STUDIES ON NICOTINAMIDE

BY

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STUDIES ON THE ACID HYDROLYSIS
OF
α-HALOGENATED PYRIDINE COMPOUNDS
During a recent investigation of the chemistry of Coenzyme I and II the methiodides of 2- and 6- fluoronicotinamides were required. Reaction of the corresponding fluoronicotinamides with excess methyl iodide under pressure, followed by recrystallization from water, was found to give only the hydroxynicotinamides. When ethyl iodide was substituted for methyl iodide no reaction whatever occurred. The corresponding bromonicotinamides were unreactive toward either halide.

It was further observed that the successful preparation of the fluoronicotinamides appeared to be dependent upon the presence of a small amount of thionyl chloride. Frequently, when all traces of thionyl chloride were removed from the acid chloride, hydrolysis occurred during the initial recrystallization from water. When this was not done, hydrolysis did not occur. Hydroxynicotinamides were also isolated as byproducts of the successful preparation of the amides. No such difficulty was observed in the preparation of the bromonicotinamides.

Because of these observations, a study of the relative ease of acid hydrolysis of various halogens when substituted in the $\beta$-position in pyridine was undertaken. Although it has been known for some time that $\alpha$- and $\gamma$-halogen on pyridine is more reactive than aromatic halogen, relatively little systematic work has been done on the subject. Skraup (1) found that $\alpha$-chloroquinoline was hydrolysed to carbo-styril by dilute acid at 120°. Later Decker (2) found that
ten minutes at the boiling point sufficed for the acid
hydrolysis of 8-nitro-2-chloroquinoline. He suggested that
the formation of the strongly positive quaternary nitrogen
was in part responsible for the ease of hydrolysis. The
results of Wibaut (3), who found that N-4-pyridyl-4-chloro-
pyridinium chloride and its bromine analogue are hydrolysed
to the corresponding pyridones by dilute acid at room tem-
perature, support this hypothesis. Rath (4) has reported
that 2-chloropyridine, 5-nitro-, 5-chloro-, and 3-chloro-5-
nitro-2-chloropyridines are all hydrolysed to the corres-
ponding pyridones by hydrochloric acid at 150°. Bobranski
(5) found that 4-chloroquinoline was converted to 4-quin-
lone on acid treatment at 150°, and Wibaut (3) reported that
2,6-dibromopyrididine could be cleaved by acid at 150° but not
at 100°.

These facts suggest that while α- and γ-halogen on
the pyridine nucleus is somewhat activated, the activation
is not as great as some authors have supposed in the past.
The electronic similarity of pyridine and nitrobenzene pro-
posed by Erlenmeyer⁶ (6) on biochemical grounds provides a
frame of reference from which to interpret the reactivity of
α- and γ-halogen. On this basis the variation in lability
becomes more intelligible. Just as the chlorine of α- or
p-nitrochlorobenzene is more reactive than that of chloro-
benzene, so is that of α- or γ-chloropyridine. Just as
the halogen of 2,4-dinitrochlorobenzene is more reactive
than that of nitrochlorobenzene, so is the halogen of
$\text{H}^+ \text{F} + \text{H}_3\text{O}^+ \rightleftharpoons \text{H}^+ \text{F}^{\cdot} \text{HOH}_2^\cdot$ 

$\text{H}_2\text{O} \rightarrow \left[ \begin{array}{c} \text{H}^+ \text{OH} \\ \text{H} \end{array} \right] \left[ \begin{array}{c} \text{H}^+ \text{OH} \\ \text{H} \end{array} \right] + \text{FH-OH}_2^\cdot + \text{H}_3\text{O}^+$
8-nitro-2-chloroquinoline more reactive than that of 2-chloroquinoline.

Although Wibaut (3) has reported the hydrolysis of 2,6-dibromopyridine to 2-bromo-6-hydroxypyridine by means of alcoholic sodium hydroxide, on the whole alkaline hydrolysis is much less readily effected, as the experimental results reported below will show. None of the fluoro- or bromonicotinic acids or picolines showed appreciable alkaline hydrolysis. This is to be expected since the activating quaternary nitrogen is present in acid but not in basic solution. In confirmation it was found that 2-iodo-3-methyl- and 2-iodo-5-methyl pyridine ethiodides were readily hydrolysed to the corresponding N-ethylpicolones in good yield by means of warm sodium hydroxide solution.

A study of the hydrolysis of a series of pyridine derivatives was undertaken to see if an explanation of the fluorine anomaly would be forthcoming. Refluxing with 6N hydrochloric acid for twenty-four hours was selected as the standard treatment. Increasing the time to forty-eight or seventy-two hours did not change the results summarized in Table I. In all cases the corresponding hydroxy-compounds were isolated. While all of the fluoroderivatives were hydrolysed, the only bromo compounds which were hydrolysed were those which contained an additional activating group. The results are consistent with the adjacent electronic mechanism. The lesser tendency of chlorine and bromine to form hydrogen bonds as well as their lower electronegativity
both operate to decrease the lability of these halogens as compared with fluorine. When, however, another labilizing group is present, as in the bromonicotinic acids or 2-chloroquinoline, hydrolysis does occur.

The same considerations do not seem to apply to γ-halogen, which is in general much more reactive. Thus moist γ-chloropyridine (7) is converted on distillation to γ-pyridon hydrochloride. Also γ-fluoropyridine (8) cannot be isolated because of its rapid conversion to N-γ-pyridyl-γ-pyridon. Presumably this is due to the greater resonance stabilization of the p-quinoidal type intermediate structure (I) as compared with the γ-quinoidal type structure (II).5

Table I

Results of Hydrolytic Experiments

<table>
<thead>
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<td>2-bromopyridine</td>
</tr>
<tr>
<td>2-fluoro-5-methylpyridine</td>
<td>2-bromo-3-methylpyridine</td>
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<tr>
<td>2-fluoronicotinic acid</td>
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<tr>
<td>6-bromonicotinic acid</td>
<td></td>
</tr>
<tr>
<td>2-chloroquinoline</td>
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The formation of the hydroxynicotinamides in the reaction of the fluoronicotinamides with methyl iodide can
be easily correlated with the above results. A considerable decomposition of methyl iodide occurs at 80° and the initial aqueous solutions are sufficiently acidic (pH \( \leq 1 \)) to effect the observed hydrolysis.

As indicated in the experimental results, an acidic byproduct was present during the first recrystallization from water. Unless precautions were taken to limit the period of heating and the amount of water to a minimum the fluoroamides would not precipitate from the acidic solution. Concentration of the solution did not give the original fluoroamides but the hydroxyamides. The acidity increases on concentration suggesting an autocatalytic hydrolysis which proceeds readily once the solution is even mildly acidic. Once freed of this acidic byproduct the fluoroamides are stable toward water. The hydroxyamides must be considered to be artifacts.

Experimental\(^6,7\)

Preparation of Intermediates: The 2- and 6-fluoro-3-methylpyridines, nicotinic acids, and nicotinamides were prepared by the method of Minor and VanderWerf (10). 2-bromo-3-methylpyridine and 2-bromo-5-methylpyridine were prepared in 87% and 80% yields, respectively, by the methods of Allen (11) for the preparation of 2-bromopyridine.

Best results were obtained when a stiff tantalum Hershberg stirrer was used, as the mixtures become quite thick during the course of the reaction. Less concentrated hydrobromic acid (40%) may be used in place of the 48% acid, but
the results are not so satisfactory (ca. 75-80% yields). The 2-bromo-3-methylpyridine boiled at 82-88° at 9 mm. Mariella (12) has reported the boiling point as 76-77° at 7 mm.

**Anal.** Calc'd for C₆H₅NBr: N, 8.2.

Found: N, 8.3, 8.5.

The 2-bromo-5-methyl-pyridine boiled at 73-77° at 8 mm.

**Anal.** Calc'd for C₆H₅NBr: N, 8.2.

Found: N, 8.3, 8.5.

**Preparation of 6-bromonicotinic acid.** Exactly 122.3 g. of 2-bromo-5-methylpyridine was added to a solution of 278 g. of potassium permanganate in 3 l. of water. The mixture was stirred under reflux for five hours. Then 15 g. of potassium permanganate was added and refluxing was continued for an additional hour. The mixture was distilled until all of the unreacted 2-bromo-5-methylpyridine (about 45 g.) was recovered. The hot solution was then filtered and the precipitated manganese dioxide was twice stirred with boiling water and refiltered. The combined filtrate and washings were concentrated to 600 ml, filtered, and acidified with concentrated hydrochloric acid. About 90-100 g. of crude acid was obtained. After recrystallization from water it melted at 193.0-193.9°.

**Anal.** Calc'd for C₆H₄O₂NBr: N, 6.9.

Found: N, 7.0, 7.1.

The crude acid may be directly converted to the amide. The acid was characterized as the methyl ester.
Esterification by means of diazomethane gave much better results than the use of methanol with either dry hydrogen chloride or sulfuric acid as catalyst. To an ether solution of 0.1 mole of diazomethane a slight excess of the acid as a slurry in ether was added gradually with shaking until the yellow color vanished. The ether solution was washed with 10% sodium carbonate solution, then with saturated brine, and distilled to give 18.1 g. (84%) of the ester boiling at 107-110° at 4 mm. An analytical sample, m.p. 108.5-110.0°, was obtained by vacuum sublimation.

**Anal.** Calc'd for C_7_ H_5_ O_2_ NBr: N, 6.5.
Found: N, 6.4, 6.3.

The 2-bromonicotinic acid was prepared in exactly the same way in the same yield. After recrystallization from water it melted at 249.1-250.4°.

**Anal.** Calc'd for C_6_ H_4_ O_2_ NBr: N, 6.9.
Found: N, 7.0, 7.1.

The acid was characterized as the methyl ester prepared in the same way as its isomer. A 92% yield of ester boiling at 95-97° at 1.4 mm was obtained. After recrystallization from dilute alcohol it melted at 107.2-108.3°.

**Anal.** Calc'd for C_7_ H_6_ O_2_ NBr: N, 6.5.
Found: N, 6.3.

6-bromonicotinamide. A mixture of 30 g. of 6-bromonicotinic acid and 300 ml. of thionyl chloride was refluxed for at least twenty-four hours. Excess thionyl chloride was removed in vacuo. The residue was added as a slurry in
dioxane or benzene to cold stirred concentrated ammonium hydroxide, stored overnight, and filtered to give 26.2 g. (87%) of the amide. After recrystallization from water it melted at 213.5-214.2°.  

