p-Phenylenediamine as a Schistosomicidal Agent and Its Condensation with Acetoacetic Ester

By I. NABIH and E. HELMY

_p_-Phenylenediamine condensed with acetoacetic ester at room temperature to give diester (I*a*). It hydrolyzed in the presence of hydrogen chloride to yield purified _p_-phenylenediamine dihydrochloride, which was found to possess schistosomicidal activity. The ester (I*a*) underwent the Mannich reaction yielding a product (I*b*) which was stable to acids and was cyclized by concentrated sulfuric acid to give 2,6 - dimethyl - 3,7 - diethylaminomethyl - 4,8 - diethoxylpyrido[2,3 - *g*]-quinoline (III).

IN OTHER studies, the authors found that the schistosomicidal agent lucanthone is oxidized through the catalytic effect of peroxidase (1). These results prompted us to choose *p*-phenylenediamine which contains a well-known oxidizable system as a starting material for the synthesis of potential schistosomicides.

Biological studies of *p*-phenylenediamine will be described and followed by an outline of preparation of certain of its derivatives.

BIOLOGICAL RESULTS

p-Phenylenediamine has previously been described as an insecticide (2), antimitotic (3), and tuberculostatic (4). Its dihydrochloride is highly soluble in water and thus lends itself well to pharmacological study.

Mice, weighing between 25 and 35 Gm., were experimentally infected with *Schistosoma mansoni* by contact with water containing cercaria from different snails to obtain mixed infection, since it has been observed that uniform infection from one source gave only one sex as noted in the adult infection. After a period of 6 to 8 weeks, stools examined for the presence of schistosome ova showed infection in most animals. Also, the excreted eggs could hatch when warm distilled water was added.

To 15 infected mice was administered orally 40 mg./Kg. body weight of *p*-phenylenediamine dihydrochloride from a freshly prepared solution (100 mg./100 ml. water). This dose was repeated after 6 hr. The daily divided dose was administered for 15 days. A part of the drug was excreted within 2 hr. of its administration to the mice. Its presence was detected through red spots formed when urine was brought in contact with a light filter paper. The density of the spots could be compared with the same formed by fresh solutions of the drug.

The number of eggs present in 24 hr. stools of treated animals was compared with the number from untreated infected mice kept as control. It was observed that the number of living eggs, determined by their hatchability, decreased gradually and was accompanied by an increase in the number of dead ova. These were dark and nonhatchable. Six weeks after the end of treatment, no more living ova and only a few dark degenerated ova were observed. The animals were dissected after 2 months. Dead worms were found in the liver blood vessels, and dark degenerated, nonhatchable ova were found in the liver tissues (Fig. 1). In control mice, living worms were present in the mesenteric veins and living hatchable ova in the liver tissues (Fig. 2).

CHEMICAL STUDIES

Knorr (5) reported that *p*-phenylenediamine condenses with acetoacetic ester when heated in a sealed tube under pressure at a temperature of 160°–170° to give *N*,*N*,'-bis(acetoacetyl)-*p*-phenylenediamine, m.p. 169° (II). In the present work, the condensation was carried out under normal pressure and at room temperature to give ethyl *p*-phenylenediamine-*N*,*N*'-bis-β-crotonate (I*a*), m.p. 132°, which was different from the Knorr compound. I*a* also was obtained by heating the reactants under atmospheric pressure at 160°–170° (6, 7).

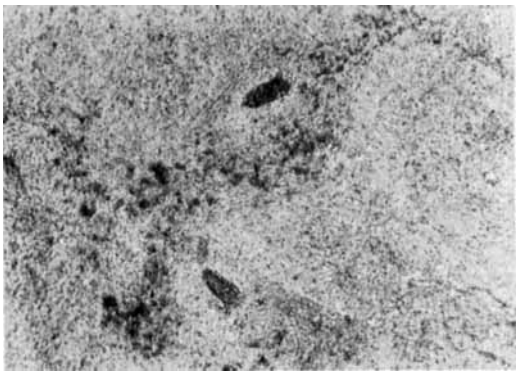

Fig. 1.—Dead schistosoma mansoni ova in mouse liver after treatment.

