Anal. Calcd. for $C_{11}H_{11}N_5$: C, 61.93; H, 5.2; N, 32.87. Found: C, 61.50; H, 5.13; N, 32.61, 32.64.

Monourethan.—Two substances may be separated from the light brown precipitate first formed on boiling the urethan with alcoholic alkali; (1) a red substance which crystallizes from 25% alcohol in orange-yellow plates which melt at 143°, and (2) a very small amount of phenylazodiaminopyridine which melts at 170–171°. The orange-yellow substance melting at 143° is the monourethan.

Anal. Calcd. for $C_{15}H_{15}O_2N_5$: C, 58.90; H, 5.30; N, 24.56. Found: C, 58.60; H, 5.32; N, 24.30, 24.38.

The monourethan may be transformed to the diurethan by boiling with alcoholic potassium hydroxide.

Summary

γ-Phenylazo-α,α'-diaminopyridine has been prepared in a manner which strictly proves its constitution.

MOSCOW, U. S. S. R. RECEIVED JANUARY 6, 1934

Note on Bacteriostatic Azo Compounds

BY I. OSTROMISLENSKY

In 1914 A. Chichibabin and O. Zeide[1] showed that pyridine behaves analogously to naphthalene when heated with sodium amide. By changing external conditions (proportion of reagents, duration and temperature of reaction) they obtained α-aminopyridine, γ-aminopyridine, and α,α'-diaminopyridine, the second of these substances being isolated by them in the form of chloroplatinate in quite insignificant amounts. Guided by the observations of Mohr[2] in 1898 they further showed that α,α'-diaminopyridine couples with diazotized amines of the aromatic series, and in particular described two azo dyes, one formed by coupling with diazotized benzidine and the other with diazotized aniline.[1]

The author, led by certain theoretical considerations, found that certain azo dyes and among them this latter one described by Chichibabin and Zeide, although of relatively negligible toxicity, exhibited a high bacteriostatic index[3] toward certain microörganisms. He then isolated in a chemically pure state eighteen new azo compounds of the pyridine series, and about 400 azo dyes of the quinoline, aniline, naphthalene and pyrazolone series, only about a quarter of which had been previously described.[4] The most promising forms of these substances were subjected to a thorough pharmacological and therapeutical study and their distribution and elimination with different methods of ingestion into the living organism investigated. The following preparations were found to be the most active and of the greatest practical value in genito-urinary infections.

TABLE I

	Preparation	Bacteriostatic index[5]
I.	Hydrochlorides of: phenylazo-α,γ-diaminopyridine plus β-phenylazo-α,α'-diaminopyridine,[1] with negligible admixture of β,β'-diphenylbisazo-α,α'-diaminopyridine[6] (Pyridium A)	1:12,000 to 1:15,000
II.	β-Phenylazo-α,α'-diaminopyridine monohydrochloride[1] (Pyridium)	1:8,000 to 1:10,000
III.	β-*p*-hydroxyphenyl-azo-α,α'-diaminopyridine (Hydroxy-pyridium)	1:9,000 to 1:11,000
IV.	*p*-Ethoxyphenylazo-2,4-diaminobenzene hydrochloride[7] (Serenium)	1:15,000 to 1:25,000
V.	Phenylene-*m*-disazo-bis-*m*-phenylenediamine hydrochloride[8]	1:20,000 to 1:25,000
VI.	β-Naphthylazo-2,4-diaminobenzene hydrochloride (m. p. of free base)	1:6,000 to 1:8,000
VII.	Phenylazo-2,4-diaminobenzene hydrochloride (Chrysoidin)	1:7,000 to 1:8,000

Monoaminoazobenzenes possess the highest bacteriostatic indexes, ranging from 1:30,000 to 1:50,000, but these compounds are very toxic.

The substances given in the table are, however, tolerated by rabbits, guinea pigs and white mice in doses of about 0.3 g. per kilogram of animal weight.

Experimental Part

Preparation No. I was obtained under the conditions described in U. S. Patent 1,680,109. The original diaminopyridine was obtained by Chichibabin's method, heating pyridine with sodium amide suspended in ***dimethylaniline.*** Fractional crystallization from water showed that two isomeric azo compounds were present. One of these was found to be β-phenylazo-α,α'-diaminopyridine. The other is a new compound. The free base melts at 203°.

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[1] Chichibabin and Zeide, *J. Russ. Phys.-Chem. Soc.*, **46**, 1216 (1914).

[2] Mohr, *Ber.*, **31**, 2495 (1898).

[3] This term designates here the maximum dilution of a substance in a nutritive medium at which the given species of microorganism ceases to multiply.

[4] U. S. Patents 1,785,327, 1,867,587, 1,680,110.

[5] Agar-agar media at a PH ranging between 6.8 and 8.4. Determined as to *S. albus*, *S. aureus* and *Str. haem.*

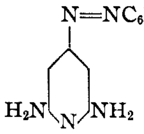
[6] U. S. Patent 1,680,109.

[7] U. S. Patent 1,785,327.

[8] U. S. Patent 1,867,587.

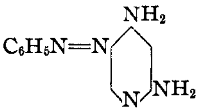
Anal.[9] Calcd. for $C_{11}H_{11}N_5$: C, 61.9; H, 5.2; N, 32.9. Found: C, 61.7; H, 5.3; N, 33.01, 32.85.

