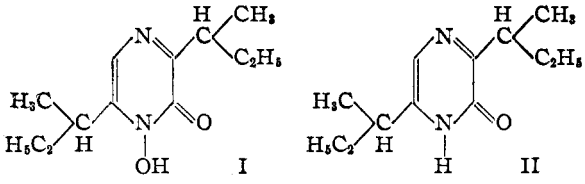
[CONTRIBUTION FROM THE DIVISION OF MEDICINAL CHEMISTRY, THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH]

# Analogs of Aspergillic Acid. I. The Tautomerism of the Hydroxypyridine-N-oxides[1]

BY ELLIOTT SHAW

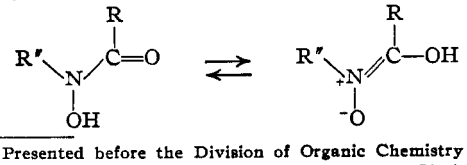
A structural study of aspergillic acid, an antibiotic produced by the mold, *Aspergillus flavus*,[2] led to the conclusion[3] that the substance is a 1-hydroxy-3,6-dialkyl-2-pyrazone (I). The acidic properties are thus attributable to a cyclic hydroxamic grouping. Like the more familiar acyclic hydroxamic acids, aspergillic acid can be reduced



I II

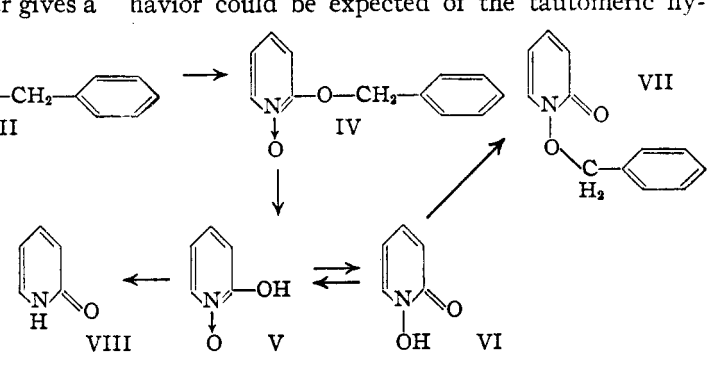
by chemical agents with loss of one oxygen atom to a neutral substance (II) which no longer gives a deep red color with ferric chloride and does not form a copper salt. Of considerable importance is the fact that this change is also accompanied by loss of antibacterial properties. The hydroxamic acid grouping, therefore, seems to be an essential feature of the molecule for biological activity, a conclusion supported by the observation that certain simpler hydroxamic acids not related to pyrazine derivatives also inhibit bacterial growth.[4]

This novel occurrence of a hydroxamic acid grouping in a natural product, together with the possibility of developing synthetically a new series of antibacterial agents, led us to undertake the preparation of cyclic hydroxamic acids. The only examples of this type encountered in the literature are a few N-hydroxy-2-quinolones obtained by a synthesis of quite limited application, *i. e.*, partial reduction of ethyl *o*-nitrocinnamates with cyclization taking place at the *o*-hydroxylamino ester stage.[5,6] It seemed likely that more general methods of preparation might be developed by a study of a postulated tautomerism of hydroxamic acid and hydroxy N-oxide structures:

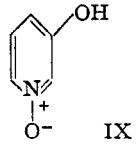


The existence of such a tetrad system has been indicated[7] in the case of N-hydroxyphthalimidine by the formation of the isomeric O-ethers.

The pyridine series was chosen for the initial studies because of the accessibility of intermediates. 2-Benzyloxypyridine (III) was prepared and, by means of perbenzoic acid, converted to its N-oxide (IV), an ether of tautomeric structure (V). The oxide dissolves in aqueous hydrochloric acid with rapid separation of benzyl chloride, and a crystalline acid, $pK$ = 5.9, corresponding to (V) or (VI) can be isolated from the solution. The acid forms a copper salt and gives a deep red color with ferric chloride—characteristic, classic properties of hydroxamic acid. However, similar behavior could be expected of the tautomeric hydroxypyridine N-oxide (V) in which the positively charged nitrogen in the amine oxide semi-polar bond would facilitate ionization by an inductive effect. For example, 3-hydroxypyridine N-oxide (IX) was prepared and found to be quite acidic, $pK$ = 6.4 compared to $pK$ = 8.6 for 3-hydroxypyridine itself. For this isomer, no tautomeric structure comparable to a hydroxamic acid (as in VI) can be formulated.