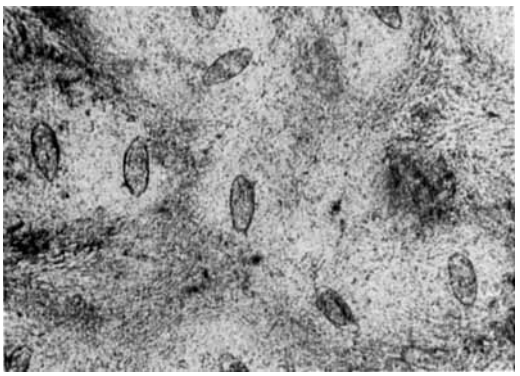

Fig. 2.—Living schistosoma mansoni ova in mouse liver without treatment.

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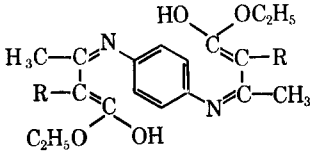

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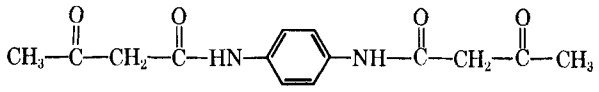


II

Ia, R = H

Ib, R = $CH_2N(C_2H_5)_2$

When a stream of hydrogen chloride gas was passed through an alcoholic solution of Ia, *p*-phenylenediamine was recovered as the dihydrochloride in quantitative yield and in high state of purity.

The ultraviolet absorption spectrum of the *p*-phenylenediamine dihydrochloride in water solution at pH 3.6 showed maxima at 283 and 235 mμ. At pH 7.2, the maxima were shifted to 304 and 238 mμ.

p-Phenylenediamine dihydrochloride was oxidized by hydrogen peroxide in the presence of peroxidase (8, 9). After oxidation, the absorption spectrum showed only one maximum at 245 mμ in acidic medium and at 256 mμ in basic medium.

In further chemical studies, Ia was found to undergo the Mannich reaction. The dihydrochloride of the product (Ib) failed to oxidize in the presence of peroxidase. The presence of the Mannich basic groupings at both side chains of Ib favored cyclization by means of concentrated sulfuric acid to give the pyridoquinoline derivative (III). Heat failed to bring about closure of Ib.

EXPERIMENTAL

All melting points were taken in open capillaries and corrected.

Ethyl p - Phenylenediamine - N,N' - bis - β-crotonate (Ia).—*Method A.*—A mixture of 10.8 Gm. (0.1 mole) of recrystallized *p*-phenylenediamine and 26 Gm. (0.2 mole) of acetoacetic ester was vigorously shaken and left to stand overnight. A reddish mass was formed. Recrystallization from aqueous alcohol gave 24 Gm. (73% yield) of Ia, m.p. 132°. [Lit. (10) m.p. 135°.]

Anal.—Calcd. for $C_{18}H_{24}N_2O_4$: C, 64.98; H, 7.25; N, 8.43. Found: C, 65.61; H, 7.14; N, 8.48.

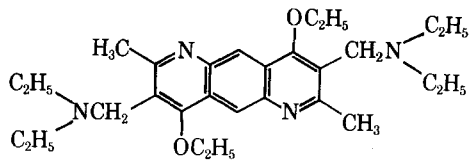
Ia is insoluble in water and alkalies and soluble in ethyl alcohol.

p - Phenylenediamine Dihydrochloride.—Ia was dissolved in chloroform and a stream of hydrogen chloride gas was passed through the solution to give a white powder. The product was crystallized from ethyl alcohol, m.p. 268°; it proved to be the *p*-phenylenediamine dihydrochloride, as shown through mixed melting point and infrared spectra.

Anal.—Calcd. for $C_6H_{10}Cl_2N_2$: C, 40.00; H, 5.56; Cl, 39.00; N, 15.55. Found: C, 40.00; H, 5.69; Cl, 39.11; N, 15.44.

Its ultraviolet absorption spectrum at pH 3.6 in water solution shows peaks at 283 and 235 mμ, while at pH 7.2 it shows maxima at 304 and 238 mμ.

Method B (6,7).—A mixture of 10.8 Gm. (0.1 mole) of crystalline *p*-phenylenediamine was heated with 26 Gm. (0.2 mole) of acetoacetic ester at reflux temperature (160°–170°) for 20 min. The mixture was cooled and the solid product was recrystallized from ethyl alcohol: yield, 28.6 Gm. (87%) of Ia, m.p. 132°. There was no depression of melting point upon admixture with Ia.



