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

Synthesis of Some Substituted Benzimidazoles, Benzotriazoles, and Quinoxalines

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The synthesis of 3,4-diamino-5-nitrotoluene and 2,3-diamino-5-nitrobenzoic acid is described. These new *o*-diamines, and also 5-chloro-3-nitro-*o*-phenylenediamine, have been subjected to ring closure with appropriate reagents to prepare the derived benzimidazoles, benzotriazoles, and quinoxalines. The derivatives have been used to continue an investigation on the inhibition of developing embryos of the frog, *Rana pipiens*, by such heterocyclic compounds.

The inhibition of the development of *Rana pipiens* by substituted benzimidazoles, benzotriazoles, and quinoxalines has been actively investigated in our laboratories for some time. The work so far completed[1] seemed to indicate that each of the three types of heterocyclic compounds elicits a different morphological response and that the introduction of a nitro group into the parent ring system increases the activity. This effect of the nitro group is diminished by the simultaneous presence of an alkoxy group. Woolley,[2] summarizing his experience with structural analogs as inhibitors of naturally occurring vitamins, suggested that replacing a methyl or carboxyl group by a chlorine atom might result in a more active compound. It seemed of interest, therefore, to prepare derivatives of benzimidazole, benzotriazole, and quinoxaline in which the nitro group was retained but in which the alkoxy group was replaced respectively by methyl, carboxyl, and chlorine. The synthesis of the desired substances required the preparation of two new substituted *o*-phenylenediamine intermediates: 3,4-diamino-5-nitrotoluene and 2,3-diamino-5-nitrobenzoic acid.

Reduction of 4-amino-3,5-dinitrotoluene[3] with alcoholic ammonium sulfide gave a 70% yield of 3,4-diamino-5-nitrotoluene. The *o*-diamine was treated with formic acid, nitrous acid, glyoxal, diacetyl, and benzil to produce respectively, 6-methyl-4-nitrobenzimidazole, 6-methyl-4-nitrobenzotriazole, 7-methyl-5-nitroquinoxaline, 5-nitro-2,3,7-trimethylquinoxaline, and 2,3-diphenyl-7-methyl-5-nitroquinoxaline. No attempt was made to isolate an acetyl derivative of the base but, when the *o*-diamine was dissolved by warming in acetic anhydride, the solution cautiously diluted with cold 3*N* hydrochloric acid, and then refluxed for one-half hour, a good yield of 2,6-dimethyl-4-nitrobenzimidazole was obtained.

2,3-Diamino-5-nitrobenzoic acid was formed in 78% yield when 2-amino-3,5-dinitrobenzoic acid[4] was warmed with aqueous ammonium sulfide and the resulting solution filtered and acidified with the acetic acid. The desired 6-nitrobenzimidazole-4-carboxylic acid, 2-methyl-6-nitrobenzimidazole-4-carboxylic acid, 6-nitrobenzotriazole-4-carboxylic acid, and 2,3-dimethyl- (and 2,3 diphenyl)-7-nitroquinoxaline-5-carboxylic acids were obtained on ring closure of the *o*-diamine with appropriate reagents as indicated in the case of 3,4-diamino-5-nitrotoluene. Attempts to prepare 7-nitroquinoxaline-5-carboxylic acid by treating the *o*-diamine with glyoxal in alcohol or glyoxal bisulfite in water resulted only in resin formation.

The chloronitro substituted benzimidazoles, benzotriazole, and quinoxalines were obtained from 5-chloro-3-nitro-*o*-phenylenediamine in a similar fashion. 6-Chloro-4-nitrobenzimidazole has previously been described by Hoover and Day.[5]

The carboxylic compounds have been found to be completely inactive against developing *Rana pipiens* embryos. The methyl-nitro derivatives of each heterocyclic type appear to be about as active as the alkoxynitro substituted compounds and the chloronitro derivatives are more active than the compounds in which only a nitro group is present. The details of the biological work will be reported elsewhere.

EXPERIMENTAL[6]

The analytical results on the compounds prepared are listed in Table I.