III IV VII VIII V VI



IX

However, in the 2-pyridyl series it is necessary to conclude from the spectroscopic data described below that the acid obtained on debenzylation of the amine oxide (IV) is not the corresponding hydroxyoxide (V) but a hydroxamic acid (VI). The tautomeric system involved was defined chemically by the preparation of the benzyl ethers corresponding to both forms, (IV) and (VII), and by their conversion on reductive debenzylation to a single acid m. p. 150° (VI).

The N-benzyloxy ether (VII) was formed as the only non-acidic product when the sodium

(1) Presented before the Division of Organic Chemistry at the 112th Meeting of the American Chemical Society, New York, N. Y., September, 1947.

(2) White and Hill, *J. Bact.*, **45**, 433 (1943).

(3) Dutcher and Wintersteiner, *J. Biol. Chem.*, **155**, 359 (1944).

(4) Dutcher, *J. Biol. Chem.*, **171**, 321 (1947).

(5) Friedlander and Ostermaier, *Ber.*, **14**, 1916 (1881); **15**, 332 (1882).

(6) Heller and Wunderlich, *ibid.*, **47**, 2889 (1914).

(7) Griffiths and Ingold, *J. Chem. Soc.*, **127**, 1689 (1925).

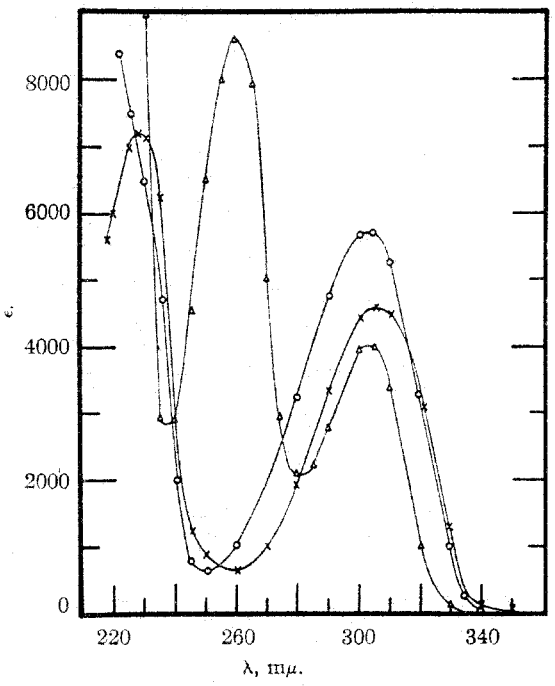



Fig. 1.—Ultraviolet absorption spectra in ethanol: -Δ-Δ-Δ-, 2-benzyloxypyridine N-oxide; -X-X-X, N-hydroxy-2-pyridone; -O-O-O-, N-benzyloxy-2-pyridone.

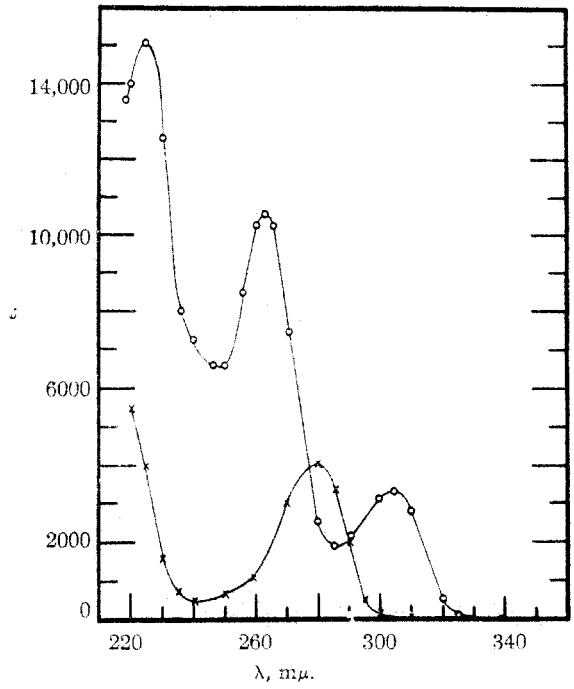
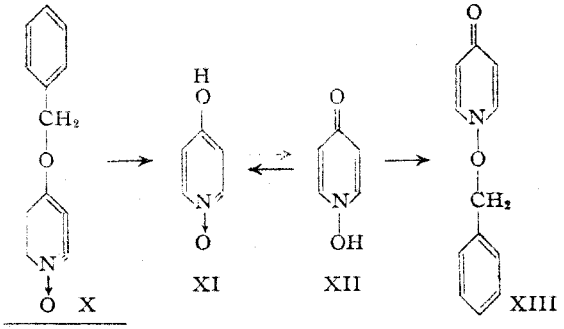