To the ortho isomer was first assigned the structure[10]



Further experimentation has shown that two isomeric diaminopyridines are formed by heating pyridine with sodium amide in dimethylaniline. These are α,α'- and α,γ-diaminopyridine.

When a mixture of α,α'- and α,γ-diaminopyridines is coupled with diazotized aniline a mixture of β-phenylazo-α,α'-diaminopyridine and phenylazo-α,γ-diaminopyridine must result. The structure of the new substance must therefore be one of the following, and the first is the more probable.



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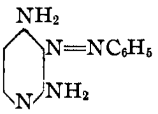

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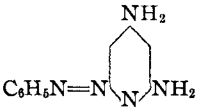


Preparation II; β-Phenylazo-α,α'-diaminopyridine and its Analogs.—When diazotized aniline is coupled with α,α'-diaminopyridine in a medium containing about 8% of free hydrochloric acid, a new dihydrochloride of β-phenylazo-α,α'-diaminopyridine is formed. It is precipitated as lustrous reddish-violet sandy crystals. This was washed with 7% hydrochloric acid and dried in the presence of fuming hydrochloric acid.

Anal. Calcd. for $C_{11}H_{11}N_5 \cdot 2HCl$: HCl, 25.47. Found: HCl, 24.91.

The yield is almost quantitative. When the dihydrochloride is heated to 100° its color changes and both hydrochloric acid molecules are split off with the production of the free base. Kept in a vacuum over caustic soda, or by the action of water, only one molecule of hydrochloric acid is split off and the monohydrochloride described by Chichibabin is obtained. The dihydrochloride may be preserved indefinitely in a sealed vessel.

α-Phenyldiazoamido-α-aminopyridine is formed by the coupling of phenyldiazonium salts with α,α'-diaminopyridines in a weakly acidified aqueous medium.[11] Prismatic yellow-brown crystals are formed. These are very soluble in aniline and pyridine and may be recrystallized from carbon tetrachloride. This substance melts at 117°. Its hydrochloride is changed to the free base on exposure to atmospheric moisture. When the substance is boiled with distilled water for a few hours it is transformed quantitatively to β-phenylazo-α,α'-diaminopyridine hydrochloride.

β-*p*-Methoxyphenylazo-α,α'-diaminopyridine.—The free base may be recrystallized from methyl alcohol or from 50% ethyl alcohol. It melts at 192°. Its bacteriostatic index is below 1:5000.

Anal. Calcd. for $C_{12}H_{13}N_5O$: N, 28.85. Found: N, 28.77.

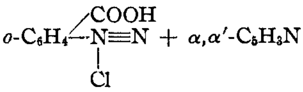
Preparation III. β-*p*-Hydroxyphenylazo-α,α'-diaminopyridine recrystallized from water using animal charcoal melts at 214–215°. Its bacteriostatic index is 1:9000 to 1:11,000.

Anal. Calcd. for $C_{11}H_{11}N_5O$: N, 30.61. Found: N, 30.49.

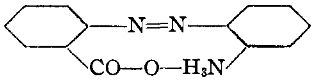
Preparation IV. β-*p*-Ethoxyphenylazo-α,α'-diaminopyridine may be recrystallized from boiling benzene or from 50% methyl alcohol. It melts at 176–177°. Its bacteriostatic index is below 1:5000.

Anal. Calcd. for $C_{13}H_{15}N_5O$: N, 27.49. Found: N, 27.59.

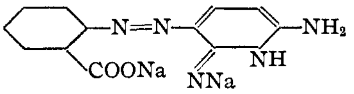
α,α'-Diaminopyridine azophenyl-*o*-carboxylic acid is obtained from diazotized anthranilic acid and α,α'-diaminopyridine in the presence of hydrochloric acid. On the addition of sodium acetate to the coupling mixture amorphous dark red flakes of inner salt I are precipitated. This is almost insoluble in cold water and difficultly soluble in boiling water. Its solution is neutral to Congo paper and to litmus. It is moderately soluble in dilute hydrochloric acid and forms an intense cherry-red solution. The inner salt I also dissolves in pyridine, dilute ammonia and alkalies.



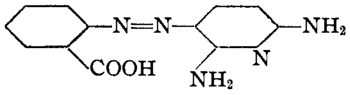
$$o\text{-}C_6H_4{<}^{COOH}_{N\equiv N(Cl)} + \alpha,\alpha'\text{-}C_5H_3N(NH_2)_2 + HCl \longrightarrow$$



I



II



III

The disodium salt II is formed from the corresponding free acid under all conditions, regardless of the quantity of caustic soda used in the reaction.

When aqueous solutions of the salts are acidified with an excess of mineral acids diaminopyridine-azophenyl-*o*-carboxylic acid of normal structure III separates in a free state. If organic acids are used or a deficiency of mineral acid, the inner salt I separates out. When a neutral solution of the inner salt is treated with calcium chloride in aqueous solution no sediment is formed but the brownish-yellow color of the salt changes to a pure golden yellow, and there can be no doubt that a water soluble calcium salt is formed in the reaction.

The normal acid III is almost insoluble in ether but dissolves readily in nitrobenzene and in aniline. When the substance is heated in a capillary tube to 270° the acid does not melt but apparently decomposes, gradually changing to greenish-yellow in color.[10]

NEW YORK CITY

RECEIVED FEBRUARY 20, 1934

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[9] These analyses were made at my request by Dr. V. L. Burger.

[10] Ostromislensky, U. S. Patent 1,680,109.

[11] U. S. Patents 1,820,483 and 1,724,305.