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Pushkavera and Agivalova¹) reported the oxidative ring cleavage of phenazine and they established in this reaction 2,3-quinoxalinedicarboxylic acid in 80% yield. The authors inquired into this reaction more closely and further attempted the oxidation of 1- and 2-methoxyphenazine under the same condition. The result is reported herein together with the syntheses of quinoxaline derivatives related structurally to the pyrazinecarbonamide which was found to possess an antitubercular activity.

In the case of phenazine, the oxidation product was obtained similar to that of Pushkavera et al. as a crude acidic substance, but this was separated into two substances by treating with boiling methanol. The methanol solution gave 2,3-quinoxalinedicarboxylic acid (I) of m.p. 190° (decomp.) in 70% yield and a small amount of another acidic substance which was insoluble in methanol. The same oxidation of 1- and 2-methoxyphenazine was found to give the same methanol-insoluble product in yields of 45% and 24%, respectively. This compound did not melt at 340°, was insoluble in organic solvents, soluble in sodium hydrogen carbonate solution with frothing, and the U.V. spectrum showed $\lambda_{\text{max}}^{0.1%NaOH}$ 288 m$\mu$. Further characterization has not been made as yet.

Some of the synthesis of quinoxalinedicarboxylic acid derivatives are described.

As in usual manner, when (I) was heated with three volumes of acetic anhydride, 2,3-quinoxalinedicarboxylic anhydride (II) was obtained. This affords half-ester (III) by methanolation, which when heated at 150° in vacuo, decarboxylated to form 2-methoxy-carbonylquinoxaline (IV) in a good yield. When (IV) was dissolved in methanol saturated

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2) O. Hinsberg, F. König: Ber., 27, 2185 (1894).
with ammonia, 2-quinoxalinecarbonamide (V) deposited as colorless needles. From (IV) also 2-quinoxalinecarboxyhydrizide (VI) was obtained by the action of 60% hydrazine hydrate, while hydroxamic acid (VII) was obtained by heating (IV) with hydroxylamine. According to the method of Phillips, half-amide of 2,3-quinoxalinedicarboxylic acid (VIII) and 2-amino-quinoxaline-3-carboxylic acid (IX) were prepared from (II).

The results of antitubercular activities of the compounds (V), (VI), and (VII) thus obtained are summarized in Table I.

<table>
<thead>
<tr>
<th>Table I. Antibacterial Activity against M. tuberculosis H₃Rv</th>
<th>Minimum concentration inhibiting growth (γ/cc)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>(V) CONH₂</td>
<td>&lt;100</td>
</tr>
<tr>
<td>(VI) CONNH₂</td>
<td>&lt;100</td>
</tr>
<tr>
<td>(VII) CONHOH</td>
<td>500</td>
</tr>
<tr>
<td>INAH</td>
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</table>

* Modified Dubos medium. Incubation period: 14 days at 37°C.

The authors are grateful to Prof. Ishidate for his encouragement. Their thanks are also due to Mr. E. Kimura, Mr. M. Endo, and Miss T. Oshibe for microanalysis, to Dr. M. Tsuruoka for antitubercular test, and to Mr. S. Nakajima for molecular weight determination.

**Experimental**

**Oxidation of Phenazine**—Phenazine (3.7 g.) was dissolved in 15% HCl (100 cc.), and to this was added the excess of 30% NaOH solution with vigorous stirring. To the homogeneous suspension of phenazine, a solution of KMnO₄ (22.5 g.) in water (360 cc.) was added dropwise, keeping the temperature at 80–90° for 4 hrs., and stirring was further continued for 1 hr. Then the reaction mixture was filtered and the filter cake was washed well with hot water. The filtrate was concentrated under reduced pressure to about 80 cc., and acidified with conc. HCl on cooling. The deposited product was washed with a little amount of cold water and dried. It weighed 3.1 g. in 80% yield. This was boiled with methanol and separated from the insoluble substance. The methanol solution gave 2.7 g. of 2,3-quinoxalinedicarboxylic acid (I) of m.p. 190° (decomp.) as colorless needles (60% MeOH).

The substance which was insoluble in MeOH was recrystallized from water to colorless prisms (0.4 g.). This did not melt at 340° and showed U.V. \( \lambda_{\text{max}}^\text{MeOH} \) 288 m\( \mu \) (log e 0.425), mol. wt., 158.6 (by titration).

**Oxidation of 1-Methoxyphenazine**—1-Methoxyphenazine (4 g.) was oxidized by the same manner as above, the acidic substance (1.6 g.) which did not melt at 340° was obtained as colorless prisms, which showed U.V. \( \lambda_{\text{max}}^\text{MeOH} \) 288 m\( \mu \) (log e 0.370), mol. wt., 158.3. 1.75 g. of 1-methoxyphenazine was recovered.