**Anal.** Calc'd for C₆H₅ON₂Br: N, 13.9.  
**Found:** N, 13.8.

2-bromonicotinamide. This product, prepared in 80% yield in the same manner as its isomer, exhibited an unusual melting point behavior. It softened appreciably at 140°, resolidified at 147°, melted at 171-172°, resolidified at 175-176° and finally decomposed at about 260°.  

**Anal.** Calc'd for C₆H₅ON₂Br: C, 35.8; H, 2.5.  
**Found:** C, 35.5; H, 2.0.

2-fluoropyridine. This product was prepared in good yield according to the method of Roe & Hawkins (8). 2-fluoropyrimidine could not be successfully prepared in this manner. 2-chloro-pyridine, 2-bromopyridine and 2-chloroquinoline were Eastman products.

Reaction of the fluoro- and bromonicotinamides with ethyl iodide. In each case a 2-g. sample of amide was heated with 25 ml. of ethyl iodide for twelve hours at 110° in a sealed tube. The ethyl iodide was then removed by evaporation and the residue recrystallized from water. In all cases the original amide was recovered in good yield.

Reaction of the amides with methyl iodide. These reactions were carried out in a sealed tube at 80°. Both bromo-amides were recovered unchanged. The behavior of the
fluoro-amides was quite different. The 2-fluoro-amide dissolved in the methyl iodide on heating but later separated out again as a red oil. Immediate evaporation after solution had occurred gave only the original amide. After twelve hours of heating the methyl iodide was removed by distillation, the residue was taken up in water (very soluble) and extracted with ether to remove free iodine. The solutions were concentrated somewhat, cooled and the crystalline product filtered off. The compound was recrystallized from water. The solubility was much lower at this stage. The behavior of 6-fluoronicotinamide was similar except that it did not dissolve in the methyl iodide. The 2-hydroxy-amide sintered at 265° and melted at 270.1-272.0°.

**Anal.** Calc'd for C₆H₅O₂N₂: C, 52.1; H, 4.3; N, 20.3.

Found: C, 51.9; H, 4.1; N, 20.9.

Acid hydrolysis gave the known 2-hydroxy nicotinic acid melting at 260.0-261.2°, with gas evolution, after recrystallization from water. Phillips (13) reported 256°.

**Anal.** Calc'd for C₆H₅O₃N: C, 51.7; H, 3.6; N, 10.1.

Found: C, 51.4; H, 3.3; N, 10.1.

It was further identified as the methyl ester, m.p. 152.1-153.3°, prepared by the method of Kirpal (14) who reported m.p. 153°. The 6-hydroxy-amide melted at 313.0-314.4°.
Acid hydrolysis gave the known 6-hydroxynicotinic acid, which sintered at 305-308° and melted at 309°. Von Pechman (15) reported 303° as the m.p.

The acid was further identified as the methyl ester, m.p. 166.1-167.5°, prepared by the method described by Meyer (16), who reported 164° as the m.p.

Preparation of 2- and 6-fluoronicotinamides. When the preparation was carried out exactly as described by Minor and VanderWerf (10) the fluoroamides are easily obtained. Working up of the mother liquors gave a small amount of the corresponding hydroxyamides. Frequently, however, when the last traces of thionyl chloride were removed by the repeated addition and vacuum distillation of dry benzene the hydroxyamides were the sole products. Judging from the solubility behavior, the actual hydrolysis seemed to occur during the first recrystallization from water. The pH of the freshly prepared solution was 6.4. If the solution was concentrated, the pH dropped to 3.9 within a short time. Although on smaller scale runs (ca. 2 g.) the fluoroamides did not hydrolyse during the brief period required to concentrate the solution, during the much longer period required for large runs (ca. 70 g.) when carried out on the steam bath
hydrolysis did occur. If solution was effected with a minimum of water and prompt cooling employed, pure fluoroamides were obtained in good yield.

Acid hydrolysis of the compounds in Table I. One gram samples of each compound were refluxed for twenty-four hours with 10 ml. of 6N hydrochloric acid. Results were as follows:

Bromo- and fluoronicotinic acids. On cooling, the hydroxynicotinic acids crystallized. The acids, after recrystallization from water, were identified by melting point and mixed melting point.

2-chloroquinoline. The product was 2-hydroxyquinoline, m.p. after recrystallization from alcohol 199.0-200.1°, which separated in quantitative yield from the acid solution. Morgan (17) reported the m.p. as 199-200°.

2-fluoro-, 2-chloro-, 2-bromo-, 2-fluoro-3-methyl-, 2-fluoro-5-methyl-, 2-bromo-5-methyl-, 2-bromo-5-methyl-pyridines. The acid solutions were made alkaline with sodium carbonate. In all cases except that of the fluoro-derivatives the solutions were repeatedly extracted with ether. Chloroform was used for the fluoro-derivatives. The extracts were washed, dried over sodium sulfate, and concentrated. The chloro- and bromo-derivatives were recovered unchanged in almost quantitative yield. From 2-fluoropyridine, 2-pyridone, b.p. 290-295° (730 mm.), m.p. 106-107°, was obtained in 60% yield. From the fluoropicolines, the corresponding picolones were obtained in 66%
yield. 3-methylpyridon-2,9 after sublimation in vacuo, melted at 138.0-139.5º. Seide (18) reported 140º as the m.p.

**Anal.** Calc'd for C₆H₇ON: N, 12.8.

Found: N, 12.7, 12.8.

3-methyl-pyridon-2, after recrystallization from benzene, melted at 183.0-184.1º.

**Anal.** Calc'd for C₆H₇ON: N, 12.8.

Found: N, 12.8.

**Alkaline hydrolysis studies.** Two gram samples of 2-fluoro-3-methylpyridine and 2-bromo-3-methylpyridine were refluxed with 10 ml. of 25% sodium hydroxide solution in water or 50% alcohol for four days. No appreciable reaction occurred in any case, the starting material being recovered in each instance.
SUMMARY

1. A study has been made of the reactions of methyl and ethyl iodides with 2- and 6-bromo- and 2- and 6-fluoronicotinamides. N-alkylation could not be effected in any instance; however, after treatment with methyl iodide, the fluoronicotinamides underwent hydrolysis to the corresponding hydroxynicotinamides during the course of the subsequent isolation procedure. This hydrolysis was catalysed by acidic products formed during the attempted methylation reaction.

2. Comparative studies have shown that fluorine substituted in the $\alpha$-position on the pyridine nucleus is more labile toward acid-catalysed hydrolysis than either chlorine or bromine.

3. A mechanism which accounts for the comparative ease of acid-catalysed hydrolysis of $\alpha$-fluoropyridines is proposed.
FOOTNOTES

1. N-diethyl m-nitrobenzamide was found to possess the same analeptic action as the corresponding pyridine derivative. Also o-sulfanilamido-nitrobenzene was found to possess antibacterial activity comparable to that of sulfapyridine.

2. Such a similarity has also been proposed on purely chemical grounds. See "Organic Chemistry of Nitrogen" by Sidgwick, Oxford University Press, 2nd Ed., 1937, p. 522.

3. The experimental details will be found in Part II of the thesis.

4. Such an acid catalysis has recently been reported for the benzyl fluorides by Miller and Bernstein (J. Am. Chem. Soc., 70, 3600 (1948)).

5. See Waters (9) for a discussion of the significance of the transition state in substitution reactions.

6. All melting points corrected, all boiling points uncorrected.

7. All analyses by Clarke Microanalytical Laboratories, Urbana, Illinois, unless starred. Starred analyses are by Arlington Laboratories, Fairfax, Virginia.

8. The fact that neither the fluoro- nor the bromonicotinic acids hydrolyzed during the concentration of these alkaline solutions is evidence for the absence of any rapid alkaline hydrolysis reaction.

9. This compound has also been isolated as a byproduct of
the preparation of 2-fluoro-3-methylpyridine by the procedure of Minor and VanderWerf (10).
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EXCHANGE REACTIONS
OF
< -HALOGENATED PYRIDINES
During the course of a study of the chemistry of Coenzyme I and II, the need arose for a series of 3-methyl-2- and 6-halogenated pyridine methiodides. Unlike the corresponding substituted nicotinamides, studied in the first part of this thesis, the halogenated α-picoline reacted readily with methyl iodide.

Both 2-fluoro- and 6-fluoro-3-methylpyridine gave the rather unstable fluorine substituted methiodides in good yield. On the other hand, 2-bromo- and 6-bromo-3-methylpyridine gave quantitative yields of the corresponding iodo-methiodides when treated with methyl iodide for twelve hours at 90°. Even when the reaction was carried out for a shorter period (four hours) at a lower temperature (50°), the only products isolated, although in inferior yield, were the iodo-methiodides. While this type of halogen exchange reaction is not new, having been reported in the reaction of 2-chloropyridine(1) and of 2-chloroquinoline(2) with methyl iodide, it was stated in each of these cases that prolonged treatment at 100° was required.

Since the order of reactivity for the fluoro- and bromo-derivatives was the reverse of that reported in the first part of this thesis for the acid hydrolysis of 2- and 6-halogenated nicotinamides, it was decided to study the reaction further.

In our investigation, 2-fluoro-, 2-chloro-, 2-bromo-, and 3-bromopyridine and 2-chloroquinoline were treated with methyl iodide at 90° for twelve hours, and all but
2-fluoro- and 3-bromopyridine gave the corresponding iodo-
methiodides. When treated at lower temperatures (circa 50°C)
2-chloro- and 2-bromopyridine and 2-chloroquinoline still
gave the iodo-methiodides, although in much poorer yield.
In the case of 2-chloropyridine, some chloro-methiodide was
formed but it could not be completely separated from the
iodo-methiodide even by repeated fractional crystallization.
All of the above-mentioned halogenated pyridines save
3-bromo- and 2-fluoropyridine and 2-fluoro-5-methylpyridine
were also treated with ethyl iodide at 90°C for twelve hours.
Here again the iodo-ethiodides were the only products iso-
lated, except in the case of 2-fluoro-3-methylpyridine,
which gave the expected, though rather unstable, fluoro-
ethiodide. 2-chloroquinoline reacted to only a limited
extent. Prolonged treatment did not increase the yields
appreciably. From a consideration of molecular models
steric hindrance seems to be the critical factor in this
case. The lack of reactivity of collidine with methyl
iodide is further evidence of the importance of steric fac-
tors.