III

Anal.—Calcd. for $C_{18}H_{24}N_2O_4$: N, 8.43. Found: N, 8.58.

Catalytic Oxidation of p-Phenylenediamine Dihydrochloride with Hydrogen Peroxide in the Presence of Peroxidase (11).—*At pH 3.6.*—To 3.5 Gm. of the salt dissolved in 44 ml. of distilled water (pH of solution, 3.6), 3 ml. of hydrogen peroxide (0.147 mole) was added, followed by the addition of 3 ml. of peroxidase solution (3 Gm. in 100 ml. of water). After reaction, the ultraviolet absorption spectrum was traced; it showed a single maximum at 245 mμ, with no change in pH value.

At pH 7.2.—To 3.5 Gm. of the salt dissolved in 40 ml. of distilled water, 3 ml. (0.147 mole) of hydrogen peroxide, 3 ml. of phosphate buffer (pH 7.2), and 3 ml. of peroxidase solution (3 mg. %) were respectively added. The ultraviolet absorption spectrum was determined after the completion of reaction; it showed a single maximum at 256 mμ, with no change in pH value.

Ethyl p - Phenylenediamine - N,N' - bis - (α-diethylaminomethyl-β-crotonate) (Ib) (12).—A 3.2-Gm. quantity of paraformaldehyde mixed with 40 ml. of diethylamine was dissolved in 50 ml. of absolute alcohol. Then a solution of 1.48 Gm. of compound Ia dissolved in 10 ml. absolute alcohol was added. The mixture was refluxed for 3 hr. Solvent and excess amine were removed by distillation, and the residual solid was recrystallized from chloroform to give 1.2 Gm. (56% yield) of yellow crystalline product (Ib), m.p. 182°.

Anal.—Calcd. for $C_{28}H_{46}N_4O_4$: N, 11.14. Found: N, 10.98.

The product was insoluble in water and alkalies, but soluble in mineral and organic acids. It formed a dihydrochloride, m.p. 270°, which did not react with hydrogen peroxide in presence of peroxidase as did *p*-phenylenediamine dihydrochlor de.

2,6 - Dimethyl - 3,7 - diethylaminomethyl - 4,8-diethoxypyrido[2,3-g]quinoline (III).—*Method A.*—A 2-Gm. quantity of Ib was dissolved in 10 ml. of concentrated sulfuric acid, and the mixture heated on a water bath for 30 min., poured in ice water, and neutralized by sodium carbonate. The resulting precipitate was recrystallized from a xylene–alcohol mixture to yield 1.45 Gm. (78%) of III, m.p. 300°.

Anal.—Calcd. for $C_{28}H_{42}N_4O_2$: N, 12.01. Found: N, 12.10.

Method B.—A 2-Gm. quantity of Ib was added to hot paraffin oil and heated to 230° for 30 min. After cooling, the paraffin oil was decanted and the solid residue was triturated with petroleum ether to remove traces of paraffin oil. Then it was recrystallized from chloroform, m.p. 185°, and mixed melting point proved to be recovered starting material.

REFERENCES

(1) Nabih, I., and ElSheikh, M., Abstracts of Papers, 150th Meeting, American Chemical Society, Atlantic City, N. J., September 1965.
(2) Swingle, M., Phillips, M., and Gehan, J., *U. S. Dept. Agr., Bur. Entomol. Plant Quaranline*, July 1944, 621.
(3) Paramentier, R., *Compt. Rend. Soc. Biol.*, **143**, 585 (1949).
(4) Block, H., *et al.*, *Helv. Chim. Acta*, **30**, 539(1947).
(5) Knorr, L., *Ber.*, **17**, 541(1884).
(6) Backeberg, O., *J. Chem. Soc.*, **1935**, 1568.
(7) Jacini, G., and Bertulli, N., *Gazz. Chim. Ital.*, **69**, 111(1939).
(8) Heiduschha, A., and Goldstein, E., *Arch. Pharm.*, **254**, 584(1916).
(9) Szent-Gyorgyi, A., *Biochem. Z.*, **150**, 195(1924).
(10) Bangdiwala, B., and Desai, C., *J. Indian Chem. Soc.*, 31, 688(1954); through *Chem. Abstr.*, **50**, 1008g(1956).
(11) Mann, F., and Saunders, B., "Practical Organic Chemistry," Longmans, London, England, 1960, p. 520.
(12) Blicke, F. F., *Org. Reactions*, **1**, 303(1947).

