the reaction proceeding as a simple reversal of eq. 1. More devious routes might well introduce randomization.

The conclusion reached from these results is that the predominant product of this enzymatic reaction *in vitro* is an isomer or isomers of DL-allothreonine. Indeed, this may be the only product. However DL-threonine (or L- or D-threonine) could be present in rather large proportion and not be detected. The specific stereoisomer(s) produced awaits future recrystallization, microbiological experiments, etc., in order to determine its configuration.

IV. Discussion

Inasmuch as allothreonine has not been identified in biological material, it obviously is impossible to relate this compound to present schemes of intermediary metabolism. The present findings do suggest the possible natural occurrence of this diastereoisomer, however.[25]

It is worth reiterating that no serious attempt has been made in this present work to identify D- or L-threonine except as a large component. The compound might well escape detection if present in less than approximately 30% of the total amount of threonine. Indeed suggestive of the probability of this occurrence, but in lower forms of life, is the previously cited evidence obtained in yeast.[11-13,26]

(25) Relative to metabolic utilization, if not metabolic occurrence, D-allothreonine was used by certain mutant microörganisms in the biosynthesis of isoleucine and threonine [H. E. Umbarger and E. A. Adelberg, *J. Biol. Chem.*, **192**, 883 (1951)]. Therefore, this enzyme present in certain lower forms may well serve the production of a precursor of certain amino acids in such microörganisms. However, in the rat it is well established that only one isomer, L-threonine, is utilized for growth purposes [H. D. West and H. E. Carter, *ibid.*, **122**, 611 (1938)].

(26) A more remote possibility is suggested by a *Neurospora crassa* mutant which revealed properties which may be explained by an alternate metabolic pathway for the synthesis of threonine [E. A. Adelberg, C. A. Coughlin and R. W. Barratt, *J. Biol. Chem.*, **216**, 425 (1955)]. This is suggested inasmuch as this double mutant was blocked as follows: homoserine ⊣→ threonine ⟶ α,β-dihydroxy-β-ethylbutyric acid ⊣→ α-keto-β-ethylbutyric acid. It, in minimal medium, synthesized threonine at a slow rate. That one strain of this species, at least, has the enzyme in question, has been reasonably well authenticated by Wagner and Bergquist (footnote 15). However, glycine did not stimulate production of α,β-dihydroxy-β-ethylbutyric acid. Compare also discussions of the possibility of L-threonine synthesis by this condensation reaction in *Escherichia coli* [J. O. Meinhart and S. Simmonds, *J. Biol. Chem.*, **213**, 329 (1955)] and in *Clostridium kluyveri* [N. Tomlinson, *ibid.*, **209**, 597 (1954)].

Also suggestive is the fact that L-allothreonine was cleaved at approximately seven times the rate that L-threonine was split by the enzyme concerned[5] (prepared from rat liver).[14] So it may well be true that the kinetics of the synthetic reaction, favors allothreonine formation in some such proportion. Furthermore, in the corresponding reversible model reaction[10] both threonine and allothreonine are produced and in approximately comparable amounts. Since both are pyridoxal catalyzed, a somewhat similar relationship should be expected for the enzymatic reaction.

The essentiality of threonine for mammals implies that synthesis of L-threonine by such a route, if it does indeed occur, is of a relatively small quantity compared to the daily growth requirement. Perhaps the availability of acetaldehyde may limit the utility of this reaction in animals. By the same token in yeast, the reaction may be of importance as a synthetic mechanism because acetaldehyde is a major metabolite.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY, AND THE FRANCIS DELAFIELD HOSPITAL]

Some New o-Phenylenediamines and the Related Benzimidazoles, Benzotriazoles and Quinoxalines

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The synthesis of 3,4-diamino-5-nitrophenetole and 3,4-diamino-2-nitroanisole is described. These *o*-diamines have been used to prepare the related benzimidazoles, benzotriazoles and quinoxalines which were needed to continue an investigation of the inhibition of developing *R. pipiens* embryos by such heterocyclic compounds. Derivatives of 3,4-diamino-2-nitroanisole have served as useful reference compounds for the solution of several problems of structure.