It was thought of interest to determine at what point
the halogen exchange occurred. A careful fractionation of
the unreacted base in the low temperature treatment of
2-chloropyridine and 2-chloroquinoline with methyl iodide
indicated the complete absence of any iodopyridine or iodo-
quinoline; all of the 2-chloropyridine or 2-chloroquinoline
could be accounted for as methiodide or unreacted
2-chloropyridine or 2-chloroquinoline. Since the exchange did not occur at this point it seemed likely that it occurred after methiodide formation. To test this hypothesis, the authentic bromo-methiodides, prepared by the reaction of the corresponding bromopyridines with methyl sulfate, followed by treatment with potassium iodide, were treated with methyl iodide for twelve hours at 90°. After several recrystallizations the iodo-methiodides were isolated in fair yield. The results were confirmed by mixed melting point. Although 2-bromopyridine methiodide gave 2-iodopyridine methiodide when ethyl iodide was employed, a lower conversion was obtained with 2-bromo-5-methylpyridine methiodide. The cause of this is uncertain. It was further found that 2-bromopyridine methiodide was converted to the corresponding iodo-compound by refluxing with potassium iodide in methyl ethyl ketone solution.

These results suggest the following mechanism: Since excess alkyl halide is present in all cases the bromide ion would be substantially converted to methyl bromide and not be detected in the pyridinium compound. The reversal of the order of reactivity found for the substituted nicotinamides is believed to be due to the difference in the nature of the media in the two cases. Nucleophilic displacement of a ring fluorine atom in a pyridinium compound appears to proceed less readily than a similar displacement of a chlorine or bromine atom unless, as in the case in acid solution, conditions favor the solvation of the fluorine through hydrogen
\[
\text{Me}^+ \text{I}^- \xrightleftharpoons{\text{MeI}} \text{Me} \text{Br}^- \xrightleftharpoons{\text{MeI}} \text{Me} \text{I}^+ \\
\text{Br}^- + \text{MeI} \xrightleftharpoons{} \text{MeBr} + \text{I}^-
\]
bonding. A similar generalization is indicated for benzyl fluoride according to the recent work of Miller and Bernstein(3).

To test the hydrolysis mechanism proposed in the first part of this thesis, three of the compounds were subjected to acid hydrolysis and two to alkaline hydrolysis. Both 2-bromo-3-methylpyridine ethiodide and 2-bromo-5-methylpyridine ethiodide, when refluxed for one hour with 25% sodium hydroxide solution, gave good yields of the corresponding N-ethylpicolones. Acid hydrolysis of the above compounds as well as of 2-iodopyridine methiodide did not proceed so smoothly. N-methylpyridon-2 was obtained in good yield from 2-iodopyridine methyl iodide. The other two compounds gave largely complex iodinated products of somewhat uncertain nature. A fair yield of 1-ethyl-5-methylpyridon-2 was obtained from 2-iodo-5-methylpyridine ethiodide but no pyridon from the 2,3 isomer. The results confirm our predictions on the importance of the quaternary nitrogen for the hydrolysis. The formation of iodinated products is probably due to direct iodination by molecular iodine formed by oxidation during the refluxing. Some free iodine was detected in the reflux condenser. The rapid hydrolysis in alkaline solution probably arises from the greater hydrolytic power of the hydroxyl ion once the quaternary nitrogen is present.

In connection with our other work 3-methylpyridine methiodide, 3-cyanopyridine methiodide, and 5-methylthiazole methiodide were also prepared. Very carefully purified
3-methylpyridine was used in the preparation of the methiodide, which was obtained as a yellow-orange solid melting at 79.1-80.2°. Murrill(4) had previously reported the compound as a liquid.

The spectra of all the compounds save 2-fluoro-3-methylpyridine ethiodide were measured in the region between 230 and 400 m/. All of the compounds were studied at a concentration of 0.1 mg/ml. in distilled water as solvent. Substitution of halogen in the α- or α'-positions of 3-methylpyridine methiodide causes a shift of about 20 m/ in the principal absorption peak without appreciably shifting the preceding minimum. The thiazole derivative showed no such absorption peak but only a gradual decrease in intensity from an initial peak at 230 m/.

Experimental

Intermediates. The bromo-α-picoline were prepared as described in the first part of the thesis. The fluoro-α-picolines were prepared by the method of Minor, Hawkins, VanderWerf, and Roe(5). The fluoropyridine was prepared by the method of Roe and Hawkins(6). 5-methylthiazole was prepared according to the excellent directions of McLean and Muir(7). The β-picoline was a highly purified sample prepared by Hoffman and VanderWerf(8) in this laboratory. 3-bromo- and 3-cyanopyrididine were obtained through the kindness of the Dow Chemical Company. 2-chloro- and 2-bromopyridines and 2-chloroquinoline were obtained from the Eastman Kodak Company.
Figure I

C = 0.1 mg./ml.  - - - 2-fluoropyridine methiodide,  - - - 2-fluoro-3-methylpyridine methiodide,  - - - 2-fluoro-5-methylpyridine methiodide
Figure II

![Graph showing the absorption spectra of various pyridine methiodides.](image)

\[ C = 0.1 \text{ mg./ml.} \]

- **Solid line**: 2-bromopyridine methiodide
- **Dashed line**: 3-bromo-2-methylpyridine methiodide
- **Dotted line**: 2-bromo-3-methylpyridine methiodide
- **Dashed-dotted line**: 2-bromo-5-methylpyridine methiodide
Figure III

$C = 0.1 \text{ mg./mL.}$  
- - 2-iodo-pyridine methiodide,  
- - 2-iodo-3-methylpyridine methiodide,  
- - 2-iodo-5-methylpyridine methiodide.
Figure IV

C = 0.1 mg./ml.  
- - - 2-iodo-pyridine ethiodide, —— 2-iodo-3-methylpyridine ethiodide, —— 2-iodo-5-methylpyridine ethiodide.
$C = 0.1 \text{ mg./ml.}$ 5-methylthiazole methiodide, --- 3-methylpyridine methiodide, --- 3-cyanopyridine methiodide.
All of the reactions with methyl and ethyl iodides were carried out in capped citrate bottles. Two sets of reaction conditions were employed. A.) Treatment at 90° for twelve hours. B.) Treatment at 50° for four hours. Since the same procedure was employed in all cases only a single example will be given for each procedure. The melting points and analytical data for the individual compounds are given in Table I.

Method A. A mixture of 15 g. of 2-bromo-5-methylpyridine and 50 ml. of ethyl iodide was heated for twelve hours at 90°. A crystalline precipitate started forming within an hour. The bottle was then cooled, opened and the contents filtered off on the Buchner. The precipitate was thoroughly washed with dry ether. A 28 g. (98%) yield of almost pure material was obtained. Concentration of the filtrate gave a little more product to give a quantitative yield. After one recrystallization from alcohol-water-ether, the 2-iodo-5-methylpyridine ethiodide had a melting point of 214.5-215.1°, unchanged by repeated recrystallization. The addition of ether to the hot alcohol-water solution facilitated the recrystallization for the more soluble members of the series. The exact excess of alkyl halide did not appear to be critical. In all cases save 2-idoquinoline ethiodide, better than 90% yields were obtained consistently.

Method B. A solution of 3 g. of 2-bromopyridine in 15 ml. of methyl iodide was heated for four hours at 50°. The cooled solution was then filtered and the precipitate and
the mother liquor worked up separately. The 2 g. precipitate was practically pure 2-iodopyridine methiodide, m.p. 207.5-208.8°. The mother liquor was concentrated and then diluted with anhydrous ether to give an additional 0.6 g. of product having the same melting point.

In the case of 2-chloropyridine the fraction isolated from the mother liquor could be resolved into pure 2-iodopyridine methiodide and a yellow amorphous fraction, whose analysis corresponded to a 75-25 mixture of 2-chloropyridine methiodide and 2-iodopyridine methiodide.

Anal. Calc'd for 75% C₆H₇Cl and 25% C₆H₇I₂: C, 25.8; H, 2.5; N, 5.0; Hal, 66.8.

Found: C, 25.4; H, 2.7; N, 5.2; Hal, 66.7.
### Table I

<table>
<thead>
<tr>
<th>Compounds</th>
<th>M.P.  1°</th>
<th>Formula</th>
<th>Calc'd</th>
<th>Found</th>
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<tr>
<td>Pyridine Methiodides</td>
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<td></td>
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<tr>
<td>3-Me-</td>
<td>79.1--80.2</td>
<td>C₇H₁₀NI</td>
<td>5.9</td>
<td>6.0</td>
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<tr>
<td>3-Br-</td>
<td>159.1-159.9</td>
<td>C₆H₇NBrI</td>
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<tr>
<td>3-CN-</td>
<td>194.5-196.1</td>
<td>C₇H₇N₂I</td>
<td>11.4</td>
<td>11.4</td>
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<tr>
<td>2-Br-</td>
<td>198.5-199.6</td>
<td>C₆H₇NBrI</td>
<td>4.7</td>
<td>4.4</td>
</tr>
<tr>
<td>2-Me-3-Br-</td>
<td>203.5-205.0</td>
<td>C₇H₈NBrI</td>
<td>4.5</td>
<td>4.1</td>
</tr>
<tr>
<td>2-Me-5-Br-</td>
<td>203.8-204.5</td>
<td>C₇H₈NBrI</td>
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<tr>
<td>2-I-</td>
<td>209.5-210.1</td>
<td>C₆H₇NI₂</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2-Me-3-I-</td>
<td>210.5-211.2</td>
<td>C₇H₉NI₂</td>
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<tr>
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<td>211.1-212.2</td>
<td>C₇H₉NI₂</td>
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<td>Pyridine Ethiodides</td>
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<tr>
<td>2-I-</td>
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<td>C₈H₁₁NI₂</td>
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<td>C₈H₁₁NFI</td>
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<td>2-Iodoquinoline derivatives</td>
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<tr>
<td>N-Me-</td>
<td>210.1-211.0</td>
<td>C₁₀H₁₀NI₂</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N-Et-</td>
<td>201.0-201.5</td>
<td>C₁₁H₁₂NI₂</td>
<td>3.4</td>
<td>3.5</td>
</tr>
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<td>5-Me-Thiazole Methiodide</td>
<td>67.1--68.3</td>
<td>C₅H₈NSI¹⁰</td>
<td>2.8</td>
<td>2.9</td>
</tr>
</tbody>
</table>
Footnotes for Table I

1. All of the compounds save the 5-methylthiazole, \( \beta \)-picoline, and 2-fluoropyridine derivatives darkened at about 10° below their melting points. Best results were obtained when the melting point tubes were put in a bath preheated to within 10° of the melting point. Fairly rapid heating is necessary if distinct melting points are to be obtained.

2. Murrill (4) reported this compound as an oil.

3. Decker and Kaufmann (9) report the melting point as 146°.

4. Fischer (1) reports the melting point as 207°.

5. This compound is difficult to purify and was analysed in the form of its periodide, steel blue crystals, m.p. 49.5-50.2°. The periodide is readily prepared by treating the methiodide in alcoholic solution with an equimolar amount of iodine as described by Murrill (4).