3,4-Diamino-5-nitrotoluene (I). To 200 ml. of freshly prepared 6% ammonium sulfide, there was added 200 ml. of ethanol and 6 g. (0.03 mole) of 4-amino-3,5-dinitrotoluene. The mixture was refluxed for 30 min., diluted with 200 ml. of water, and kept at 5° for several hours. The precipitate was collected, washed with water, and extracted with 400 ml. of hot 0.5*N* hydrochloric acid. When the cooled acid extract was neutralized with concd. (28%) aqueous ammonia, there separated 3.5 g. (70%) of 3,4-diamino-5-nitrotoluene. The diamine crystallized from 25% ethanol as long, red needles, m.p. 152–154°.

6-Methyl-4-nitrobenzimidazole (II). A mixture of 500 mg. (3 mmoles) of I and 1.5 ml. of 98% formic acid in 25 ml. of

(1) K. Liecke, M. Engelman, and S. Graff, *J. Embryol. Exp. Morph.*, **5**, 368 (1957).

(2) D. W. Woolley, the Harvey Lectures, 1945–46, p. 212.

(3) C. L. Jackson and M. H. Ittner, *Am. Chem. J.*, **19**, 6 (1897).

(4) P. Cohn, *Monatshefte fuer Chemie*, **22**, 387 (1901).

(5) J. R. E. Hoover and A. R. Day, *J. Am. Chem. Soc.*, **77**, 4324 (1955).

(6) Melting points are uncorrected.

TABLE I

ANALYTICAL DATA OF COMPOUNDS SYNTHESIZED

Compd. No.	Formula	Carbon, %		Hydrogen, %		Nitrogen, %		Chlorine, %	
		Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found
I	$C_7H_9O_2N_3$	50.30	50.16	5.39	5.37	25.15	25.38		
II	$C_8H_7O_2N_3$	54.24	54.25	3.96	4.27	23.73	23.96		
III	$C_9H_9O_2N_3$	56.54	56.48	4.71	4.66	21.99	22.17		
IV	$C_7H_6O_2N_4$	47.19	47.30	3.37	3.58	31.46	31.64		
V	$C_9H_7O_2N_3$	57.14	57.34	3.70	3.86	22.22	22.51		
VI	$C_{11}H_{11}O_2N_3$	60.83	61.13	5.07	5.16	19.35	19.42		
VII	$C_{21}H_{15}O_2N_3$	73.90	73.61	4.40	4.42	12.32	12.28		
VIII	$C_7H_7O_4N_3$	42.64	42.92	3.55	3.73	21.32	21.22		
IX	$C_8H_5O_4N_3$	46.38	46.25	2.42	2.51	20.29	20.28		
X	$C_9H_7O_4N_3$	48.87	49.12	3.17	3.26	19.00	18.73		
XI	$C_7H_4O_4N_4$	40.38	40.24	1.92	2.24	26.92	26.75		
XII	$C_{11}H_9O_4N_3$	53.44	53.54	3.64	3.94	17.00	16.93		
XIII	$C_{21}H_{13}O_4N_3$	67.92	68.14	3.50	3.81	11.22	11.27		
XIV	$C_6H_3O_2N_4Cl$					28.21	27.89	17.88	17.80
XV	$C_8H_6O_2N_3Cl$					19.86	20.10	16.78	16.72
XVI	$C_8H_4O_2N_3Cl$					20.05	19.90	16.95	16.89
XVII	$C_{10}H_8O_2N_3Cl$					17.68	17.81	14.95	14.86
XVIII	$C_{20}H_{12}O_2N_3Cl$					11.62	11.35	9.81	9.81

3*N* hydrochloric acid was refluxed for 1 hr. The resulting yellow solution was diluted with 75 ml. of hot water, clarified with Darco, and neutralized with 28% ammonium hydroxide solution. After cooling, the yellow precipitate was collected, washed with water, and dried in air. The yield was 465 mg. (87.6%). The 6-methyl-4-nitrobenzimidazole was recrystallized from ethanol, yellow needles, m.p. 288–290°.