Among a number of benzimidazoles, benzotriazoles and quinoxalines previously reported,[1,2] several have shown interesting properties as inhibitors of developing *R. pipiens* embryos. The most active of these were 4-methoxy-6-nitrobenzimidazole, 4-methoxy-6-nitrobenzotriazole and 5-methoxy-7-nitroquinoxaline.[3] Isomers of these heterocyclic compounds, in which the nitro and methoxy groups were interchanged, exhibited an approximately equal level of activity.[4] It seemed of interest, therefore, to investigate whether the activity of such compounds might be enhanced or diminished by changing the methoxy group to ethoxy and changing the relative configuration of the nitro and methoxy groups to *ortho* rather than *meta*. The synthesis of the desired substances required the preparation of two new substituted *o*-phenyl-

(1) H. Gillespie, M. Engelman and S. Graff, THIS JOURNAL, **76**, 3531 (1954).

(2) H. Gillespie, M. Engelman and S. Graff, *ibid.*, **78**, 2445 (1956).

(3) K. Liedke, M. Engelman and S. Graff, *J. Exp. Zool.*, **127**, 201 (1954).

(4) K. Liedke, private communication.

enediamine intermediates, 3,4-diamino-5-nitrophenetole and 3,4-diamino-2-nitroanisole.

4-Amino-3,5-dinitrophenetole was obtained by nitration of an alcoholic suspension of *p*-toluenesulfonyl-*p*-phenetidide according to the procedure of King and Beer[5] for the preparation of 3,5-dinitro-4-*p*-toluenesulfonamidoanisole and hydrolysis of the intermediate 3,5-dinitro-4-*p*-toluenesulfonamidophenetole. This procedure was more effective than the hydrolysis of the nitration product of 3-nitro-4-*p*-toluenesulfonamidophenetole.[6] Reduction of the 4-amino-3,5-dinitrophenetole by ammonium sulfide gave a 70% yield of 3,4-diamino-5-nitrophenetole. The *o*-diamine was treated with formic acid, nitrous acid, glyoxal and diacetyl to give, respectively, 6-ethoxy-4-nitrobenzimidazole, 6-ethoxy-4-nitrobenzotriazole, 7-ethoxy-5-nitroquinoxaline and 2,3-dimethyl-7-ethoxy-5-nitroquinoxaline. Amino derivatives were prepared by catalytic reduction of the nitro group.

Attempts to obtain 3,4-diamino-2-nitroanisole by ammonium sulfide reduction of 4-amino-2,3-dinitroanisole yielded only resinous products. The *o*-diamine, however, was isolated readily as the hydrochloride on treating the aminodinitroanisole in alcohol with the calculated quantity of stannous chloride and hydrochloric acid for the reduction of one nitro group. The product was shown to be an *o*-diamine by reaction with benzil to give 2,3-diphenyl-6-methoxy-5-nitroquinoxaline. The desired 5-methoxy-4-nitrobenzimidazole, 5-methoxy-4-nitrobenzotriazole, 6-methoxy-5-nitroquinoxaline and 2,3-dimethyl-6-methoxy-5-nitroquinoxaline were obtained by ring closure of the *o*-diamine with appropriate reagents as indicated in the case of 3,4-diamine-5-nitrophenetole. The 5-methoxy-4-nitrobenzotriazole obtained in this fashion was identical with that prepared earlier[7] by nitration of 1-acetyl-5-methoxybenzotriazole, thus confirming the previous assignment of structure. Hydrolysis of the methoxynitrobenzimidazole and benzotriazole by 48% hydrobromic acid gave the corresponding hydroxy compounds.

Meldola and Eyre[8] obtained 2,3,4-triaminoanisole in the form of a crude hydrochloride by complete reduction of 4-amino-2,3-dinitroanisole with tin and hydrochloric acid. The triamine condensed with benzil to yield an aminomethoxydiphenylquinoxaline, m.p. 215°. This product could be either 5-amino-6-methoxy- or 5-amino-8-methoxy-2,3-diphenylquinoxaline. A definite assignment of structure at that time was not possible for lack of reference compounds. Catalytic reduction of 2,3-diphenyl-6-methoxy-5-nitroquinoxaline gave the 5-amino-6-methoxy derivative having m.p. 156–157°. To obtain the 5,8-isomer, 4-acetamido-2,3-dinitroanisole was reduced by hydrogen at atmospheric pressure in the presence of palladium-on-carbon. The crude 4-acetamido-2,3-diaminoanisole thus produced reacted with benzil to give 5-acetamido - 2,3 - diphenyl -8 - methoxyquinoxaline which was hydrolyzed by alcoholic hydrogen chloride to the free amino compound, m.p. 214–215°. The product obtained by Meldola and Eyre must have been 5-amino-2,3-diphenyl-8-methoxyquinoxaline.