6. This compound, though initially white, turns yellow within a few hours but does not seem to decompose on standing.

7. This compound is completely decomposed within two to three weeks. It is even more unstable in solution.

8. Roser (2) has reported the melting point as 213°.

9. Roser (2) has reported the melting point as 220°. This compound could not be obtained in better than 30-35% yield despite prolonged treatment with ethyl iodide.

10. This compound could only be induced to crystallize with difficulty and was therefore analysed in the form of
its periodide, magnificent orange-red needles, melting at 75.1-76.0°.
Isolation of chloropyridine and chloroquinoline from reaction mixtures: Exactly 15 g. of 2-chloroquinoline was heated with methyl iodide as described in Method B. Only 1 g. 2-iodoquinoline methiodide was obtained as yellow crystals, m.p. 210.1-211.5°. Fractional distillation of the ether filtrate gave 14.5 g. of 2-chloroquinoline, b.p. 119-121/4 mm. Similarly treated, 12 g. of 2-chloropyridine gave 5.8 g. of 2-iodopyridine methiodide and 9.1 g. of 2-chloropyridine, b.p. 163-166°/740 mm.

Authentic bromopyridine methiodides: A mixture of 6.88 g. (0.04 m) of 2-bromo-5-methylpyridine and 6.5 ml. of redistilled methyl sulfate was heated on a steam bath overnight. Excess methyl sulfate was then removed in vacuo. The residue was dissolved in 12 ml. of water and extracted with two 5 ml. portions of chloroform to remove the small amount of insoluble material. A twofold excess of potassium iodide was then added with vigorous shaking. The yellow precipitate (11 g., 90%) was filtered off and recrystallized repeatedly to give white needles, m.p. 203.8-204.5° (de-comp.). 2-bromopyridine and 2-bromo-5-methylpyridine were treated similarly to give the corresponding bromo-methiodides in about the same yield.

Reaction of bromo-methiodides with methyl iodide. One gram samples of each of the above bromo-methiodides and 10 ml. of methyl iodide were heated overnight at 90°. The products were filtered off and recrystallized as usual. All of the products melted within 1° of the values found for the
iodo-methiodides, gave no depression in mixed m.p. with authentic samples of iodo-methiodides, and gave a depression in mixed m.p. with the original bromo-methiodides.

Reaction of 2-bromo-pyridine methiodide and ethyl iodide: A mixture of 1 g. of 2-bromopyridine methiodide and 10 cc. of ethyl iodide was heated for forty-eight hours at 90°. The product was isolated as described above. After two recrystallizations, it melted at 207.1-208.3°. Under similar conditions 2-bromo-5-methylpyridine methiodide was only very partially converted to the corresponding iodo-compound.

Reaction of 2-bromopyridine methiodide and potassium iodide: A mixture of 0.6 g. of 2-bromo-methiodide and 2 g. of potassium iodide in 30 ml. of methyl ethyl ketone was refluxed for forty-eight hours. After cooling, a quantitative yield of iodo-methiodide was filtered off and recrystallized in the usual manner. It melted at 210.5-211.0° after one recrystallization.

Alkaline hydrolysis experiments: A mixture of 10 g. of 2-bromo-3-methylpyridine ethiodide and 50 ml. of 25% sodium hydroxide solution was refluxed for one hour. A red oil started to separate within \( \frac{1}{2} \) hour. The oil was extracted with chloroform, washed with saturated salt solution, dried over Drierite and distilled in vacuo to give 2.4 g. (75%) of 1-ethyl-3-methyl-2-pyridone, b.p. 92-95°/2 mm., \( n^{25}_D \) 1.5401.

**Anal.** Calc'd for C\(_8\)H\(_{11}\)ON: N, 10.2.

Found: N, 10.4.
Similarly 10 g. of 2-bromo-5-methylpyridine ethiodide gave 2.0 g. (62%) of 1-ethyl-5-methyl-2-pyridone, boiling at 109-112°/1.5 mm., n\(\text{D}_{25}\) 1.5413.

**Anal.**  Calc'd for C\(_8\)H\(_{11}\)ON:  N, 10.2.

Found:  N, 10.5.

**Acid Hydrolysis experiments:** A mixture of 5 g. of 2-iodopyridine methiodide and 40 ml. of 6N hydrochloric acid was refluxed for twenty-four hours. The solution was concentrated on the steam bath, neutralized with solid sodium carbonate and extracted with chloroform. The extract was dried and distilled to give 1.3 g. (80%) of 1-methyl-2-pyridone, b.p. 90-110°/2 mm. The oil in anhydrous ether solution was treated with anhydrous hydrogen chloride to give 1-methyl-2-pyridone hydrochloride, m.p. 167-168° after one recrystallization from alcohol-ether. Neundlinger(10) has reported the melting point as 166°.

A mixture of 10 g. of 2-iodo-5-methylpyridine ethiodide and 50 ml. of 6N hydrochloric acid was refluxed for twenty-four hours. The solution was concentrated and extracted with ethyl acetate to remove a highly iodinated product of uncertain composition. The solution was then extracted exhaustively with chloroform. The extract was dried and distilled to give 1.7 g. (47%) of 1-ethyl-5-methyl-2-pyridone, b.p. 111-114°/2 mm., and considerable high boiling residue.

When treated similarly, 2-iodo-3-methylpyridine ethiodide gave only complex iodinated products. Concentration
to about 10 ml. precipitated 3.7 g. of orange crystals melting at 103.1-104.2°. The product contains a large amount of iodine but no definite structure could be deduced for the compound. The same compound could be isolated by neutralization of the acid solution with sodium carbonate. Further concentration of the solution gave 5.1 g. of white crystals, m.p. 174.0-174.5°, of unknown structure, containing chlorine as well as iodine and nitrogen. In these latter two cases free iodine separated out in the condenser.
SUMMARY

1. A series of substituted pyridine methiodides have been prepared.

2. $\alpha$-substituted chlorine and $\alpha$-substituted bromine in pyridine methiodides have been found to exchange readily with iodine in alkyl halide solution. $\alpha$-substituted fluorine does not undergo this reaction under similar conditions.

3. A mechanism has been proposed to account for these results.

4. The mechanism proposed earlier for the hydrolysis of $\alpha$-substituted halogen in pyridine methiodides has been further substantiated.
FOOTNOTES

1. All melting points are corrected; all boiling points are uncorrected.

2. All analyses are by the Clark Microanalytical Laboratories, Urbana, Illinois.
REFERENCES

THE OXIDATION
OF SOME $\beta$-SUBSTITUTED
PYRIDINE ALKIODIDES
Although the role of nicotinamide in Coenzyme I and II has long been known(1), there still exists doubt as to whether the structure of the reduced form is I or II, i.e., whether the 2- or the 6- position is the active one.

On the basis of biochemical oxidation experiments, Knox and Grossman(2) suggested the 6- position as the active center. They were able to isolate 1-methyl-2-pyridone-5-carbonamide from human urine following ingestion of N-methylnicotinamide chloride. The same compound was also obtained by the action of a rabbit liver quinine oxidizing enzyme on N-methylnicotinamide chloride. Huff(3) provided further support for this argument when he found that trigonelline acid sulfate or N-methylnicotinamide chloride could be oxidized by ferricyanide in alkaline solution to 1-methyl-2-pyridone-5-carboxylic acid.

The present work was undertaken to determine whether the nature of the 5-substituent would affect the orientation of the chemical oxidation. While our investigation was in progress, Wiegand and Holman(4) reported the isolation of 1-methyl-2-pyridone-3-carbonamide from the low temperature ferricyanide oxidation of N-methylnicotinamide iodide, as well as the isolation of 1-methyl-2-pyridone-5-carboxylic acid from the acidified solution. We therefore extended our investigation to include a study of the apparent discrepancy between the results of Huff and Wiegand and Holman.

RESULTS

We have found that chemical oxidation of 3-methylnpyri-
dine methosulfate, 3-methylpyridine ethiodide, 3-bromopyridine methiodide, and N-methyl nicotinamide iodide occurred predominantly in the 2-position. 3-cyanopyridine methiodide appeared to be oxidized in both positions, whereas nicotinic acid methosulfate was oxidized predominantly in the 6-position.

The last-named compound, when oxidized by the ferri-cyanide procedure of Huff(3) or with alkaline hydrogen peroxide, gave only 1-methyl-2-pyridone-5-carboxylic acid, melting at 240.5-241.2° in good agreement with reported values. The acid was further identified as the amide, m.p. 211.0-211.5°, undepressed on mixed melting point with samples generously provided by Drs. Knox and Holman.

Evidence that the product obtained by the low temperature oxidation of 3-cyanopyridine methiodide, by the method of Decker and Kaufmann(5), was a mixture of cyano-pyridones was afforded by the wide range over which it boiled and melted. Repeated recrystallization did not resolve the mixture into the isomeric forms. There was isolated a very small amount of a compound, m.p. 159.1-159.8°, which was resistant to alkaline hydrolysis. Attempted hydrolysis of the original mixture to the amide by the method of Spaeth and Koller(6) was unsuccessful. Hydrolysis to the acid by the method of the same authors gave a fair yield of pure 1-methyl-2-pyridone-5-carboxylic acid. None of the 2,3 isomer could be isolated from the residue despite the wide melting range of the original nitrile.
The oxidation of N-methylnicotinamide iodide at 0°C gave the same 1-methyl-2-pyridone-3-carbonamide reported by Wie- gand and Holman(4). The identity was confirmed by mixed melting point with an authentic sample prepared from 2-hydroxynicotinic acid and with one kindly provided by Dr. Holman, and by a depression of the melting point upon mixing with 1-methyl-2-pyridone-5-carbonamide.

β-picoline methosulfate when oxidized by Fargher and Furness' (7) method gave 1,3-dimethyl-2-pyridone. The identity was confirmed by comparison of the boiling point, melting point, and melting point of the hydrochloride with those of authentic samples of the two isomeric dimethyl pyridones, prepared by the alkylation of the hydroxypicolines according to the general procedure of Rath(8). The method of Fehman and Balzer(9) in which the hydroxy compound is heated under pressure with alkyl halide and that of Meyer(10) using diazomethane as alkylating agent failed to give isolable amounts of product.