**Oxidation of 2-Methoxyphenazine**—2-Methoxyphenazine (2 g.) was oxidized and the acidic substance (0.4 g.) was obtained. m.p. >340°, U.V. \( \lambda_{\text{max}}^\text{MeOH} \) 288 m\( \mu \) (log e 0.286), mol. wt., 158.8. In this reaction, starting material (0.2 g.) was recovered. Because of the difficulty of burning on analysis, the molecular formula of this compound was not determined.

**2,3-Quinoxalenedicarboxylic Anhydride (II)**—Prepared by heating (I) with 3 vols. of Ac₂O. m.p. 254° (decomp.).

**2-Methoxycarbonyl-3-quinoxalinecarboxylic Acid (III)**—A solution of anhydride (II) (1.5 g.) in dehyd. MeOH (4 cc.) was refluxed on a water bath for 1 hr. MeOH was removed under reduced pressure and the remaining substance solidified. By recrystallization from MeOH, 1.2 g. of colorless needles of m.p. 151° (decomp.) was obtained. *Anal.* Calcd. for C₁₁H₈O₄N₂: C, 56.89; H, 3.44; N, 12.06. Found: C, 56.83; H, 3.11; N, 11.68.

**Methyl 2-Quinoxalinecarboxylate (IV)**—(III) (1.2 g.) was heated at 150–160° in vacuo, the reaction mixture melted with frothing. After cooling, crystal mass was obtained and purified by sublimation.

Colorless needles (1 g.) of m.p. 111–112° were obtained. *Anal. Calcd. for C₃₇H₃₇O₁₂N₂: C, 63.83; H, 4.25; N, 14.89. Found: C, 63.99; H, 4.44; N, 14.35.*

2-Quinoxalinecarboxamide (V) — (IV) (1 g.) was dissolved in MeOH (30 cc.), saturated with ammonia, and kept standing for 12 hrs. Colorless needles deposited out from the solvent. m.p. 200° (from MeOH). *Anal. Calcd. for C₃₇H₃₇O₁₂N₂: C, 62.42; H, 4.06; N, 24.27. Found: C, 62.26; H, 4.16; N, 23.95.*

2-Quinoxalinecarboxyhydrazide (VI) — To the solution of (IV) (0.5 g.) in MeOH (10 cc.) was added hydrazine hydrate (60%, 4 cc.). The mixture was refluxed on a water bath for 2 hrs. On cooling, crystalline solid separated out and purified from 60% MeOH to colorless needles of m.p. 208°. *Anal. Calcd. for C₃₇H₃₇O₁₂N₂: C, 57.44; H, 4.25; N, 29.78. Found: C, 57.56; H, 4.43; N, 29.83.*

2-Quinoxalinehydroxamic Acid (VII) — Metallic Na (0.5 g.) was dissolved in MeOH (30 cc.), and to this a solution of hydroxylamine hydrochloride (0.8 g.) in MeOH (20 cc.) was added, and sodium chloride separated was filtered off. To this filtrate, (IV) was added and boiled for 30 mins. After the reaction, MeOH was removed, and the remaining substance was washed with water and purified from MeOH to give colorless needles of m.p. 190° (decomp.). *Anal. Calcd. for C₃₇H₃₇O₁₂N₂: C, 57.14; H, 3.70; N, 22.22. Found: C, 57.59; H, 4.43; N, 21.97.*

Summary

Oxidation of phenazine gave 2,3-quinoxalinedicarboxylic acid in the yield of 70%, together with small amount of another acidic substance. The latter was obtained by the similar oxidation of 1- and 2-methoxyphenazine in the yields of 45% and 24%, respectively. From quinoxalinedicarboxylic acid, 2-quinoxaline-carbonamide, -carboxyhydrazide, and -hydroxamic acid were prepared and their antitubercular activities tested.

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The new cardiotonic glycoside, glucodigifucoside, isolated from the seeds of Digitalis purpurea, is a diglycoside with digitoxigenin as the aglycone and glucose and fucose as the sugars, as described in the preceding paper.¹ In Part IV of this series, enzymatic decomposition of gitostin³ with an enzyme obtained from a snail (Euhadra quaesita Deshayes) was described and the same enzymatic method was adopted in the examination of the structure of glucodigifucoside.

Glucodigifucoside was treated for 5 days by the usual method with the enzyme solution, prepared from the snail enzyme powder treated with acetate buffer (pH 5.4), and digitoxigenin was obtained, but not a monoglucoside formed from glucodigifucoside. Examination by paper chromatography of the sugar portion formed by this enzymatic hydrolysis indicated spots for glucose and fucose, giving identical Rf values as those of the sugar portion obtained by acid hydrolysis as reported in the preceding paper. This has shown that the enzyme had also hydrolysed fucose.

Reichstein and others used their Schneckenferment, obtained from Weinberg-schnecke, to numerous kinds of glycoside and the sugars hydrolyzed reported in the literature are all glucose.⁴ In the enzymatic decomposition of cheiroside-A (uzarigenin-

¹ Hirakawabashi, Sumida-ku, Tokyo (岡野淳二).
² Part VI: This Bulletin, 5, 272(1957).