Fig. 2.—Ultraviolet absorption spectra in ethanol: -X-X-X, 3-hydroxypyridine; -O-O-O-, 3-hydroxypyridine N-oxide.

salt of the acid m. p. 150° was treated with benzyl chloride. This ether differs from its isomer (IV) not only in lack of basicity, but also in its resistance to hot, aqueous hydrochloric acid. That it is the more stable isomer is indicated by its isolation, in small quantities, from the hydrochloric acid debenzylation of (IV) apparently due to an isomerization of (IV) to (VII).

In the ultraviolet region, the spectra of the acids (V–VI) in ethanol shows two maxima ($\lambda$ = 228 m$\mu$, $\epsilon$ = 7,200 and $\lambda$ = 305 m$\mu$, $\epsilon$ = 4,600) corresponding rather well to those in the spectrum of 2-pyridone ($\lambda$ = 227 m$\mu$, $\epsilon$ = 7,300 and $\lambda$ = 300 m$\mu$, $\epsilon$ = 5,000). 2-Pyridone is itself considered[8,9] to be in the amide rather than in the hydroxypyridine form, preferably expressed[9] as a dipolar modification. Additional evidence in favor of a hydroxamic acid structure (VI) for the acid is found in a comparison of its spectrum with those of the benzyl ethers of the tautomeric forms shown in Fig. 1. The absorption characteristics for the acid differ markedly from those of the amine oxide (IV) and are in better agreement with the spectrum of the N-benzyloxy isomer (VII). The maximum at 260 m$\mu$ for 2-benzyloxypyridine N-oxide (Fig. 1) is apparently related to the N-oxide configuration. A similar peak is found in the spectrum of 3-hydroxypyridine N-oxide (Fig. 2) at 263 m$\mu$, both substances in ethanol. Further, pyridine N-oxide, measured conveniently in water by neutralizing the hydrochloride prepared by the method of Meisenheimer[10] absorbs mainly at 255 m$\mu$, $\epsilon$ = 9,000; when measured also in water, the spectrum of 3-hydroxypyridine N-oxide shows a shift of the maximum at 263 m$\mu$ (ethanol) to 255 m$\mu$ and, like pyridine N-oxide, exhibits diminishing intensity, as the *p*H drops from 5 to 1, without a change in the wave length of the maximum. Specific absorption in this region is significantly absent from the spectrum in water or ethanol of the acid m. p. 150°, on observation in agreement with the assignment of a hydroxamic acid (VI) rather than a pyridine oxide structure (V) to the substance.

To complete the study of the isomeric hydroxypyridine N-oxides, the 4-pyridyl analog was prepared. As in the 2-pyridyl series, tautomerism with a hydroxamic acid structure was anticipated (XI–XII). 4-Benzyloxypyridine was converted to the N-oxide (X) which, when reduced cata-




(8) Specker and Gawrosch, *Ber.*, **75**, 1345 (1942).
(9) Arndt and Kalischek, *ibid.*, **63**, 587 (1930).
(10) Meisenheimer, *ibid.*, **59**, 1848 (1926).

lytically, gave an acid m. p. 238–240°, $pK$ = 5.9. A new benzyl ether, undoubtedly XIII was obtained from the sodium salt of the acid on treatment with benzyl chloride. A comparison of the ultraviolet absorption spectra of the acid (XI–XII) and the isomeric benzyl ethers offers no information as to the state of the tautomerism since all three substances show maximum absorption in the same region, 268–270 mμ (Fig. 3).[11]

Of the three isomeric pyridine acids described, only N-hydroxy-2-pyridone has significant antibacterial activity.