β-picoline ethiodide reacted by Decker's (5) method gave a low yield of 1-ethyl-3-methyl-2-pyridone. Most of the product was a very high boiling material of unknown composition. It is possible that this material is an iodinated product; as this compound is known to be iodinated in acid solution1. The identity of the oxidation product was confirmed by comparison of the boiling point and uv spectra (Fig. I) with those of authentic samples prepared from the corresponding hydroxypicolines.
C = 0.4 mg./ml.  
- - - - 1-ethyl-3-methyl-2-pyridone obtained by oxidation,  
- - - - 1-ethyl-3-methyl-2-pyridone,  
- - - - ethyl-5-methyl-2-pyridone.
Figure II

C = 0.05 mg./ml.  
- - - - 1-methyl-3-bromo-2-pyridone,

1-methyl-5-bromo-2-pyridone.
A fair yield of 1-methyl-3-bromo-2-pyridone, boiling over a range, was obtained by oxidation in the same manner. On prolonged cooling a small amount of crystalline material, insufficient for a melting point determination, separated from the lower boiling fractions. The boiling point, melting point, and uv spectra (Fig. II) of the compound differed from the values obtained for an authentic sample of the 2,5 isomer prepared in the usual manner from 2-amino-5-bromopyridine. A more convenient procedure for the preparation of this useful intermediate than that of Case(11) was developed. The identity of the oxidation compound was confirmed by bromination to the known 1-methyl-3,5-dibromo-2-pyridone. The melting point and mixed melting point agreed completely with that of a sample prepared by brominating 1-methyl-5-bromo-2-pyridone. The rates of reaction for the two isomers differed markedly. Attempts to prepare 1-methyl-3-bromo-2-pyridone failed. Methyl 3-bromocoumalinic ester, prepared in equally indifferent yield by the method of von Pechman (12) or by diazomethane esterification was quantitatively converted to 1-methyl-3-bromo-5-carboxamethoxy-2-pyridone with excess methylvamine solution. The acid, obtained by alkaline hydrolysis, could not be decarboxylated to the desired 1-methyl-3-bromo-2-pyridone, either by pyrolysis in the presence of copper as described by Case(11) or by vacuum pyrolysis, to the desired product even though evolution of carbon dioxide did occur in both cases. Possibly the
Figure III

\[
\begin{align*}
\text{R} & \quad \text{R} \\
\text{R} & \quad \text{R} \\
\text{R} & \quad \text{R}
\end{align*}
\]

1 \quad + \quad 1 \quad \text{R} \\
\text{R} & \quad \text{R} \\
\text{R} & \quad \text{R}

2 \quad \text{R} \\
\text{R} & \quad \text{R} \\
\text{R} & \quad \text{R}
Figure IV

\[
\begin{align*}
\text{Me} & \quad \text{Me} \\
\text{Me} & \quad \text{Me} \\
\text{Me} & \quad \text{Me} \\
\text{Me} & \quad \text{Me} \\
\end{align*}
\]
Figure V

\[
\text{OH} \quad \text{N} \quad \text{H} \\
\text{~} \quad \text{Me} \\
\text{OH} \\
\text{H}
\]
molecule enters into condensation reactions at the reactive carbon bromine bond.

**DISCUSSION**

The results at first glance are rather confusing. A consideration of the mechanism, however, provides a possible explanation of the results. As Knox and Grossman(2) have pointed out, the reaction proceeds in two steps; 1) formation of the pseudo-base and 2) dehydrogenation of the pseudo-base to the pyridone (Fig. III). The orientation of pseudo-base formation, therefore, would be the principal determining factor of the configuration of the oxidation product. On the other hand, if the presence of a substituent renders one of the pseudo-bases less reactive than the other, the fact that one of the pseudo-bases is formed more readily than the other may not necessarily be decisive in determining the orientation of the oxidation product. The 2-position appears to be favored when a $\beta$-substituent capable of entering into resonance with the two double bonds remaining after pseudo-base formation is present. As can be seen from the adjacent diagram, more resonance structures can be written when pseudo-base formation occurs in the 2-position (Fig. IV) than when it occurs in the 6-position (Fig. V).

All of the compounds which gave 2,3-substituted products contained a group capable of entering into resonance with the conjugated system of the ring. The $\beta$-methyl group is capable of interacting via the Baker-Nathan effect.
Figure VI

\[
X^+ \text{Me} \overset{\circ}{\text{OH}^-} \rightarrow \overset{\circ}{\text{OH}} \text{Me} \overset{\circ}{\text{X}} \overset{\circ}{\text{OH}^-} \overset{\circ}{\text{X}} \text{Me} \overset{\circ}{\text{OH}^-} \rightarrow \overset{\circ}{\text{X}} \text{Me} \overset{\circ}{\text{O}^-} \overset{\circ}{\text{H}} \text{Me} \overset{\circ}{\text{X}} \text{Me} \overset{\circ}{\text{OH}^-} \rightarrow \overset{\circ}{\text{X}} \text{Me} \overset{\circ}{\text{O}^-} \overset{\circ}{\text{H}} \text{Me} \overset{\circ}{\text{X}} \text{Me}
\]

\[
H^- + 2 \text{Fe(CN)}_6^{\text{III}} + \text{OH}^- \rightarrow 2 \text{Fe(CN)}_6^{\text{II}} + \text{H}_2\text{O}
\]
Resonance interaction of a bromine atom with a system of double bonds is generally accepted. (Consider the accepted structure of vinyl bromide and the general decrease in bond length for a C-halogen bond adjacent to a carbon-carbon double bond.) The ability of a carbonyl group such as that present in the nicotinamide derivative to interact is also well known.

In order to explain the two cases in which oxidation occurred in the 6-position, a critical examination of the following suggested mechanism (Fig. VI) is necessary. If $X$ is capable of interacting with the hydroxyl hydrogen so as to decrease its acidity, then step A would be hindered. Accordingly, formation of the 2,3-substituted pyridone would be decreased and formation of the 2,5-substituted pyridone would be favored.

In the case of the N-methylnicotinate ion the carboxylate ion would tend to inhibit pseudo-base formation in the 2-position because of its electrostatic repulsion on the approaching hydroxyl ion. In addition, a consideration of Fisher-Hirschfelder molecular models suggests that from the standpoint of spatial relationships the type of interaction mentioned above could occur$^2$. The combined effect seems sufficient to explain the absence of 2,3-substituted acid.

An examination of molecular models of the pseudo-base from 1-methyl-3-cyanopyridine hydroxide suggests that from the standpoint of spatial considerations the highly negative nitrile nitrogen would be able to interact with the hydroxyl hydrogen to decrease its acidity and accordingly favor
formation of the 2,5-substituted products.

On the basis of these results it is suggested that considerable caution should be exercised in applying the results of purely chemical studies to problems of biochemical reactivity. In particular steric effects which do not seem to be critical in these chemical studies might very well be decisive in determining the orientation of products obtained by enzymatic action.

Experimental\textsuperscript{3,4}

\textbf{1-methyl-2-pyridone-5-carboxylic acid:} A mixture of 50 g. of nicotinic acid and 75 ml. of redistilled methyl sulfate was heated at 100° for one hour with stirring. The mixture was then cooled, diluted with 100 ml of water and extracted with chloroform.

A.) One half of the solution was neutralized to pH 6 and then 10 g. of sodium hydroxide added. After ½ hour a solution of 182 g. of potassium ferricyanide in 550 ml. was added dropwise over the course of an hour. After an additional 3/4 hour the solution was adjusted to pH 3.4 and chilled overnight. A crude yield of 12 g. was obtained. A small amount of blue inorganic material could not be conveniently removed by recrystallization from water. If the finely ground acid was extracted in a Soxhlet with anhydrous ether, however, a complete separation could be effected. After removal of the ether the residue was recrystallized from water to give pure 1-methyl-2-pyridone-5-carboxylic acid, m.p. 239.5-240.5°.
B.) The other half of the solution was neutralized with sodium hydroxide and mixed with 100 ml. of alcohol and 50 ml. of 50% sodium hydroxide solution. The resulting deep red solution was warmed to 50° and 200 ml. of 30% hydrogen peroxide added over a period of 3/4 hour. A vigorously exothermic reaction occurred. The solution was then acidified with sulfuric acid to pH 3.5 and chilled overnight. The voluminous precipitate was filtered off, dried, ground finely, and thoroughly extracted with ether in the Soxhlet. A 7 g. yield of acid, m.p. 240.5-241.5°, was isolated.

1-methyl-2-pyridone-5-carbonamide:-- A mixture of 1.36 g. of the above acid and 15 ml. of thionyl chloride was refluxed for one hour. The thionyl chloride was removed in vacuo and the residue shaken with 3 ml. cold concentrated ammonia solution. After cooling for one hour, the precipitate was filtered off and recrystallized from water; its melting point (211.0-211.5°) was essentially undepressed when it was mixed with an authentic sample (m.p. 209.0-211.5°) kindly furnished by Dr. Knox or with a sample (m.p. 204.0-206.5°) sent us through the courtesy of Dr. Holman.

1-methyl-3 & 5-cyano-2-pyridones:-- To an ice-cooled solution of 72.5 g. of 3-cyanopyridine methiodide in 200 ml. of water, solutions of 200 g. of potassium ferricyanide in 550 ml. of water and 90 g. of potassium hydroxide in 100 ml. of water were added simultaneously with vigorous stirring over a one hour period. The solution was then saturated with potassium carbonate in the cold. After removal
of the precipitated potassium ferrocyanide, the solution was exhaustively extracted with 1:1 ether-chloroform. The combined extracts were washed, dried, and concentrated. The residue was distilled in vacuo to give 5 g. (14%) of yellow 1-methyl-cyano-2-pyridone, b.p. 180-200°/2 mm. Spaeth(6) reported a b.p. of 243°/18 mm. for 1-methyl-3-cyano-2-pyridone. The same results were obtained when sodium chloride was used as the saturating agent. After one recrystallization from alcohol, the product sintered at 112-114°, semi-melted at 117-125°, and gave a clear melt at 142°. Repeated recrystallization gave a very small amount of material melting at 159.0-159.8°. Analysis indicated a very low nitrogen content. Attempted hydrolysis to the amide by the method of Spaeth and Koller(6) gave only a small amount of the 159° compound. Hydrolysis to the acid, on the other hand, was successful. A solution of 2 g. of crude product in 10 g. of sodium and 200 ml. of anhydrous methyl alcohol was refluxed for seventy-two hours while a stream of nitrogen was bubbled through the solution. The solution was concentrated in vacuo, acidified, and evaporated to dryness in vacuo. The powdered residue was repeatedly extracted with anhydrous alcohol. The extract was concentrated and the residue recrystallized from water to give 0.6 g. of crude acid, m.p. 220-230°. After one recrystallization from water it melted at 239.5-240.8°, and was undepressed on mixed melting point with an authentic sample of acid. Recrystallization of the residue gave only a small amount of additional 2,5-substi-
1-methyl-2-pyridone-3-carbonamide:—A solution of 61 g. of nicotinamide methiodide[13] in 175 ml. of water was cooled to 0° and stirred vigorously. Solutions of 60 g. of potassium hydroxide in 70 ml. of water and 175 g. of potassium ferricyanide in 500 ml. of water were added simultaneously over a 1½ hour period. The solution was stirred for an additional 3/4 hour, saturated with salt, and filtered. The filtrate was exhaustively extracted with chloroform. The extract was washed, dried, and concentrated to dryness. The crude material, 5.3 g. (15%), after recrystallization from water, melted at 217.1-218.2°; mixed m.p. with an authentic sample, 219.5-220.1°. A sample of Dr. Holman's material, m.p. 217-218°, gave the same mixed melting point with the authentic sample.