Nitration of 3,4-diacetamidoanisole in acetic acid with concentrated nitric acid yielded a mononitro derivative, m.p. 224–227°. This could be any one of three compounds: namely, 3,4-diacetamido-2-nitroanisole (A), 3,4-diacetamido-5-nitroanisole (B) or 4,5-diacetamido-2-nitroanisole (C). Attempts to prepare A and B by acetylation of 3,4-diamino-2-nitroanisole and 3,4-diamino-5-nitroanisole, respectively, led to mixtures difficult to purify. These mixtures undoubtedly contained some of the monoacetyl derivatives along with the diacetyl derivatives and also traces of 2-methylbenzimidazoles. Phillips[9] has shown that mono- and diacetyl derivatives of *o*-diamines readily cyclize to 2-methylbenzimidazoles. The structure of the nitration product was elucidated by the preparation of the isomeric 2-methylbenzimidazoles. A modification of Phillips' procedure for the preparation of 2-substituted benzimidazoles was employed to convert 3,4-diamino-2-nitroanisole to 5-methoxy-2-methyl-4-nitrobenzimidazole, m.p. 204–205°, and 3,4-diamino-5-nitroanisole yielded 6-methoxy-2-methyl-4-nitrobenzimidazole, m.p. 226–227°. When the nitration product was heated with hydrochloric acid, it cyclized to a 2-methylbenzimidazole, m.p. 172–174°. This must be 5-methoxy-2-methyl-6-nitrobenzimidazole, since the melting point eliminates the other two possibilities. The nitration product is therefore 4,5-diacetamido-2-nitroanisole (C).

All of the benzimidazoles, benzotriazoles and quinoxalines here reported are being biologically screened against various test systems. The results of the biological work will be reported elsewhere.

Experimental[10]

3,5-Dinitro-4-*p*-toluenesulfonamidophenetole.—A stirred suspension of 39 g. of *p*-toluenesulfonyl-*p*-phenetidide[11] in 150 ml. of ethanol was treated dropwise during a 2-hr. period with 85 ml. of concentrated (d. 1.4) nitric acid. The suspended material dissolved when approximately one-third of the acid had been added, and shortly thereafter the dinitro derivative began to separate. After all the acid had been added, the mixture was stirred for 1 hr. at room temperature, refluxed for 1 hr. and cooled. The product was collected, washed with 50% ethanol and recrystallized from 1.5 liters of 50% acetic acid. The 3,5-dinitro-4-*p*-toluenesulfonamidophenetole separated as light yellow needles melting at 161–163°. The yield was 30 g. (60%).

Anal. Calcd. for $C_{15}H_{15}O_7N_3S$: N, 11.02; S, 8.40. Found: N, 10.86; S, 8.30.

4-Amino-3,5-dinitrophenetole.—A solution of 20 g. of 3,5-dinitro-4-*p*-toluenesulfonamidophenetole in 50 ml. of sulfuric acid (90%) was kept at room temperature overnight, poured onto ice and diluted with water to one liter. The bright red precipitate of 4-amino-3,5-dinitrophenetole was collected and washed with water. It crystallized as needles (10.7 g., 90%) from hot 50% acetic acid (500 ml.); m.p. 136–139° (lit.[6] m.p. 138–139°).

3,4-Diamino-5-nitrophenetole.—To 260 ml. of freshly prepared 10% ammonium sulfide, there was added 520 ml. of ethanol and 11.4 g. (0.05 mole) of 4-amino-3,5-dinitrophenetole. The reaction mixture was kept at 50–60° and mechanically stirred for 2 hr. After cooling, the crude

(5) F. E. King and R. J. S. Beer, *J. Chem. Soc.*, 792 (1945).