## Experimental[12]

**2-Benzyloxypyridine N-Oxide (IV).**—2-Benzyloxypyridine (37 g.) was added to a chloroform solution of perbenzoic acid (1.5 equivalents) and the mixture left standing at room temperature in a loosely stoppered flask. After three days the solution was washed successively with aqueous sodium carbonate and water, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The crystalline residue was thinned with a mixture of ethyl acetate and hexane for filtration, yielding 18 g. (45%) of product m. p. 99–102°. This material was satisfactory for the next step. An analytical sample was obtained by recrystallization from ethyl acetate and hexane and melted at 103–106°.

*Anal.* Calcd. for $C_{12}H_{11}O_2N$: C, 71.62; H, 5.51; N, 6.96. Found: C, 71.66; H, 5.74; N, 6.88.

**N-Hydroxy-2-pyridone (VI).**—(A) By hydrochloric acid debenzylation: 2-Benzyloxypyridine N-oxide (4.3 g.) was boiled for ten minutes with 15 ml. of 20% hydrochloric acid under a reflux condenser provided with a take-off to remove benzyl chloride. The turbid solution was then taken to dryness *in vacuo* and recrystallized from benzene containing a small amount of methanol. There was obtained 1.6 g. (68%) of product melting at 145–147°. A recrystallization from ethyl acetate brought the m. p. to 149–150°.

*Anal.* Calcd. for $C_5H_5O_2N$: C, 54.05; H, 4.54; N, 12.61. Found: C, 53.94; H, 4.62; N, 12.55.

N-Hydroxy-2-pyridone gives a deep red color with ferric chloride in alcohol solution or a precipitate in aqueous solution. The copper salt melts at 298° (dec.).

*Anal.* Calcd. for $(C_5H_4O_2N)_2Cu$: N, 9.87. Found: N, 9.73.

From the mother liquor in the above debenzylation a small neutral fraction was obtained by combining the benzene and ethyl acetate filtrates, extracting acidic material with aqueous alkali, and concentrating the organic layer. Colorless crystals were obtained, m. p. 83–84°, isomeric with the starting material.

*Anal.* Calcd. for $C_{12}H_{11}O_2N$: C, 71.62; H, 5.51; N, 6.96. Found: C, 71.56; H, 5.60; N, 6.97.

(B) By catalytic reduction: 2-Benzyloxypyridine N-oxide (25 g.) was shaken in 100 ml. of ethanol with 750 mg. of palladium catalyst (5% on charcoal) at an initial pressure of 50 lb. of hydrogen. The reduction was complete in about five minutes. Concentration of the filtered solution gave 9.5 g. (69%) of N-hydroxy-2-pyridone, m. p. 150°.

**N-Benzyloxy-2-pyridone (VII).**—N-Hydroxy-2-pyridone (3.25 g.) was added to a solution of sodium (0.75 g.) in 45 ml. of ethanol. The resultant suspension of sodium salt was refluxed with benzyl chloride (4.18 g.) for three hours. The filtrate was concentrated to a small volume, taken up in chloroform, and washed with dilute sodium hydroxide solution to remove unreacted starting acid. The dried organic layer on evaporation left a crystalline neutral residue of 3.8 g. (70%) melting at 85–86°. The melting point was unchanged by recrystallization from ethyl acetate and hexane. This material is identical with that obtained from the mother liquors of the hydrochloric acid debenzylation of 2-benzyloxypyridine N-oxide as described above.

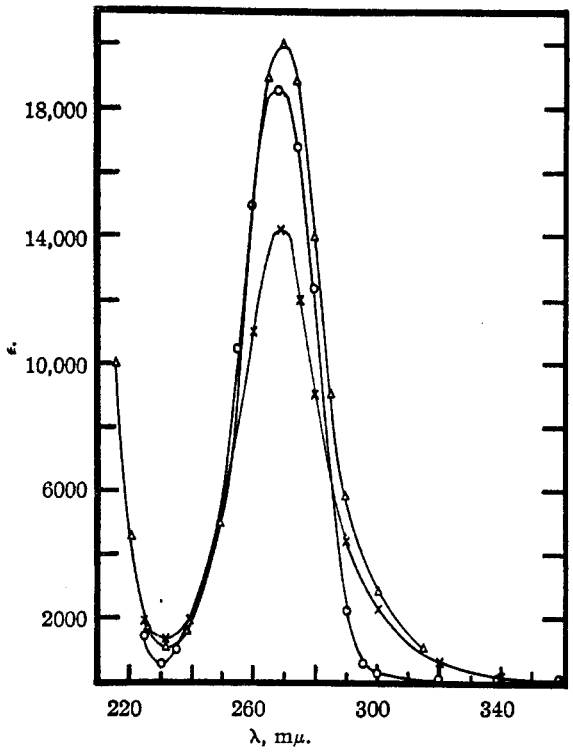



Fig. 3.—Ultraviolet absorption spectra in ethanol: -Δ-Δ-Δ-, 4-benzyloxypyridine N-oxide; X-X-X, N-hydroxy-γ-pyridone or tautomer; -O-O-O, N-benzyloxy-γ-pyridone.

