Summary

1. An ultracentrifugal study has been made of the orange seed globulin, pomelin.

2. Pomelin was found to consist of three components, the sedimentation constants of which are 2.2, 11.38 and 16.75 × 10^{-13}.

3. Half-normal solutions of potassium chloride, potassium bromide and potassium iodide were found to extract the same relative amounts of the three components.

4. The component of sedimentation constant 11.38, the chief constituent of pomelin, was found to be stable throughout the *P*H range 3.5–12. Acid denaturation is complete at *P*H 3.3. In the alkaline region beyond *P*H 12 the molecules are broken up into smaller fragments which coalesce to a coherent precipitate when the *P*H of the solution is brought back to the neutral range. The component of sedimentation constant 16.75 begins to decompose at *P*H 10.3 in the alkaline region. Acid denaturation is complete at *P*H 3.3.

5. Pomelin was found to be readily denatured in solutions of low salt content.

6. A sample of dried pomelin gave evidence of denaturation.

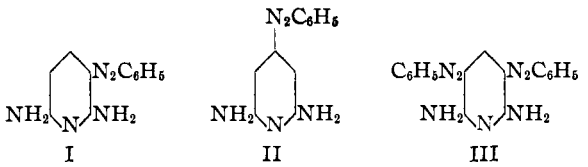
UPSALA, SWEDEN RECEIVED FEBRUARY 13, 1934

[CONTRIBUTION FROM THE CHEMICAL LABORATORY, ACADEMY OF SCIENCES U. R. S. S., MOSCOW]

γ-Phenylazo-α,α′-diaminopyridine

BY A. E. CHICHIBABIN AND E. D. OSSETROWA

Ivan Ostromislensky[1] by coupling diazotized aniline with α,α′-diaminopyridine obtained as the chief product β-phenylazo-α,α′-diaminopyridine (formula I). This substance had been obtained much earlier in the same way by Chichibabin and Zeide.[2] In addition to this main product Ostromislensky mentions two other substances to which he ascribes the formulas II and III.



I II III

There is nothing improbable in the formation of a substance having formula III. It is, however, highly improbable that a substance having formula II should be formed under these circumstances because so far as we know there is not a single instance among the prodigious number of coupling reactions which have been investigated where the azo group enters the meta position. Moreover, the orienting influences would make such an entry particularly improbable in the pyridine series.

In this situation we have undertaken to prepare a substance having formula II by a series of unambiguous reactions, as follows.

γ-Chlorodipicolinic acid IV was treated with phenylhydrazine to form γ-phenylhydrazodipicolinic acid V. This was oxidized by atmospheric oxygen to give γ-phenylazodipicolinic acid VI. The methyl ester of this acid was treated with hydrazine, which served to reduce the azo group. The γ-phenylhydrazo-α,α′-dihydrazidopyridine VII formed was oxidized by atmospheric oxygen to give γ-phenylazo-α,α′-dihydrazidocarbopyridine VIII. This was treated with nitrous acid to form γ-phenylazo-α,α′-dihydrazidocarbopyridine IX, which was converted to the diurethan X by boiling in alcoholic solution. If the boiling is not continued for a sufficient length of time the monourethan XI may also be obtained. The saponification of the diurethan X gives the desired γ-phenylazo-α,α′-diaminopyridine II. The properties of this substance differ from those indicated by Ostromislensky for the substance II.

Numerous experiments were made in our laboratory on the action of diazotized aniline on α,α′-diaminopyridine. With an excess of diazotized aniline the compound III is formed, together with other products, and has the properties of the compound described as such in the patent. Numerous attempts to prepare III without an excess of diazotized aniline indicated that it either is not formed at all or is formed only in an insignificant quantity. β-Phenylazo-α,α′-diaminopyridine may be obtained quite pure from one crystallization. In spite of numerous prepara-

(1) U. S. Patent 1,680,109 (Serial No. 97,771); *Chem. Zentr.*, I, 1026 (1929).

(2) Chichibabin and Zeide, *J. Russ. Phys.-Chem. Soc.*, **46**, 1216 (1914); *Chem. Zentr.*, I, 2, 1069 (1915).

tions of this latter compound it was not possible to discover the presence either of a substance which possessed the properties indicated by Ostromislensky for the substance II or of the substance with the properties of the true γ-phenylazo-α,α'-diaminopyridine.

Experimental Part

γ-Phenylhydrazodipicolinic Acid.—Ten grams of γ-chlorodipicolinic acid[3] and 10–13 cc. of phenylhydrazine was heated for one hour on the oil-bath at 130–133° in a flask closed with a stopper which was provided with a tube which had been drawn down to a capillary. The reaction product was dissolved by heating on the water-bath with a 5% sodium hydroxide solution. The solution was cooled and washed with ether to remove excess phenylhydrazine. Unchanged γ-chlorodipicolinic acid was precipitated with acetic acid. The solution was filtered and phenylhydrazodipicolinic acid was precipitated by the cautious addition of hydrochloric acid. A yield of 40–50% was obtained. On standing the mother liquors deposited 1 g. of crystals which melted with decomposition at 22° after recrystallization from alcohol. γ-Phenylhydrazodipicolinic acid crystallizes well from alcohol in light yellow needles or small prisms. The crystals contain one molecule of alcohol which may be removed by heating at 150–160°. When closely heated the alcohol is driven off and the acid melts at 231°.