(6) F. Reverdin and L. Furstenberg, *J. prakt. Chem.*, [2] **88**, 321 (1913).

(7) H. Gillespie, M. Engelman and S. Graff, THIS JOURNAL, **78**, 1651 (1956).

(8) R. Meldola and J. V. Eyre, *J. Chem. Soc.*, **81**, 988 (1902).

(9) M. A. Phillips, *ibid.*, 2393 (1928).

(10) All melting points are corrected.

(11) F. Reverdin and P. Crepieux, *Ber.*, **34**, 3002 (1901).

product was collected and washed successively with 25-ml. portions of carbon disulfide and 50% ethanol. The *o*-diamine was recrystallized from ethanol (400 ml.). It separated as dark red needles having m.p. 144–146°. The yield was 7.0 g. (70%).

Anal. Calcd. for $C_8H_{11}O_3N_3$: C, 48.73; H, 5.59; N, 21.32. Found: C, 48.81; H, 5.78; N, 21.13.

2,3-Diphenyl-7-ethoxy-5-nitroquinoxaline.—A mixture of 579 mg. (3 mmoles) of 3,4-diamino-5-nitrophenetole, 630 mg. of benzil and 500 mg. of anhydrous sodium acetate in 150 ml. of ethanol was refluxed for 3 hr. The dark red solution was concentrated to 50 ml. and cooled. The 2,3-diphenyl-7-ethoxy-5-nitroquinoxaline (381 mg.) crystallized from ethanol (30 ml.) as light yellow needles, m.p. 142–144°.

Anal. Calcd. for $C_{22}H_{17}O_3N_3$: C, 71.16; H, 4.85; N, 11.32. Found: C, 71.33; H, 4.70; N, 10.99.

6-Ethoxy-4-nitrobenzimidazole.—To 1.5 g. of 3,4-diamino-5-nitrophenetole in 20 ml. of 3 *N* hydrochloric acid, there was added 1 ml. of 98% formic acid. After refluxing for 0.5 hr., another 1-ml. portion of formic acid was added and heating was continued for 30 minutes more. The hot solution was diluted with boiling water to 200 ml. and filtered. The filtrate was made slightly alkaline by cautious addition of concentrated (28%) aqueous ammonia. When cold, the crude 6-ethoxy-4-nitrobenzimidazole was collected and washed with water. The yield was 1.5 g. The compound crystallized from ethanol (400 ml., Nuchar) as tiny bright yellow needles melting at 268–269°. A sample for analysis was sublimed at 210–225° under reduced pressure.

Anal. Calcd. for $C_9H_9O_3N_3$: C, 52.17; H, 4.35; N, 20.29. Found: C, 52.29; H, 4.37; N, 19.83.

4-Amino-6-ethoxybenzimidazole.—A solution of 420 mg. (2 mmoles) of 6-ethoxy-4-nitrobenzimidazole in 200 ml. of ethanol was stirred in the presence of 90 mg. of palladium-on-carbon (10%) and hydrogen at one atmosphere pressure for 3 hr. when reduction was complete. The catalyst was removed by filtration and the filtrate concentrated to dryness *in vacuo* in the absence of air. This procedure was employed for all other catalytic reductions which follow. The 4-amino-6-ethoxybenzimidazole (340 mg., 96%) was recrystallized from a small volume (6 ml.) of water; white crystals, m.p. 144–145°.

Anal. Calcd. for $C_9H_{11}ON_3$: C, 61.01; H, 6.21; N, 23.73. Found: C, 61.17; H, 6.50; N, 23.80.

6-Ethoxy-4-nitrobenzotriazole.—A solution of 1 g. of 3,4-diamino-5-nitrophenetole in 100 ml. of hot 2 *N* sulfuric acid was cooled to 5°. The sulfate of the base separated. Keeping the temperature at 5°, there was added gradually with stirring, a solution of 500 mg. of sodium nitrite in 5 ml. of water. After standing overnight at room temperature, the black product (1.01 g.) was collected and washed with water. It was finely powdered and heated under reflux for 1.5 hr. with 250 ml. of 50% ethanol. The hot solution was treated with Nuchar and filtered. After cooling, the 6-ethoxy-4-nitrobenzotriazole (648 mg., 61%) was collected and recrystallized to constant m.p. 240–242°.