Synthesis of Two 4,5-Dialkyl Isosydnones

By EDWARD B. ROCHE* and LEMONT B. KIER

The syntheses of two new dialkyl isosydnones are described. These represent the first nonbenzenoid derivatives to be reported. Spectral data for each compound are also given.

THIS LABORATORY has been interested in the study of *meso*-ionic compounds as possible therapeutic agents for several years. The first *meso*-ionic sydnone system I was synthesized in 1935 by Earl and Mackney (1). The authors have previously reported (2–6) the synthesis and pharmacological activity of a number of 3-monosubstituted and 3,4-disubstituted derivatives of this system. Both the monoalkyl and the dialkyl substituted syndnones exhibited potent central nervous system stimulatory properties (2–4). Further work on a possible mechanism of action and important medicinal chemical features of this system is currently underway in this laboratory.

As a logical extension of these investigations, a study of the medicinal aspects of a system isomeric with the sydnones was warranted.

The synthesis of ψ-2,4-dihydro-4,5-diphenyl-2-keto-1-oxa-3,4-diazole (II*a*) has been reported by Hashimoto and Ohta (7). (The system II shall be referred to by the name *isosydnone*.) This system is isomeric with the sydnone ring I in that the pseudolactone function has been reversed in structure II. An earlier report by Hoegerle (8) disclosed the synthesis of a fused ring *meso*-ionic system III from the reaction of *N*-aminopyridone-(2) with phosgene. This system contains the isosydnone nucleus which was first obtained as a monocyclic system II*a* in 1961(7).

Hashimoto (7) achieved the synthesis of compound II*a* by the method of Hoegerle (8), in which 1-benzoyl-1-phenylhydrazine was treated with phosgene in chloroform solution in the presence of potassium carbonate. The product was characterized through its infrared spectrum and degradative reactions in both acid and base. Further attempts to prepare other derivatives of this nucleus (II) were unsuccessful, with the exception of preparing a small amount of compound II*b*. However, the amount obtained was insufficient to afford complete characterization.

Recently, Ainsworth (9) has reported the preparation of 4-methyl-5-phenylisosydnone II*b* in good yield, as well as a study of the nature of the reaction. The method of synthesis differed from that employed by Hashimoto (7) in that the hydrochloride salt of the acylhydrazine was used in solution in dioxane, and the reaction was completed under reflux conditions rather than at -7 to $-10°$.

Both of the isosydnone derivatives, II*a* and II*b*, reported contain a phenyl ring which can aid in stabilizing the *meso*-ionic system through π-electron delocalization. For the purpose of studying the medicinal chemical aspects of the isosydnones (II), it was desirable to synthesize derivatives in which both R and R′ are alkyl substituents. This was also necessary for correlation with the medicinal activity of the alkyl-sydnones (2–6). With this purpose in mind, this preliminary report discloses the synthesis of two new dialkyl isosydnones, II*c* and II*d*.

The problem of obtaining 1-acylated hydrazines was overcome to a large degree by acylating the alkylhydrazine in a high dilution of ether with the appropriate acid anhydride (10). This procedure gives a mixture of 1-acylhydrazine and 1,2-di-acylhydrazine which can be separated by fractional distillation to provide the 1-acylhydrazine in good yield.

EXPERIMENTAL

4,5-Dimethylisosydnone (IIc).—1-Acetyl-1-methylhydrazine (IV) (0.19 mole) was dissolved in 2 L. of cold chloroform containing 300 Gm. of anhydrous potassium carbonate. The mixture was cooled to $-10°$ in a propylene glycol–dry ice bath. With vigorous stirring, a steady stream of phosgene was introduced into the mixture for a period of 15 min. The mixture was allowed to warm to room temperature while stirring. After refluxing for 0.5 hr. to expel the excess phosgene, the insoluble solids were filtered out, and the chloroform solution was concentrated *in vacuo* to give a red oil. Vacuum distillation of the oil yielded 4 Gm. of a colorless oil

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* National Institutes of Health Predoctoral Fellow.