Preparation of an authentic sample of the 2,3 amide:—A solution of 5 g. of 2-hydroxynicotinic acid[6] and 4.4 g. of potassium hydroxide in 20 ml. of water was evaporated to dryness. The residue was powdered and heated with 50 ml. of anhydrous methyl alcohol and 6 ml. of methyl iodide at 90° for five hours in a pressure bottle[12]. The solution was cooled, filtered, and concentrated on the steam bath. The residue was hydrolysed by refluxing with 13 ml. of 10% sodium hydroxide solution for two hours. The solution was then cooled and acidified with hydrochloric acid. The mixture was refrigerated overnight and filtered to give 4 g. of crude acid. A sample, recrystallized from water, melted at
184.5-185.1°. Spaeth(6) reported the melting point as 183°. A mixture of 1.2 g. of the acid and 15 ml. of thionyl chloride was refluxed for one hour. Excess thionyl chloride was removed in vacuo and the residue shaken with 3 ml. of concentrated ammonium hydroxide, and cooled for one hour. The precipitate (1 g.) was removed by filtration and recrystallized from water to give short needles, m.p. 219.0-219.5°. After a second recrystallization, the pure 1-methyl-2-pyridone-3-carbonamide melted at 219.5-220.1°.

1,3-dimethyl-2-pyridone:- A mixture of 25 g. of β-picoline and 50 g. of methyl sulfate was heated on the steam bath overnight and then diluted with 150 ml. of water. A solution of 170 g. of potassium ferricyanide in 350 ml. of water was cooled in ice and vigorously stirred, while the methosulfate solution and a solution of 90 g. of potassium hydroxide in 100 ml. of water were added simultaneously over the course of one hour. The solution was stirred for an additional 3/4 hour, saturated with potassium carbonate, and filtered. The precipitate was thoroughly washed with benzene and the filtrate repeatedly extracted with benzene.

The extract was washed, dried, concentrated, and the residue was vacuum distilled to give 13.8 g. (41%) of 1,3-dimethyl-2-pyridone, b.p. 86-88°/1.45 mm., n^25_D 1.55387. The hydrochloride was prepared by passing anhydrous hydrogen chloride into an ether solution of the base, melted at 120.1-121.2° after recrystallization from anhydrous alcohol-ether. The melting point was not depressed when the compound was mixed
with the hydrochloride of an authentic sample of the 2,3-

isomer, but dropped to 75-83° when the hydrochloride of the

2,5-isomer was added.

**Authentic 1,3-dimethyl-2-pyridone:** The compound was

prepared by the alkylation of 2-hydroxy-3-methylpyridine

prepared by the method of Seide(15) except that evaporation

of the solution to dryness before extraction with chloroform

was found unnecessary. Concentration of the chloroform

solution gave a quantitative yield of crude material.

Attempted alkylation with methyl iodide at 90°, or with
diazo methane in ether-dioxane solution gave only the origi-
nal starting material. Rath's(8) method, on the other hand,
proved successful. To a refluxing solution of 15.9 g. of

2-hydroxy-3-methylpyridine and 9.7 g. of potassium hydroxide

in 200 ml. of anhydrous ethanol, an excess of methyl iodide

in ethanol solution was added dropwise over a two hour peri-

d with stirring. The solution was stirred for an additional
two hours, then cooled, filtered, and concentrated on the

steam bath. The residue was diluted with chloroform, refil-

tered to remove additional potassium iodide, and again con-

centrated. Vacuum distillation of the residue gave 12 g.

(68%) of 1,3-dimethyl-2-pyridone, b.p. 83-84°/1.3 mm., n^25D

1.5525. The hydrochloride, prepared as described above,
melted at 120.2-121.6°.

**Anal.** Calc'd for C₇H₉NO: N, 11.4.

**Found:** N, 11.3.

**Authentic 2,5-isomer:** Crude 2-hydroxy-5-methylpyridine
was prepared by Seide's (14) method from 2-amino-5-methylpyridine (Reilly Coal Tar and Chemical Company) in 87% crude yield. Methylation as described above gave 13.8 g. (78%) of 1,5-dimethyl-2-pyridone, b.p. 109-111°/1.7 mm., n\textsubscript{25} 1.5565, and m.p. 36.9° (cooling curve). The hydrochloride melted at 159.5-160.2°.

\textit{1-ethyl-5-methyl-2-pyridone:—} A mixture of 24 g. of \(\beta\)-picoline and 75 ml. of ethyl iodide was heated overnight at 90° in a pressure bottle. The mixture was cooled, filtered, and the precipitate washed with ether. The residue (61 g.) was taken up in 200 ml. of water and cooled to 0°. Simultaneously, solutions of 150 g. of potassium ferricyanide in 350 ml. of water and 60 g. in 70 ml. of water were added dropwise with vigorous stirring over the course of one hour. The mixture was stirred for an additional 3/4 hour and then saturated with potassium hydroxide. The precipitate was removed by filtration and both the solution and the precipitate were thoroughly extracted with chloroform. The extract was washed, dried, and concentrated. The residue was vacuum distilled to give 8.0 g. (23.8%) of 1-ethyl-5-methyl-2-pyridone, b.p. 95-96°/2.5 mm. Saturation with potassium carbonate resulted in a lower yield.

\textit{Authentic 1-ethyl-3-methyl-2-pyridone:—} A solution of 15.9 g. of 2-hydroxy-3-methylpyridine and 9.7 g. of potassium hydroxide in 200 ml. of absolute ethanol was treated with excess ethyl iodide as described above. Vacuum distillation gave 12.6 g. (62%) of 1-ethyl-3-methyl-2-pyridone, b.p. 92-
$94^\circ/2.2$ mm., $n^{25D} 1.5411$.

**Anal.** Calc'd: N, 11.4.

**Found:** N, 11.4.

**Authentic 1-ethyl-5-methyl-2-pyridone:** Exactly 15.9 g. of 2-hydroxy-5-methylpyridine was reacted as described above to give 12.4 g. (60%) of 1-ethyl-5-methyl-2-pyridone, b.p. 104-106$^\circ/1.7$ mm., $n^{25D} 1.5415$.

**Anal.** Calc'd: N, 10.3.

**Found:** N, 10.5.

**1-methyl-3-bromo-2-pyridone:** A mixture of 33 g. of 3-bromopyridine and 50 ml. of methyl iodide was heated overnight at $90^\circ$. A quantitative yield of crude material was obtained. The product was dissolved in 175 ml. of water, cooled to $0^\circ$, and stirred vigorously. Solutions of 135 g. of potassium ferricyanide in 400 ml. of water and 60 g. of potassium hydroxide in 70 ml. of water were added simultaneously over a $2^{1/2}$ hour period. The solution was stirred for several additional hours, saturated with potassium carbonate, and filtered. Both the precipitate and the solution were thoroughly extracted with chloroform. The extract was washed, dried, and concentrated. Vacuum distillation yielded 10.6 g. (27%) of material, careful fractionation of which gave a small fraction boiling between 110-118$^\circ/0.45$ mm. and a much larger fraction boiling at 122-127$^\circ/0.45$ mm. On re fractionation it boiled at 120-125$^\circ/0.43$ mm.

**Preparation of an authentic sample of 1-methyl-5-bromo-2-pyridone:** A.) Preparation of 2-amino-5-bromopyridine.
To a solution of 20 g. of 2-aminopyridine in 100 g. of 20% sulfuric acid, 13 ml. of bromine was added over a period of one hour. The solution was stirred overnight, diluted with 150 ml. of water, and neutralized with solid sodium carbonate. After cooling, the precipitate (38 g.) was removed by filtration, dried, extracted twice with skellysolve B, and recrystallized from benzene to give 21.7 g. (59%) of 2-aminopyridine, m.p. 132-135°. Case(11) reported a m.p. of 138°.

B.) 2-hydroxy-5-bromopyridine:— The 2-aminopyridine prepared above was treated by the method of Seide (14) to give 11.0 g. (50%) of 2-hydroxy-5-bromopyridine, m.p. 162-166°, after one recrystallization from water. Tschitschbabin(15) reported 177-178°.

C.) Exactly 15.0 g. of 2-hydroxy-5-bromopyridine was methylated as described above to give 12.3 g. (73%) of 1-methyl-5-bromo-2-pyridone, b.p. 126-131°/1.6 mm., which solidified at once to a solid, m.p. 62.1-63.0°. Rath(8) reported the melting point as 53°. Methylation with diazomethane in ether-dioxane solution failed.

1-methyl-3,5-dibromo-2-pyridone:— A.) From the oxidation compound. To a cooled solution of 2.2 g. of 1-methyl-3-bromo-2-pyridone in 10 ml. of glacial acetic acid, 0.7 ml. of bromine was added with stirring. The product began to precipitate after 3/4 hour. After an additional hour, 30 ml. of water was added with vigorous shaking. The white precipitate (1.7 g.) was removed by filtration, washed
thoroughly, and recrystallized from alcohol-water. It melted at 182.1–183.2°; no depression resulted upon admixture with a sample of the dibromide prepared from 1-methyl-5-bromo-2-pyridone. Decker(5) reported the melting point as 178°.

B. Preparation from 1-methyl-5-bromo-2-pyridone. A solution of 2.0 g. of the 2,5 derivative was treated as described above, to give 1.3 g. of the same product, m.p. 182.1–183.2°.

Attempted preparation of an authentic sample of 1-methyl-3-bromo-2-pyridone:– Methyl 3-bromocoumalinate was prepared in 31% yield by the method of von Pechman(12) or in 29% yield by esterification with diazomethane in alcohol solution. In both cases a considerable amount of an intractable oil was formed.