Anal. Calcd. for $C_8H_8O_3N_4$: C, 46.15; H, 3.85; N, 26.94. Found: C, 46.35; H, 4.04; N, 26.87.

4-Amino-6-ethoxybenzotriazole.—Catalytic reduction of 970 mg. (4.8 mmoles) of 6-ethoxy-4-nitrobenzotriazole in 200 ml. of ethanol yielded 800 mg. (96%) of crude 4-amino-6-ethoxybenzotriazole. A solution of the crude product in 20 ml. of hot 1 *N* sulfuric acid was boiled briefly with Darco and filtered. The free base precipitated on making the filtrate slightly alkaline (*p*H 8.0) by adding concentrated (28%) aqueous ammonia. It was recrystallized from hot water and separated as pink plates which had m.p. 120–122°, with previous shrinking at 88°. Analysis was done on a sample dried at 110°.

Anal. Calcd. for $C_8H_{10}ON_4$: C, 53.93; H, 5.62; N, 31.46. Found: C, 54.20; H, 5.50; N, 31.84.

7-Ethoxy-5-nitroquinoxaline.—A mixture of 2 g. of 3,4-diamino-5-nitrophenetole, 46.5 ml. of absolute ethanol and 3.5 ml. of a 30% aqueous solution of glyoxal was refluxed for 1 hr. and the hot solution filtered. On cooling there was obtained 930 mg. of crude 7-ethoxy-5-nitroquinoxaline. It was recrystallized from ethanol and separated as yellow needles, m.p. 142–144°. The yield was 611 mg. (27.5%).

Anal. Calcd. for $C_{10}H_9O_3N_3$: C, 54.79; H, 4.11; N, 19.17. Found: C, 54.95; H, 3.78; N, 19.26.

5-Acetamido-7-ethoxyquinoxaline.—Two millimoles (440 mg.) of 7-ethoxy-5-nitroquinoxaline in 150 ml. of ethanol was catalytically reduced as previously described. The product was a brown oil which would not crystallize. The oil was dissolved in 5 ml. of hot acetic anhydride. The solution was refluxed for 15 minutes and poured onto ice. After standing overnight, there was collected 211 mg. of 5-acetamido-7-ethoxyquinoxaline. It was recrystallized from 25% ethanol; white needles, m.p. 190–191°.

Anal. Calcd. for $C_{12}H_{13}O_2N_3$: C, 62.34; H, 5.63; N, 18.18. Found: C, 62.37; H, 5.58; N, 18.00.

2,3-Dimethyl-7-ethoxy-5-nitroquinoxaline.—A suspension of 1 g. of 3,4-diamino-5-nitrophenetole in 50 ml. of 25% acetic acid was stirred and heated on the steam-bath while a solution of 528 mg. of diacetyl in 4 ml. of 10% acetic acid was added dropwise during 20 minutes. On cooling the resultant solution, there separated 1.08 g. of 2,3-dimethyl-7-ethoxy-5-nitroquinoxaline which was collected and washed with water. The compound could be recrystallized from either 50% acetic acid or 50% ethanol, m.p. 151–153°. The yield was 822 mg. (66%).

Anal. Calcd. for $C_{12}H_{13}O_3N_3$: C, 58.30; H, 5.26; N, 17.00. Found: C, 58.26; H, 5.37; N, 16.91.

3,4-Diamino-2-nitroanisole Hydrochloride.—To a solution of 13.6 g. (60 mmoles) of stannous chloride in 100 ml. of ethanol, there was added 4.2 g. (20 mmoles) of 4-amino-2,3-dinitroanisole.[8] The base was kept in suspension by stirring while adding dropwise 25 ml. of concentrated hydrochloric acid. The temperature of the reaction mixture gradually rose to 45°. From the resulting clear, red solution, the monohydrochloride of 3,4-diamino-2-nitroanisole separated on cooling as a felted mass of yellow needles. The crude product was collected, washed with ethanol and recrystallized from 3 *N* hydrochloric acid.

Anal. Calcd. for $C_7H_9O_3N_3 \cdot HCl$: N, 19.14; Cl, 16.13. Found: N, 19.28; Cl, 16.03.