2,6-Dimethyl-4-nitrobenzimidazole (III). Three millimoles (501 mg.) of I was dissolved in 4.5 ml. of acetic anhydride by warming on the steam bath. To the cooled solution, there was slowly added 15 ml. of 3*N* hydrochloric acid. The mixture was refluxed for 30 min., diluted with 30 ml. of hot water, boiled briefly with Darco, and filtered. The filtrate was neutralized with strong ammonium hydroxide solution and cooled. There was precipitated 541 mg. (94%) of 2,6-dimethyl-4-nitrobenzimidazole. The compound crystallized from 25% ethanol as a felted mass of tiny, colorless needles, m.p. 238–240°.

6-Methyl-4-nitrobenzotriazole (IV). A suspension of 501 mg. (3 mmoles) of I in 25 ml. of 3*N* hydrochloric acid was stirred at room temperature and treated during the course of 10 min. with a solution of 282 mg. (4 mmoles) of sodium nitrite in 5 ml. of water. Stirring was continued for 1 hr. The 6-methyl-4-nitrobenzotriazole was collected, washed with water and recrystallized from ethanol (Nuchar), m.p. 277–278°. The yield was 405 mg. (75.8%).

7-Methyl-5-nitroquinoxaline (V). A mixture of 835 mg. (5 mmoles) of I and 2.0 g. (7.5 mmoles) of glyoxal bisulfite in 100 ml. of hot water was stirred and heated on the steam-bath for 2 hr. The hot, red solution was filtered by gravity to remove a red resin and the cooled filtrate was made basic by the addition of 10% sodium hydroxide solution. After being kept at 5° for several hours, the tan precipitate was collected, washed with water, and dried *in vacuo* at room temperature. The yield was 648 mg. (69.3%). The 7-methyl-5-nitroquinoxaline was recrystallized from 25 ml. of 50% ethanol (Nuchar), m.p. 134–135°.

2,3,7-Trimethyl-5-nitroquinoxaline (VI). A mixture of 501 mg. (3 mmoles) of I and 363 mg. (4.2 mmoles) of diacetyl in 25 ml. of ethanol was refluxed for 1 hr. The resulting solution was clarified with Nuchar and cooled. The 2,3,7-trimethyl-5-nitroquinoxaline separated as short, almost colorless needles, m.p. 150–151°. The yield was 514 mg. (79%).

2,3-Diphenyl-7-methyl-5-nitroquinoxaline (VII). A mixture of 501 mg. (3 mmoles) of I and 700 mg. (3.3 mmoles) of benzil in 25 ml. of glacial acetic acid was heated on the steam bath for 30 min. To the resulting solution hot water was added to incipient turbidity. The mixture was boiled briefly with Darco and filtered. On cooling the filtrate, there separated 937 mg. (91.6%) of 2,3-diphenyl-7-methyl-5-nitroquinoxaline. It crystallized from ethanol as short needles, m.p. 172–173°.

2,3-Diamino-5-nitrobenzoic acid (VIII). To 150 ml. of freshly prepared 6% ammonium sulfide, there was added 9.1 g. (0.04 mole) of 2-amino-3,5-dinitrobenzoic acid.[4] The mixture was heated with stirring on the steam bath for 1 hr. and then boiled to remove the bulk of the ammonia with hot water being added occasionally to keep the original volume. The hot solution was filtered to remove sulfer. After cooling to 5°, the filtrate was acidified with acetic acid. The precipitate of 2,3-diamino-5-nitrobenzoic acid was filtered off, washed with water, and recrystallized from 75% ethanol. The compound, which separates as dark, red needles, decomposes above 245°. The yield was 6.8 g. (78%).

6-Nitrobenzimidazole-4-carboxylic acid (IX). A mixture of 985 mg. (5 mmoles) of VIII and 1.02 g. (15 mmoles) of sodium formate in 15 ml. of 3*N* hydrochloric acid was refluxed for 1 hr., treated with Darco, and filtered. From the filtrate on cooling, there separated 874 mg. (85.3%) of rhombic, orange crystals of 6-nitrobenzimidazole-4-carboxylic acid. The compound decomposes above 300°. An analytical sample was recrystallized from ethanol.

