazomethyl) - 1 - methylpyridinium iodide alone or in the presence of an acid catalyst. Room temperature alone was sufficient to effect a slow dehydration. Reaction of the carbinolamine product with aqueous base in alcohol yielded a red solution which on neutralization with acid also yielded the same phenylhydrazone (in water yielded a red precipitate, presumably a hydrate of the conjugate base of the desired phenylhydrazone.

Anal. Calcd. for $C_{13}H_{13}N_3 \cdot 2.9H_2O$: C, 59.3; H, 7.1. Found: C, 59.3; H, 6.6).

Lenart[12] reported that when 2-formyl-1-methylpyridinium iodide phenylhydrazone was recrystallized from water, hydrate formation took place. This hydration does not alter the ultraviolet wave-length bands of maximum absorption and should not be mistaken for a carbinolamine product. The hydrate is bright orange and turns orange-red on heating. It was also found that the unhydrated hydrazone is strongly fluorescent under an ultraviolet lamp.

2-(Acetamidohydroxymethyl)-1-methylpyridinium iodide (IX). To 5.9 g. (0.01 mole) of molten acetamide was added 1.0 g. (0.004 mole) of 2-formyl-1-methylpyridinium iodide. The mixture was heated on a steam bath until complete solution was evident, then cooled to room temperature. Stirring with 100 ml. of 1:1 ether-acetone followed by filtration gave 1.0 g. (32%) of a colorless solid m.p. 143–146° dec.

Anal. Calcd. for $C_9H_{13}IN_2O_2$: C, 35.1; H, 4.3; N, 9.1. Found: C, 35.1; H, 4.3; N, 9.1.

Acknowledgment. The authors wish to express their gratitude to the Analytical Research Branch of the Research Directorate, U. S. Army Chemical Warfare Laboratories, for the analyses here reported.

ARMY CHEMICAL CENTER, MD., AND NEWARK, DEL.