3,4-Diamino-2-nitroanisole.—Concentrated (28%) aqueous ammonia was cautiously added to a solution of 537 mg. of 3,4-diamino-2-nitroanisole hydrochloride in 30 ml. of hot water until the *p*H was approximately 8. The free base separated, on cooling, as a felted mass of dark red needles. The 3,4-diamino-2-nitroanisole was recrystallized from 25 ml. of water, m.p. 116–117°. The yield was 366 mg. (85%).

Anal. Calcd. for $C_7H_9O_3N_3$: C, 45.90; H, 4.92; N, 22.95. Found: C, 46.01; H, 4.86; N, 23.02.

2,3-Diphenyl-6-methoxy-5-nitroquinoxaline.—A mixture of 366 mg. of 3,4-diamino-2-nitroanisole hydrochloride, 180 mg. of anhydrous sodium acetate and 335 mg. of benzil in 25 ml. of 50% ethanol was heated under reflux for 1 hr. The resultant solution, on cooling, deposited 508 mg. (85%) of 2,3-diphenyl-6-methoxy-5-nitroquinoxaline which was recrystallized from ethanol (100 ml.), m.p. 216–217°.

Anal. Calcd. for $C_{21}H_{15}O_3N_3$: C, 70.59; H, 4.20; N, 11.77. Found: C, 70.72; H, 4.10; N, 11.77.

5-Methoxy-4-nitrobenzotriazole.—Ten millimoles (2.2 g.) of 3,4-diamino-2-nitroanisole hydrochloride was suspended by stirring in 100 ml. of ice-cold 1 *N* hydrochloric acid while a solution of 800 mg. of sodium nitrite in 5 ml. of water was being added dropwise. After 1 hr., the crude 5-methoxy-4-nitrobenzotriazole was collected and washed with water. It was recrystallized from ethanol (300 ml.), m.p. 237–238°. The yield was 1.5 g. (80%).

Anal. Calcd. for $C_7H_6O_3N_3$: C, 43.29; H, 3.09; N, 28.87. Found: C, 43.50; H, 3.13; N, 29.42.

5-Hydroxy-4-nitrobenzotriazole.—A solution of 938 mg. (4.8 mmoles) of 5-methoxy-4-nitrobenzotriazole in 10 ml. of hydrobromic acid (48%) was refluxed for 5 hr., cooled and diluted with 50 ml. of water. The precipitate was collected, dissolved in 25 ml. of 1 *N* sodium hydroxide and the solution filtered. A yellow precipitate of 5-hydroxy-4-nitrobenzotriazole (786 mg., 90%) was obtained when the filtrate was acidified with acetic acid. The compound decomposed at about 210°. A sample for analysis was sublimed at 200° under reduced pressure.

Anal. Calcd. for $C_6H_4O_3N_3$: C, 40.00; H, 2.22; N, 31.11. Found: C, 40.23; H, 1.98; N, 31.54.

5-Methoxy-4-nitrobenzimidazole.—This compound was obtained in almost quantitative crude yield from 1.83 g.

(10 mmoles) of 3,4-diamino-2-nitroanisole by the procedure described for 6-ethoxy-4-nitrobenzimidazole. The 5-methoxy-4-nitrobenzimidazole was recrystallized from 250 ml. of 50% ethanol, m.p. 245–247°.

Anal. Calcd. for $C_8H_7O_3N_3$: C, 49.74; H, 3.63; N, 21.76. Found: C, 50.05; H, 3.67; N, 22.23.

5-Hydroxy-4-nitrobenzimidazole.—Three millimoles (549 mg.) of 5-methoxy-4-nitrobenzimidazole was dissolved in a hot mixture of 5 ml. of acetic acid and 5 ml. of hydrobromic acid (48%). The solution was refluxed for 5 hr., diluted with 50 ml. of boiling water, treated with Darco and filtered. The hot filtrate was made slightly alkaline (*p*H about 8.0) by adding concentrated (28%) aqueous ammonia. After cooling, the yellow precipitate of 5-hydroxy-4-nitrobenzimidazole was collected and washed with water. The yield was 525 mg. (97%). It was recrystallized from boiling water. The compound decomposed at about 220°. A sample for analysis was sublimed at 200–210° under reduced pressure.