2-Methyl-6-nitrobenzimidazole-4-carboxylic acid (X). Two millimoles (394 mg.) of VIII was dissolved by warming in 4.5 ml. of acetic anhydride. To the cooled solution, there was cautiously added 15 ml. of 3*N* hydrochloric acid. The mixture was refluxed for 30 min. and clarified with Darco. The filtrate was concentrated to dryness *in vacuo* at 50° and the residue dissolved in 20 ml. of 0.5*N* sodium hydroxide. When the alkaline solution was acidified with acetic acid, there was obtained 272 mg. (61.5%) of 2-methyl-6-nitrobenzimidazole-4-carboxylic acid. The compound dissolved in hot ethanol (60 ml.) and separated on cooling as a colorless powder which does not melt below 300°.

6-Nitrobenzotriazole-4-carboxylic acid (XI). This compound was prepared by the procedure described for IV. From 333 mg. (1.7 mmoles) of VIII, there was isolated 313 mg. (88.4%) of 6-nitro-benzotriazole-4-carboxylic acid. The compound was dissolved in 75 ml. of hot 25% ethanol, treated with Nuchar, and the solution filtered. From the filtrate on cooling, it separated as a colorless powder which decomposes at about 300°.

2,3-Dimethyl-7-nitroquinoxaline-5-carboxylic acid (XII). The procedure employed for VI was followed. From 394 mg. (2 mmoles) of VIII and 288 mg. (3.3 mmoles) of diacetyl in 75 ml. of ethanol, the yield of 2,3-dimethyl-7-nitroquinoxa-

line-5-carboxylic acid was 352 mg. (71.3%); fine, grayish needles. m.p. 222–224° dec.

2,3-Diphenyl-7-nitroquinoxaline-5-carboxylic acid XIII. Two millimoles (394 mg.) of VIII treated with an equivalent quantity of benzil in 25 ml. of glacial acetic acid as described for VII, yielded 526 mg. (70.8%) of 2,3-diphenyl-7-nitroquinoxaline-5-carboxylic acid in the form of yellow plates, m.p. 235–236°. A sample for analysis was recrystallized from methanol.

6-Chloro-4-nitrobenzotriazole XIV. A solution of 940 mg. (5 mmoles) of 5-chloro-3-nitro-*o*-phenylenediamine in 100 ml. of hot 1*N* sulfuric acid was treated with Darco and filtered. The sulfate of the base which separated on cooling the filtrate was kept in suspension by vigorous stirring while a solution of 450 mg. of sodium nitrite in 5 ml. of water was added over a period of 10 min. Stirring was continued for 1 hr. The 6-chloro-4-nitrobenzotriazole was collected, washed with water, and recrystallized from 50% ethanol (75 ml.), m.p. 238–239°. The yield was 619 mg. (62.3%).

6-Chloro-2-methyl-4-nitrobenzimidazole XV. Three millimoles (563 mg.) of 5-chloro-3-nitro-*o*-phenylenediamine, treated with acetic anhydride and then hydrochloric acid as described for III, yielded 548 mg. (86.3%) of 6-chloro-2-methyl-4-nitrobenzimidazole; it was recrystallized from benzene, m.p. 229–230°.

7-Chloro-5-nitroquinoxaline (XVI). To a solution of 940 mg. (5 mmoles) of 5-chloro-3-nitro-*o*-phenylenediamine in 50 ml. of hot ethanol, there was added 1.5 ml. of a 30% aqueous solution of glyoxal. The solution was refluxed for 1 hr., treated with Nuchar, and filtered. On cooling the filtrate, the 7-chloro-5-nitroquinoxaline crystallized as long, yellow needles, m.p. 174–175°. The yield was 690 mg. (65.8%).