**Reduction of N-Hydroxy-2-pyridone to 2-Pyridone.**—Red phosphorus (1 g.) and iodine (0.5 g.) were left standing for ten minutes in 25 ml. of glacial acetic acid. N-Hydroxy-2-pyridone (2 g.) in 30 ml. of glacial acetic acid was added and the solution refluxed for two and one-half hours. The reaction was worked up although the reduction was not yet complete judging by the persistence of a ferric chloride color test. The filtrate was taken to dryness and the residue extracted with boiling ethyl acetate. The addition of picric acid (5.5 g. in 50 ml. of alcohol) precipitated 2.5 g. of picrate (43%) identical with the picrate prepared from an authentic sample of 2-pyridone and melting at 170–172°.

*Anal.* Calcd. for $C_{11}H_8O_8N_4$: C, 40.75: H, 2.49; N, 17.28. Found: C, 40.75; H, 2.60; N, 17.31.

**3-Hydroxypyridine N-Oxide (IX).**—3-Hydroxypyridine oxidized quite readily with perbenzoic acid (1.5 equivalents in chloroform), the product crystallizing out overnight, m. p. 188–190°, (65%). Recrystallization from methanol raised the m. p. slightly to 189–191°.

*Anal.* Calcd. for $C_5H_5O_2N$: C, 54.05; H, 4.54; N, 12.61. Found: C, 53.93; H, 4.68; N, 12.54.

**4-Benzyloxypyridine.**—4-Pyridylpyridinium dichloride[13] (65 g.) was dissolved in 150 ml. of hot benzyl alcohol and added to a solution of sodium (13 g.) in 250 ml. of benzyl alcohol. The suspension was refluxed for four hours, poured into water, and extracted with ether. Fractionation of the residue obtained from concentration of the dried ether extract gave an oil b. p. 147–160° (4 mm.). On redis-

(11) The coöperation of Dr. Nettie H. Coy of the Division of Development, E. R. Squibb and Sons, in providing the spectroscopic measurements cited in this paper is acknowledged.

(12) All melting points are uncorrected. Microanalyses were carried out by Mr. J. F. Alicino.

(13) Koenigs and Greiner, *Ber.*, **64**, 1049 (1931).

tillation there was obtained 11.5 g. (22%) of product, b. p. 155–160° (4 mm.). The free base could be crystallized from hexane, m. p. 55–56°.

*Anal.* Calcd. for $C_{12}H_{11}ON$: C, 77.81; H, 5.98; N, 7.56. Found: C, 78.22; H, 5.84; N, 7.42.

A picrate of the base melted at 150–151°.

*Anal.* Calcd. for $C_{12}H_{11}ON \cdot C_6H_3O_7N_3$: C, 52.18; H, 3.45. Found: C, 52.22; H, 3.40.

**4-Benzyloxypyridine N-Oxide (X).**—The oxide was prepared from the base by means of perbenzoic acid as described above for the 2-isomer and melted at 178–179°.

*Anal.* Calcd. for $C_{12}H_{11}O_2N$: C, 71.62; H, 5.51; N, 6.96. Found: C, 71.57; H, 5.42; N, 6.62.

The amine oxide formed a picrate readily in alcoholic solution, m. p. 123–124°.

*Anal.* Calcd. for $C_{12}H_{11}O_2N \cdot C_6H_3O_7N_3$: C, 50.24; H, 3.30. Found: C, 49.84; H, 3.29.

**N-Hydroxy-4-pyridone (XI–XII).**—4-Benzyloxypyridine N-oxide (1 g.) was catalytically reduced with 150 mg. of palladium (5% on charcoal) as described for the 2-isomer. The filtrate was concentrated to a small volume, depositing 345 mg., 68%, m. p. 243–244°. The acid gives no precipitate with ferric chloride but produces an orange color. The preparation of this compound by another method has been described[14] but no m. p. is given.