Anal. Calcd. for $C_7H_5O_3N_3$: C, 46.93; H, 2.79; N, 23.46. Found: C, 47.18; H, 3.00; N, 23.07.

2,3-Dimethyl-6-methoxy-5-nitroquinoxaline.—A mixture of 1 g. (11.6 mmoles) of diacetyl and 1.83 g. (10 mmoles) of 3,4-diamino-2-nitroanisole in 200 ml. of 35% ethanol was refluxed for 1 hr. The solution, on cooling, deposited 2.14 g. (92%) of crude 2,3-diphenyl-6-methoxy-5-nitroquinoxaline. It was recrystallized from 35% ethanol (Nuchar) and separated as tiny, almost colorless needles, m.p. 145–146°.

Anal. Calcd. for $C_{11}H_{11}O_3N_3$: C, 56.66; H, 4.72; N, 18.03. Found: C, 57.03; H, 4.41; N, 18.33.

5-Amino-2,3-dimethyl-6-methoxyquinoxaline.—The catalytic reduction of 466 mg. (2 mmoles) of 2,3-dimethyl-6-methoxy-5-nitroquinoxaline in 50 ml. of ethanol by the usual procedure required 2 hr. A solution of the crude product in 50 ml. of hot 0.5 *N* hydrochloric acid was treated with Darco and filtered. On adding concentrated (28%) aqueous ammonia to the filtrate until slightly alkaline (*p*H 8.0), the 5-amino-2,3-dimethyl-6-methoxyquinoxaline precipitated. It recrystallized from 60 ml. of water and separated as yellow needles, m.p. 135–136°. The yield was 209 mg. (50%).

Anal. Calcd. for $C_{11}H_{13}O_3N_3$: C, 65.02; H, 6.40; N, 20.69. Found: C, 65.05; H, 6.23; N, 20.92.

5-Amino-2,3-diphenyl-6-methoxyquinoxaline.—One millimole (357 mg.) of 2,3-diphenyl-6-methoxy-5-nitroquinoxaline suspended in 75 ml. of ethanol was catalytically reduced in the usual manner. The crude 5-amino-2,3-diphenyl-6-methoxyquinoxaline was recrystallized from 50 ml. of ethanol (Nuchar), m.p. 156–157°. The yield was 266 mg. (81%).

Anal. Calcd. for $C_{21}H_{17}ON_3$: C, 77.07; H, 5.19; N, 12.84. Found: C, 77.27; H, 5.11; N, 12.82.

5-Acetamido-2,3-diphenyl-8-methoxyquinoxaline.—Two millimoles (510 mg.) of 4-acetamido-2,3-dinitroanisole[8] in 50 ml. of ethanol was reduced in the usual manner in 4 hr. The catalyst was removed by filtration. To the alcoholic solution of 4-acetamido-2,3-diaminoanisole, there was added 420 mg. (2 mmoles) of benzil, and the mixture was refluxed for 1 hr. On cooling, there was obtained 427 mg. (58%) of 5-acetamido-2,3-diphenyl-8-methoxyquinoxaline. It was recrystallized from ethanol (Nuchar) and separated as bright yellow needles, m.p. 236–237°.

Anal. Calcd. for $C_{23}H_{19}O_2N_3$: C, 74.80; H, 5.15; N, 11.38. Found: C, 75.10; H, 4.91; N, 11.10.

5-Amino-2,3-diphenyl-8-methoxyquinoxaline.—To a mixture of 27 ml. of absolute ethanol and 3 ml. of concentrated hydrochloric acid, there was added 376 mg. (1.02 mmoles) of 5-acetamido-2,3-diphenyl-8-methoxyquinoxaline. The acetamido compound gradually dissolved when the mixture was refluxed for 1 hr. The solution was concentrated to dryness *in vacuo*. The residue was washed with 3% aqueous ammonia and recrystallized from ethanol (30 ml., Nuchar). The 5-amino-2,3-diphenyl-8-methoxyquinoxaline (275 mg., 84%) separated as tiny reddish-orange needles, m.p. 214–215°.

Anal. Calcd. for $C_{21}H_{17}ON_3$: C, 77.07; H, 5.19; N, 12.84. Found: C, 77.12; H, 5.24; N, 12.92.