Anal. Calcd. for $C_{13}H_{11}O_4N_3 + C_2H_5OH$: C, 56.40; H, 5.37; C_2H_5OH, 14.43. Found: C, 56.03; H, 5.59; C_2H_5OH, 14.55. Calcd. for $C_{13}H_{11}O_4N_3$: C, 57.11; H, 4.07; N, 15.42. Found: C, 57.10; H, 4.23; N, 15.60.

γ-Phenylazodipicolinic Acid.—A current of air was passed through a weakly alkaline solution of the phenylhydrazo acid, which was warmed on the water-bath, for six hours. The acid was then precipitated with hydrochloric acid. γ-Phenylazodipicolinic acid crystallizes from 80% acetic acid in brown-red plates which contain one-half of a molecule of acetic acid. The crystals lose acetic acid and melt at 270° with decomposition when slowly heated in a capillary tube.

Anal. Calcd. for $C_{13}H_9O_4N_3 + {}^1/_2\ C_2H_4O_3$: acetic acid, 9.09. Found: 8.95. Dried substance, calcd. for $C_{13}H_9O_4N_3$: N, 15.49. Found: 15.61, 15.33.

The Methyl Ester of γ-Phenylazodipicolinic Acid.—The methyl ester may be obtained by treating the silver salt of phenylazodipicolinic acid with methyl iodide. The silver salt was precipitated by the addition of silver nitrate solution to a solution obtained by dissolving the acid in a minimum quantity of dilute sodium hydroxide. The silver salt separates as a rose-colored powder. It is necessary to use twice the theoretical amount of silver nitrate solution, otherwise a basic or complex salt is formed. This was indicated by the analysis of the silver salt; calculated for the neutral salt, 44.32% Ag; found, 53.60 and 53.95.

The silver salt obtained from 1.75 g. of pure γ-phenylazodipicolinic acid was added to 1.8 cc. of methyl iodide and 50 cc. of dry toluene. The mix ure was heated for two hours at 60–75° and then heated for three hours on a boiling water-bath. On cooling 0.2 g. of the ester separated. The toluene was concentrated and gave 0.55 g. more of the ester. The unchanged azo acid was separated by extraction with alkali and subsequent precipitation with acid. The methyl ester of γ-phenylazodipicolinic acid crystallizes from methyl alcohol in brilliant orange-red prisms which melt at 175°.

Anal. Calcd. for $C_{15}H_{13}O_4N_3$: N, 14.05. Found: 14.27, 14.03, 14.30.

Dihydrazide of γ-Phenylazodipicolinic Acid.—Three grams of the methyl ester and 3 cc. of distilled hydrazine hydrate (6 mols to each mol of ester) were heated for one hour at 200°. The product was colorless, probably the dihydrazide of phenylhydrazodipicolinic acid. On recrystallization the product became red, due to oxidation and the formation of the hydrazide of phenylazodipicolinic acid. The product was filtered and washed with alcohol to remove the excess hydrazine hydrate; 2.8 g. or 93% of the theoretical quantity of the crude product was obtained. This dihydrazide was recrystallized from boiling water. Spheroids of brick-red needles are formed. After four crystallizations the product melted at 228°.

Anal. Calcd. for $C_{13}H_{13}O_2N_7$: N, 32.78. Found: 32.97.

Diazide of γ-Phenylazodipicolinic Acid.—0.59 g. of the dihydrazide was dissolved in one liter of boiling water and then cooled to −5°; 0.4 g. of sodium nitrate in solution was added and then a solution containing 5.8 cc. of concentrated hydrochloric acid was introduced below the surface of the solution with stirring. The diazide was purified by recrystallization from absolute ether. The diazide melts at 110° without giving off gas. When heated on a spatula over a gas flame effervescence may be noticed.

Diurethan.—2.8 g. of a recrystallized diazide was boiled for one and one-half hours with 55 cc. of absolute alcohol. The nitrogen evolved was measured; 340 cc. (20°, 752 mm.) was obtained; this is 78% of the theoretical amount. On cooling the alcohol solution 0.65 g. of the diurethan was obtained; 30 cc. of alcohol was removed and a further crop of 1.05 g. of the diurethan was obtained. The yield of 1.7 g. is 57% of the theoretical. The diurethan crystallizes from 50% alcohol in brilliant orange-red aggregates of microscopic quadrilateral pyramids which melt at 182°.

Anal. Calcd. for $C_{17}H_{19}O_4N_5$: N, 19.60. Found: 19.83.

α,α'-Diamino-γ-phenylazopyridine.—1.85 g. of the diurethan was refluxed for four hours with a solution of 2 g. of potassium hydroxide in 40 cc. of absolute alcohol. At the end of ten minutes a light brown powder began to separate. As the heating continued the insoluble material became dark red. The solution was cooled and carbon dioxide was introduced for fifteen minutes. The product was evaporated on the water-bath almost to dryness. Strong ammonia was then added and the mixture boiled for a period of time. On cooling 1.1 g. of red crystals separated. This material was recrystallized from benzene and γ-phenylazo-α,α'-diaminopyridine was obtained in scarlet flat needles which melted at 170–171°.

(3) E. Königs and Jaschke, *Ber.*, **54**, 1351 (1921).

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°.

(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 *P*H 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.