7-Chloro-2,3-dimethyl-5-nitroquinoxaline (XVII). Five millimoles (940 mg.) of 5-chloro-3-nitro-*o*-phenylenediamine in 75 ml. of 50% ethanol treated with 605 mg. (7 mmoles) of diacetyl as outlined for VI gave 815 mg. (68%) of 7-chloro-2,3-dimethyl-5-nitroquinoxaline; pale, yellow needles, m.p. 140–141°.

7-Chloro-2,3-diphenyl-5-nitroquinoxaline (XVIII). When a mixture of 563 mg. (3 mmoles) of 5-chloro-3-nitro-*o*-phenylenediamine and 700 mg. (3.3 mmoles) of benzil in 25 ml. of glacial acetic acid was processed as described for VII, a yield of 882 mg. (81.4%) of 7-chloro-2,3-diphenyl-5-nitroquinoxaline was obtained. The compound was recrystallized from ethanol: short, yellow needles, m.p. 184–185°.

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[CONTRIBUTION FROM THE RESEARCH AND DEVELOPMENT DIVISION, SMITH KLINE AND FRENCH LABORATORIES]

Synthesis of Phenothiazines. IV.[1-3] 10-Aminoalkyl Derivatives of 2-Substituted Phenothiazines and 2-Azaphenothiazines

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The present paper describes various 10-aminoalkyl derivatives of the following phenothiazines: 2-hydroxyphenothiazine, 2-methylthiophenothiazine, 2-methylsulfonylphenothiazine, 2-trifluoromethylsulfonylphenothiazine, 2-trifluoromethylthiophenothiazine, 2-azaphenothiazine, and 8-chloro-2-azaphenothiazine.

Paper II[2] of this series describes the preparation of 2-azaphenothiazine and 8-chloro-2-azaphenothiazine. Paper III describes the preparation of 2-hydroxyphenothiazine, 2-benzoyloxyphenothiazine, 2-methylthiophenothiazine, 2- and 4-trifluoromethylthiophenothiazine, 2-methylsulfonylphenothiazine, and 2-trifluoromethylsulfonylphenothiazine. In Tables I and II of the present paper are reported the preparation and physical properties of eighteen different 10-aminoalkyl derivatives of the substituted phenothiazine intermediates described in papers II and III. Biological data concerning these compounds will be published elsewhere.

EXPERIMENTAL[4]

The alkylations were carried out in the usual manner[1] with the following exceptions. The direct alkylation of 2-hydroxyphenothiazine was not attempted. Instead 2-benzoyloxyphenothiazine was alkylated using sodamide in xylene and the ester group was removed by basic hydrolysis during the workup. The alkylation of 2-trifluoromethylsulfonylphenothiazine with 3-(4-methylpiperazinyl)propyl chloride required 48 hr. instead of the usual 2 to 10 hr. The preparation of the β-acetoxyethyl compounds was accomplished as shown.

Preparation of 4-[3-(2-azaphenothiazin-10-yl)propyl]-1-piperazineethanol, acetate dimaleate. (Compound 17). A mixture of 15 g. of 2-azaphenothiazine,[2] 6.8 g. of sodamide, and 500 ml. of dry toluene was refluxed and stirred under a nitrogen atmosphere for 45 min. To the mixture was added a slurry of 21 g. of 3-chloro-1-(1-formyl-4-piperazinyl)propane hydrochloride and 300 ml. of dry toluene which had been previously azeotroped together for 1 hr. The mixture was cooled and 150 ml. of water was added. The toluene layer was extracted with dilute hydrochloric acid. The acid extracts were made alkaline and extracted with benzene. The benzene was evaporated to give 21 g. of an oil. The oil was dissolved in a solution of 250 ml. of ethanol, 60 ml. of water, and 7 ml. of 40% sodium hydroxide solution. The mixture

(1) Paper I. P. N. Craig *et al.*, *J. Org. Chem.* **22**, 709 (1957).

(2) Paper II. A. J. Saggiomo *et al.*, *J. Org. Chem.* **23**, 1906 (1958).

(3) Paper III. E. A. Nodiff *et al.*, *J. Org. Chem.*, **25**, 60 (1960).

(4) All melting points are uncorrected.