1-methyl-3-carbomethoxy-5-bromo-2-pyridone:– To 200 ml. of 18% methylamine solution exactly 50 g. of finely powdered methyl 3-bromocoumalinate was added with shaking. A quantitative yield of the pyridone compound precipitated within a few minutes and was separated by filtration. The crude ester was hydrolysed by refluxing for four hours with excess sodium hydroxide in 50–50 water-alcohol solution. The solution was concentrated, filtered, and acidified. The crude acid (48 g.), m.p. 290°, was removed by filtration and dried. Attempted decarboxylation as described by Case(11) led only to a chloroform insoluble resin. Attempted vacuum pyrolysis gave a very high melting solid.
SUMMARY

1. The structure of the products of the oxidation of a series of N-alkylated \( \beta \)-substituted pyridines has been investigated.

2. The results of Knox and Grossman and Wiegand and Holman for the oxidation of N-methylnicotinic acid and N-methylnicotinamide have been confirmed.

3. The nature of the \( \beta \)-substituent has been shown to affect the orientation of the oxidation.

4. A mechanism has been proposed which accounts for the differences observed in the orientation of the oxidation products.

5. The danger in applying purely chemical studies to problems of biochemical reactivity is discussed.
FOOTNOTES

1. For the experimental data see part II of this thesis.

2. This type of field interaction differed from hydrogen bonding with chelation, in that the system of 2 conjugated double bonds ordinarily required for such hydrogen bonding was not present.

3. All melting points are corrected. All boiling points are uncorrected.

4. All analyses are by Clark Microanalytical Laboratory, Urbana, Illinois.

5. See part II of this thesis for the preparation of this compound.

6. See part I of this thesis for the preparation of this compound.

7. It should be noted that this procedure was not satisfactory for the oxidation of N-methylated 3-bromopyridine and nicotinamide derivatives.
REFERENCES

9. von Pechman and Balzer, Ber., 24, 3144 (1891).
12. von Pechman, Ber., 17, 2396 (1884).
NICOTINAMIDE ANTAGONISTS
In connection with our studies on the chemistry of Coenzymes I and II, a study was made of the ability of 6\(\alpha\) or \(\alpha\)' substituted nicotinamides to antagonize or to replace nicotinamide for the growth of a strain of Staphylococcus Aureus, known to require nicotinamide.

Since the biological function of nicotinamide is well known it was thought of interest to see if the bacteriological results could be correlated with the chemical and biochemical reactivity of the compounds. In particular the ability of \(N\)-alkylated derivatives to be reduced to \(2\)-dihydro- forms and the ability of the compounds to enter into the biosynthesis of TPN as reported by Altman and Evans (1) was studied.

RESULTS

Although Kligler(2) has implied that both nicotinamide and tryptophane are required for the growth of Staphylococcus Aureus, the results given in Table I clearly demonstrate that tryptophane was not required by our strain and that 1 \(\gamma\)/ml. of nicotinamide is both the minimal amount required as well as sufficient for maximal growth.

Three of the compounds studied, 6-bromonicotinamide, 6-fluoronicotinamide, and 6-hydroxynicotinamide reversibly antagonized nicotinamide (Table II) and one of the compounds, 2-bromonicotinamide, irreversibly antagonized nicotinamide. Thiazole-5-carbonamide, an isostere of nicotinamide which had been included for purposes of comparison, reversibly antagonized nicotinamide in agreement with the
results reported by Erlenmeyer, Bloch, and Kiefer (3) for this organism.

### Table I

<table>
<thead>
<tr>
<th>Tryptophane mg/ml.</th>
<th>Nicotinamide μg/ml.</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
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</tr>
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<td>++++</td>
</tr>
<tr>
<td>0.0</td>
<td>0.5</td>
<td>--</td>
</tr>
<tr>
<td>0.0</td>
<td>0.1</td>
<td>--</td>
</tr>
<tr>
<td>1.0</td>
<td>0.0</td>
<td>--</td>
</tr>
<tr>
<td>0.5</td>
<td>0.0</td>
<td>--</td>
</tr>
<tr>
<td>0.1</td>
<td>0.0</td>
<td>--</td>
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<td>10</td>
<td>++++</td>
</tr>
<tr>
<td>1.0</td>
<td>1</td>
<td>++++</td>
</tr>
</tbody>
</table>

++++ ... 15-30% Transmission  +++ ... 30-50% Transmission  
++ ... 50-70% Transmission  + ... 70-90% Transmission

### Table II

<table>
<thead>
<tr>
<th>Drug Conc. M/μl.</th>
<th>Nicotinamide Conc. μg/ml.</th>
<th>10^-1</th>
<th>10^-2</th>
<th>10^-3</th>
<th>10^-4</th>
<th>10^-5</th>
<th>10^-6</th>
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<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>Thiazole-5-carbonamide</td>
<td>25</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>2-bromonicotinamide</td>
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<tr>
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<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
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</tr>
</tbody>
</table>
Two of the compounds, 2-fluoronicotinamide and 2-hydroxynicotinamide, were able to replace nicotinamide for this organism though a much greater drug level was required (Table III).

Table III

<table>
<thead>
<tr>
<th>Drug Conc. N/ml.</th>
<th>$10^{-2}$</th>
<th>$10^{-3}$</th>
<th>$10^{-4}$</th>
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<tbody>
<tr>
<td>Growth</td>
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<td>+++</td>
</tr>
<tr>
<td>2-hydroxynicotinamide</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>2-fluoronicotinamide</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Unfortunately, the methiodides of the bromo- and fluoronicotinamides could not be prepared \(^1\). Therefore, the reduction studies were carried out on the corresponding \(\alpha\) or \(\alpha'\) substituted 2-picoline methiodides and 5-methylthiazole methiodide. In addition the N-methyl derivatives of the two hydroxynicotinamides and thiazole-5-carbonamide methiodide were also studied. Nicotinamide methiodide and 3-cyanopyridine methiodide were included as reference compounds. The recently reported sodium borohydride technique of Mathews(4) was found to give much better results than the standard sodium hydrosulfite technique. The reduction was determined spectrophotometrically. The only compounds which seemed to be reduced were the 2- and 6- bromo-3-methylpyridine methiodides and the parent 2-bromopyridine methiodide (Fig. III). As can be seen from the accompanying figures (Figs. II, IV, V, AND VI), significant changes were not detected in any of the other compounds. The two reference
Figure I

C = 0.1 mg./ml. --- 3-cyanopyridine-methiodide, ------- 3-cyanopyridine-methiodide reduced by 1 mg./ml. of NaBH₄, --- Nicotinamide-methiodide, ---- Nicotinamide-methiodide reduced by 1 mg./ml. of NaBH₄
C = 0.1 mg./ml.  

- - - - - - - 2-fluoro-3-methylpyridine methiodide,  
2-fluoro-3-methylpyridine methiodide reduced by 1 mg. ml. of NaBH₄,  

- - - - - - - 2-fluoro-5-methylpyridine methiodide,  
2-fluoro-5-methylpyridine methiodide reduced by 1 mg. ml. of NaBH₄.
Figure III

C = 0.1 mg./ml.   --- 2-bromopyridine methiodide,   --- 2-bromopyridine methiodide reduced by 1 mg./ml. of NaBH₄,  2-bromo-3-methyl pyridine methiodide,   2-bromo-3-methyl pyridine methiodide reduced by 1 mg./ml. of NaBH₄, 2-bromo-5-methyl pyridine methiodide, 2-bromo-5-methyl pyridine methiodide reduced by 1 mg./ml. of NaBH₄,
Figure IV

C = 0.1 mg./ml.  
- - 1,3 dimethyl-2-pyridone,  
- - - 1,3 dimethyl-2-pyridone reduced by 1 mg./ml. of NaBH₄,  
- - 1,5 dimethyl-2-pyridone,  
- - - 1,5 dimethyl-2-pyridone reduced by 1 mg./ml. of NaBH₄
Figure V

C = 0.1 mg./ml. ••••• 5-methylthiazole methiodide, ——— 5-methylthiazole methiodide reduced by 1 mg./ml. of NaBH₄, —— 3-methylpyridine methiodide, —— 3-methylpyridine methiodide reduced by 1 mg./ml. of NaBH₄.
Figure VI

C = 0.05 mg./ml.  
- - - - 1-methyl-2-pyridone-3-carbonamide,  
- - - - 1-methyl-2-pyridone-3-carbonamide reduced by 1 mg./ml. of NaBH₄,  
- - - - 1-methyl-2-pyridone-5-carbonamide,  
- - - - 1-methyl-2-pyridone-5-carbonamide reduced by 1 mg./ml. of NaBH₄,  
- - - - Thiazole-5-carbonamide methiodide,  
Thiazole-5-carbonamide methiodide reduced by 1 mg. ml. of NaBH₄,
compounds (Fig. I) were both reduced readily to give products which had an absorption maximum near 340 m\(\mu\).

The ability of the substituted nicotinamides to form products absorbing in the region between 320 and 350 m\(\mu\) under the conditions employed by Evans and Altman(1) was also estimated spectrophotometrically. If the amount of reducible material formed in the blank is rated as 1 then the value for the addition of nicotinamide is 5. The two hydroxy nicotinamides have values of 1 while all of the others have values between 0 and 0.1 (Table IV).

Table IV

<table>
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<th>Compound</th>
<th>TPN Synthesis</th>
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<tr>
<td>Blank</td>
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<tr>
<td>Nicotinamide</td>
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<tr>
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<td>6-hydroxynicotinamide</td>
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<tr>
<td>Thiazole-5-carbonamide</td>
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</tr>
</tbody>
</table>

DISCUSSION

The results given in Tables I, II, and III suggest that the 6-position must have a considerable biological importance. This has already been suggested on the basis of enzymatic oxidation studies by Knox and Grossman(5). Presumably, reduction to the \(\alpha\)-dihydro-form is the step which
is inhibited. The behavior of 2-bromonicotinamide is rather surprising. The amount of inhibition is less than that of the 6-bromonicotinamide yet its inhibition is not reversed by a considerable excess of nicotinamide. We believe that this indicates that the mechanism of the inhibition of 2-bromonicotinamide differs from that of the 6-substituted compounds. The ability of 2-hydroxynicotinamide and 2-fluoronicotinamide to replace nicotinamide (Table II) is further evidence of the importance of the 6-position biologically. The results suggest that the 6-position must be open if biological activity is to be retained. The biological activity of the hydroxynicotinamides is not present in their N-methyl derivatives, both of which have been reported to be biologically inert (5,6). This further suggests that the biological activity of the parent compounds is related to their ability to react at the nitrogen atom.