*Anal.* Calcd. for $C_5H_5O_2N$: C, 54.05; H, 4.54; N, 12.61. Found: C, 54.47; H, 4.81; N, 12.20.

(14) Ost. *J. prakt. Chem.*, [2] **29**, 379 (1884).

**N-Benzyloxy-4-pyridone (XIII).**—N-Hydroxy-4-pyridone (47 mg.) was added to a solution of sodium (9.7 mg.) in alcohol and refluxed with benzyl chloride (53.6 mg.) for one hour. The mixture was partitioned between ethyl acetate and alkali. The organic layer on evaporation left a residue of 73 mg. After recrystallization from ethyl acetate and hexane, the material melted 112–113°.

*Anal.* Calcd. for $C_{12}H_{11}O_2N$: C, 71.62; H, 5.51. Found: C, 71.80; H, 5.60.

The picrate of this benzyl ether melted at 164° and depresses the melting point of the picrate of 4-benzyloxypyridine N-oxide.

*Anal.* Calcd. for $C_{12}H_{11}O_2N \cdot C_6H_3O_7N_3$: C, 50.24; H, 3.30. Found: C, 50.33; H, 3.65.

**Acknowledgment.**—The author is indebted to Mr. W. A. Lott for his interest and encouragement.

## Summary

The synthesis of N-hydroxy-2-pyridone, a cyclic hydroxamic acid, is described. The acid is in tautomeric relationship with 2-hydroxypyridine N-oxide.

Isomeric acids of the 3- and of the 4-pyridyl series also have been prepared.

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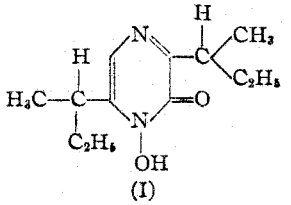
[CONTRIBUTION FROM THE DIVISION OF MEDICINAL CHEMISTRY, THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH]

# Analogs of Aspergillic Acid. II. Various Antibacterial Heterocyclic Hydroxamic Acids[1]

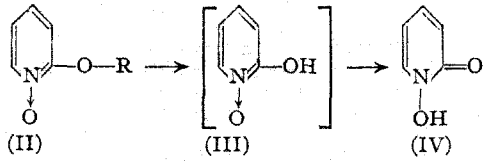
BY W. A. LOTT AND ELLIOTT SHAW

Attempts to develop synthetic methods for introducing into heterocyclic rings the hydroxamic acid grouping present in aspergillic acid (I) led to a



(I)

preparation for N-hydroxy-2-pyridone (IV).[2] The



(II) (III) (IV)

synthesis was achieved by conversion of a 2-pyridyl ether to its N-oxide (II), followed by de-alkylation. The 3- and 4-pyridyl derivatives were also prepared[2] but, of these position isomers, only N-hydroxy-2-pyridone showed antibacterial activity, an observation which encouraged the extension of synthetic work to the preparation of additional heterocyclic hydroxamic acids. New methods have been developed. The synthetic acids exceed, in some instances, the *in vitro* antibacterial activity of aspergillic acid.

The hydroxamic acid grouping in N-hydroxy-2-pyridone (IV) is quite stable chemically, resisting the action of boiling aqueous acid or cleavage of the N–O bond by catalytic reduction (Pd), ammonium sulfide, or stannous chloride. In these respects, the grouping bears no resemblance to N-oxides. It is apparent that the amine oxide (II, R = benzyl) is converted to the hydroxamic acid (IV) successfully due to a more rapid initial attack at the ether linkage, by hydrochloric acid or catalytic reduction, than at the N–O bond which subsequently gains increased resistance to reduction by a tautomeric shift (III → IV) to the hydroxamic acid structure.[2] A similar series of reactions led to a 4-methyl derivative of (IV) in good yields. However, when the substituent was bromine in the 5-position, reduction or treatment of the N-oxide (V) with hydrochloric acid led to 5-bromopyridone (VII) as the main product. In the hydrochloric acid debenzylation, for example, the ratio of pyridone to hydroxamic acid isolated (VI) was 3:1. In this case, in contrast to

(1) Presented before the Division of Organic Chemistry at the 112th Meeting of the American Chemical Society, New York, N. Y., September, 1947.

(2) Shaw, THIS JOURNAL, 71, 67 (1949).