5-Methoxy-2-methyl-4-nitrobenzimidazole.—Four millimoles (738 mg.) of 3,4-diamino-2-nitroanisole dissolved in 5 ml. of acetic anhydride was warmed on the steam-bath. To the solution there was added slowly 15 ml. of 3 *N* hydrochloric acid. The mixture was refluxed for 20 minutes, diluted with 30 ml. of hot water, boiled briefly with Darco and filtered. Concentrated (28%) aqueous ammonia was added to the hot filtrate until slightly alkaline (*p*H 8.0). After cooling, the yellow precipitate of 5-methoxy-2-methyl-4-nitrobenzimidazole was collected and washed with water. The yield was almost quantitative. It was recrystallized from 50% ethanol, m.p. 204–205°.

Anal. Calcd. for $C_9H_9O_3N_3$: C, 52.17; H, 4.35; N, 20.29. Found: C, 52.23; H, 4.09; N, 20.25.

6-Methoxy-2-methyl-4-nitrobenzimidazole.—This compound was prepared by the procedure given above from 369 mg. (2 mmoles) of 3,4-diamino-5-nitroanisole.[12] The crude product was recrystallized from 25% ethanol, m.p. 226–227°. The yield of 6-methoxy-2-methyl-4-nitrobenzimidazole was 319 mg. (77%).

Anal. Calcd. for $C_9H_9O_3N_3$: C, 52.17; H, 4.35; N, 20.29. Found: C, 52.51; H, 4.03; N, 20.35.

3,4-Diacetamidoanisole.—To a solution of 1.16 g. of 4-acetamido-3-aminoanisole[13] in 3 ml. of acetic acid, there was added 1.5 ml. of acetic anhydride. The reaction mixture became warm and the product soon began to crystallize. After standing at room temperature for 30 minutes, the almost solid mass was stirred up with 10 ml. of 1-butanol and filtered. The crude product (1.18 g., 82.5%) was recrystallized from approximately 20 ml. of water. The 3,4-diacetamidoanisole separated as short needles having a pinkish tinge, m.p. 182–184°.

Anal. Calcd. for $C_{11}H_{14}O_3N_2$: C, 59.46; H, 6.31; N, 12.61. Found: C, 59.61; H, 6.30; N, 12.50.

4,5-Diacetamido-2-nitroanisole.—One gram of 3,4-diacetamidoanisole was suspended by stirring in 5 ml. of glacial acetic acid while a mixture of 0.8 ml. of concentrated (d. 1.4) nitric acid and 1.5 ml. of acetic acid was gradually added. When approximately one-half of the mixed acids had been added, the diacetamidoanisole was almost completely in solution. The reaction mixture became warm as the remainder of the mixed acids was added and the nitro derivative began to crystallize. After all the nitric acid had been added, the mixture was stirred at room temperature for 30 minutes before pouring onto ice and diluting with water to approximately 75 ml. The yellow precipitate of 4,5-diacetamido-2-nitroanisole (1.04 g., 87%) was collected, washed with water and recrystallized from 50 ml. of 50% ethanol, m.p. 225–227°.

Anal. Calcd. for $C_{11}H_{13}O_5N_3$: C, 49.44; H, 4.87; N, 15.73. Found: C, 49.64; H, 5.12; N, 15.66.

5-Methoxy-2-methyl-6-nitrobenzimidazole.—A mixture of 2 g. of 4,5-diacetamido-2-nitroanisole and 50 ml. of 2 *N* sulfuric acid was heated under reflux until a complete solution resulted. The hot, red solution was filtered. The filtrate was diluted with an equal volume of hot water, made slightly basic by adding concentrated (28%) aqueous ammonia and kept at 5° overnight. The precipitated 5-methoxy-2-methyl-6-nitrobenzimidazole (1.4 g., 90%) was collected, washed with water and recrystallized from 25% ethanol (125 ml.) with the aid of Nuchar. The purified material was dried at 110° and had m.p. 172–174°.

The diacetamidonitroanisole also cyclized to the 2-methylbenzimidazole when heated in alcoholic potassium hydroxide.

Anal. Calcd. for $C_9H_9O_3N_3$: C, 52.17; H, 4.35; N, 20.29. Found: C, 52.01; H, 4.33; N, 19.47.

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