Unfortunately the reduction studies were highly inconclusive. Of all the compounds tested only the bromo-derivatives were reduced. The reaction of the bromo-derivatives was clearly established as reductions rather than hydrolyses, since the characteristic absorption peak of the pyridone compounds (Fig. IV) was absent. The absence of an absorption peak in the 340 m \( \mu \) region very likely arises from the fact that only 2 double bonds are present in the reduced form of the bromo-comounds and accordingly fewer resonance possibilities exist than for the reduced forms of nicotinamide methiodide and 3-cyanopyridine methiodide, which have
a conjugated system of 3 double bonds. However, the failure of some of the other compounds to be reduced is puzzling. The lack of reduction of the thiazole derivatives is in opposition to the successful reduction of ethyl thiazole-5-carboxylate methiodide to the corresponding thiazoline derivative by sodium hydrosulfite(7). Also the failure of 1-methyl-2-pyridone-3-carbonamide to be reduced is in opposition to its ability to replace nicotinamide in bacterial metabolism.

The failure of the compounds to be reduced polarographically was quite unexpected and no reasonable explanation is known.

Whereas the results of the TPN synthesis experiments suggest that all of the compounds except the two hydroxy derivatives are able to antagonize the biosynthesis occurring in the absence of added nicotinamide, no differentiation whatever appeared in the cases of the fluoronicotinamides and the hydroxynicotinamides between the isomers which supported bacterial growth and those which inhibited growth.

It is apparent from the results described above that the variation in biological activity of the various types of nicotinamides cannot be correlated with the chemical and biochemical tests which we employed, but must be attributed to more subtle distinctions.

Experimental\textsuperscript{2,3}

\textbf{Intermediates:-} The preparation of the substituted
nicotinamides is described in part I of this thesis. Thia-
zoile-5-carbonamide was prepared by the method of Erlenmeyer
and von Meyenberg(8). The preparation of the substituted
β-picoline methiodide and 3-cyanopyridine methiodide is
given in part II of this thesis. The preparation of the
isomeric N-methyl nicotinamides is given in part III of this
thesis. Nicotinamide methiodide was prepared by heating
nicotinamide with an excess of methyl iodide at 90° in a
pressure bottle overnight, and worked up as described by
Karrer(9). This procedure gave somewhat better results than
that of Karrer. Thiazole-5-carbonamide methiodide was pre-
pared in the same way from thiazole-5-carbonamide and methyl
iodide. After two recrystallizations from water-alcohol-
ether it melted at 146.1-146.8°.

Anal. Calc'd for C₅H₇ON₂SI; N, 10.7.
Found: N, 10.8.

The sodium borohydride was a generous gift of Dr. Martin B. Mathews. Adenosine triphosphate was prepared by the
procedure of Dounce(10). In our hands it gave better
results than the procedures of Kerr(11) and LePage(12).

Bacteriological experiments:- The Oxford strain of
Staphylococcus Aureous H used in penicillin assay work was
used. The basal media was that of Kligler(2) except that
tryptophane and nicotinamide were not added. The runs were
made up to a final volume of 10 ml. and continuously shaken
in a 37° incubator for a twenty-four hour period. In all
cases a 2 drop inoculum of a 42% light transmission
suspension of twice washed cells, in saline, prepared from a twenty-four hour culture, was used. Growth was estimated turbidometrically in a Coleman model 11 spectrophotometer at 540 m\(\mu\), after the culture was vigorously shaken with glass beads to produce a uniform suspension. At higher nicotinamide concentrations, in the absence of an antagonist, growth occurred in the form of large clumps in a clear medium and extreme care had to be exerted if a uniform suspension was to be obtained. Higher drug concentrations could not be studied because 10\(^{-2}\) M. is very close to solubility limit for the 6-substituted derivatives. The 2-substituted derivatives are more soluble.

Reduction studies: Spectral measurements were made in a Beckman Model DU spectrophotometer using silica cells and the ultra-violet light source throughout. An 0.15 M. phosphate buffer (pH 7.02) was used as solvent. After the spectra of the compounds in buffer had been measured, 3 mg. of sodium borohydride was added to each of the cuvettes including the blank, the solution stirred, and after gas evolution had moderated (5-10 minutes) the spectra were measured again. Tests on nicotinamide methiodide showed that the reduced form was stable for several hours in this buffer system. It is necessary to add sodium borohydride to the blank to compensate for the moderate absorption of sodium borohydride between 220 and 300 m\(\mu\). Between 300 and 400 m\(\mu\) the absorption of sodium borohydride is so slight that it can safely be ignored in this region.
Polarographic studies:— A Sargeant Herovský Model XII polarograph was used. Although the compounds were freely soluble in the buffers no sign of reduction appeared.

TPN synthesis studies:— The exact conditions of Altman and Evans were employed except that the substituted nicotinamide was added in place of nicotinamide. After the reaction was stopped by immersion in boiling water for one minute the precipitated proteins were centrifuged down. A 1 ml. aliquot of the supernatant was adjusted to pH 7 with sodium hydroxide, and diluted to 3 ml. with pH 7.02 phosphate buffer in Beckman cuvettes. Spectral measurements were made between 300 and 380 m and repeated following the addition of 3 mg. of sodium borohydride. The difference was regarded as a measure of the N-alkylated products formed.
SUMMARY

1. A series of α and α' substituted nicotinamides was tested for nicotinamide antagonist action.

2. Three of the compounds, 6-bromonicotinamide, 6-fluoronicotinamide, and 6-hydroxynicotinamide, were found to reversibly antagonize nicotinamide and one, 2-bromonicotinamide, irreversibly.

3. Two compounds, 2-fluoronicotinamide and 2-hydroxynicotinamide, were found to substitute for nicotinamide.

4. Attempts to determine the point of inhibition failed.
FOOTNOTES

1. The unsuccessful preparation of these compounds is given in part I of this thesis.

2. All melting points are corrected.

3. Analyses are by Clark Microanalytical Laboratory, Urbana, Illinois.

4. We are indebted to Mr. J. H. Fellman for valuable assistance in carrying out these bacteriological studies.
REFERENCES

6. Private communication from Dr. W. G. M. Holman.
UNCOMPLETED PROJECTS
Two projects were not carried to a successful conclusion. Studies on the determination of the reactive center in nicotinamide by reduction-oxidation studies on L or L′ deuteronicotinamide methiodide were blocked by the inability to successfully synthesize the 2 isomeric deuteronicotinamides. Two routes failed completely. Attempts to reduce 2- or 6-bromonicotinamide catalytically gave erratic results and a satisfactory reduction could not be effected. Attempts to make the deuterpicolines via reaction of the corresponding Grignard reagent with deuterium oxide failed because of our inability to prepare either the Grignard or lithium reagents from 2- and 6-bromo-3-methylpyridines. This is not surprising since a similar difficulty has been reported for 2-bromopyridine. Attempts at the catalytic reduction of the 2- and 6-bromo-3-methylpyridines gave only fair yields of picolines. In addition, the oxidative conversion gave poor yields of nicotinic acid.

The study of the ability of ATP to react with 6-iodo-glucose derivatives gave no conclusive results. Although a considerable part of the ATP was converted to ADP under the conditions employed, some conversion to triphosphate diesters was obtained. The results varied from run to run. It is possible that small variations in pH are responsible for the erratic results. A considerable part of the difficulty seems to arise from the low reactivity of the iodine in the 2 sugar derivatives which were studied; namely, 6-iodo-tetraacetylglucose and 6-iodo-α-methylglucoside. It is
possible that better results would be obtained if a more reactive sugar derivative were employed.
Many interesting possibilities for fruitful work have suggested themselves during the course of this investigation. It is possible that a 5,6-anhydrosugar derivative would possess a sufficiently high reactivity to react with ATP at a rapid rate under mild conditions so as to avoid the hydrolysis to ADP which otherwise appears to be the principal reaction. Such reactions are known to give a product having the desired configuration.

In view of the results obtained in part III of the thesis it is questionable whether a purely chemical route to the determination of the reactive center of nicotinamide would be conclusive as to which of the hydrogen atoms is the reactive center under biological conditions. The following alternate biochemical route to the determination of the reactive position of nicotinamide would eliminate this possibility of error; isolated Coenzyme I could be reduced enzymatically using deutero ethyl alcohol as substrate to give deuterated dihydro-Coenzyme I. This could then be reoxidized to Coenzyme I by conventional means. Several repetitions of this cycle would insure reasonably complete deuteration in either the 2- or 6- positions of the nicotinamide moiety of Coenzyme I. By acid hydrolysis, the labeled nicotinamide could be split off and isolated. Since, as we have shown in part III of this thesis, it is possible to oxidize N-methylnicotinamide selectively in either the 2- or the 6- position the rest of the problem would be relatively easy. Oxidation of samples of this labeled nicotinamide to
l-methyl-2-pyridone-3-carbonamide and l-methyl-2-pyridone-5-carboxylic acid would eliminate any deuterium in the 2- or 6- positions respectively. Accordingly deuterium determinations on these products would conclusively determine the position of the deuterium in the original Coenzyme I, and consequently locate the reactive center of the molecule.

In addition, the oxidation studies discussed in part III are capable of considerable extension. It would be of considerable interest to determine the orientation of the oxidation products when the oxidation was carried out electrolytically as described by Fischer and Chur(1).

Of considerable interest also would be a determination of which of the isomers is formed when the various /3-substituted pyridine methiodides used in part III are oxidized by the rabbit liver preparation of Knox(2). If the orientation is different from that obtained chemically, it would indicate that the steric or other requirements of the enzymatic reaction are capable of exerting a stronger influence over the course of the reaction than that of the compound being oxidized.

It would be useful further to find out if steric hindrance in the form of bulky groups in the 1- or 3- position is capable of changing the direction of the reaction, or whether the configuration of the product is determined solely by the normal non-steric factors.

The extreme resistance of the fluoro- and bromonicotinamides toward alkylation also remains a puzzling reaction
worthy of additional study. No reasonable explanation for the difference between these compounds and the corresponding picolines which are readily alkylated is known. It would be interesting to see if the corresponding 2- and 6- substituted-3-cyanopyridines and the corresponding esters are also resistant to methylation.

Finally, as is pointed out in part III, the question of intramolecular hydrogen-bonding in compounds in which true chelation cannot occur has never really been settled. The question could be readily settled in the following manner. The cis and trans cyclohexane cyanohydrins could be easily prepared from the known cis and trans chlorohydrins. If such hydrogen bonding is possible then the infra-red spectra of the cis-cyclohexane cyanohydrin will differ noticeably from that of the trans-cyclohexane cyanohydrin in which hydrogen bonding is impossible for spacial reasons.
REFERENCES