o-toluidine was heated to 183° in 0.5 hour, cooled, and triturated with 75 cc. of hot Skellysolve C. The decanted solvent yielded 1.0 g. of **2-(*o*-tolyl)-1,2,3,4-tetrahydro-1,3-isoquinolinedione (XIII)**, a crystalline solid, m.p. 103–106°, which was soluble in aqueous alkali.

Anal. Calcd. for $C_{16}H_{13}NO_2$: C, 76.48; H, 5.21; N, 5.56. Found: C, 76.39; H, 5.37; N, 5.59.

The residual gum was triturated with 75 ml. of hot benzene and the mixture was filtered. In the filtrate formed 0.3 g. of **N-(*o*-tolyl)-2-carboxyphenylacetamide (XIV)**, fine white crystals which melted at 183–184°.

Anal. Calcd. for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.62; N, 5.20. Found: C, 71.47; H, 5.68; N, 5.10.

Reaction with 2,6-Dimethylaniline.—A mixture of 16.2 g. (0.1 mole) of homophthalic anhydride and 12.1 g. (0.1 mole) of 2,6-dimethylaniline was heated at about 150° for 30 minutes. The original melt formed a hard solid which was triturated with ethanol and with dioxane. The crystals of **N-(2,6-dimethylphenyl)-2-carboxyphenylacetamide (XV)** which formed in the filtered solvent weighed 16 g. (57%) and melted at 235–237°.

Anal. Calcd. for $C_{17}H_{17}NO_3$: C, 72.06; H, 6.05; N, 4.94. Found: C, 71.75; H, 6.13; N, 4.95.

Reduction of N-(2,6-Dimethylphenyl)-2-carboxyphenylacetamide (XV).—A solution of 1.6 g. (0.04 mole) of lithium aluminum hydride in 0.5 liter of dry ether was treated with 5.7 g.(0.02 mole) of this amide XV and the mixture worked up as described for the homologous amide XI. There was obtained 2 g. (37%) of **N-(2,6-dimethylphenyl)-2-hydroxymethylphenylacetamide (XVII)**, an acid-insoluble solid which was recrystallized from alcohol and melted at 150–151°.

Anal. Calcd. for $C_{17}H_{19}NO_2$: C, 75.81; H, 7.11; N, 5.20; O, 11.88. Found: C, 76.02; H, 7.45; N, 5.27; O, 12.03.

The acid-soluble material in the residue from the evaporation of the ether extract was dissolved in dilute hydrochloric acid which was then shaken with benzene. The aqueous layer was basified with aqueous sodium hydroxide and the oil was extracted in benzene. Distillation gave 0.8 g. (16%) of **N-(2,6-dimethylphenyl)-2-hydroxymethyl-β-phenylethylamine (XVI)**, a fluorescent oil, b.p. 150–153° at 0.1 mm., $n^{25}D$ 1.5870.

Anal. Calcd. for $C_{17}H_{21}NO$: C, 79.96; H, 8.29; N, 5.49. Found: C, 79.99; H, 8.47; N, 5.53.

The hydrochloride salt formed in ether as colorless prisms, m.p. 185–186°.

Anal. Calcd. for $C_{17}H_{22}ClNO$: C, 69.96; H, 7.60. Found: C, 70.29; H, 7.44.

N-(2,6-Dimethylphenyl)-*o*-tolylacetamide (XVIII). Method A.—A solution of 1.3 g. of 2,6-dimethylaniline and 1.1 g. of triethylamine in 40 ml. of dry benzene was treated with 1.7 g. of *o*-tolylacetyl chloride, and the mixture was heated on steam for 0.5 hour and filtered hot. In the cooled filtrate separated 2 g. of solid which was recrystallized from 95% alcohol and from Skellysolve C. It formed fine white needles, m.p. 192°.

Anal. Calcd. for $C_{17}H_{19}NO$: C, 80.59; H, 7.56; N, 5.53. Found: C, 80.56; H, 7.71; N, 5.63.

Method B.—A solution of 1 g. of N-(2,6-dimethylphenyl)-2-hydroxymethylphenylacetamide (XVII) in alcohol was shaken over palladized charcoal under 30 lb. pressure of hydrogen until absorption was complete. The solution was filtered and the filtrate evaporated to obtain 0.9 g. of white solid, m.p. 192°. A sample recrystallized from Skellysolve C melted at 193°, not depressed by mixture with material prepared by method A.

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

Some New Benzimidazoles and Quinoxalines

BY H. B. GILLESPIE, MORRIS ENGELMAN AND SAMUEL GRAFF

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Growth antagonism by substituted benzotriazoles and benzimidazoles has been reported in earlier papers. 4-Methoxy-6-nitrobenzimidazole was found to be one of the most potent of the compounds studied. In order to pin-point the biological effect of groups, position of these groups and ring size, a number of modifications of 4-methoxy-6-nitrobenzimidazole were required. Accordingly, 2-substituted 4-methoxy-6-nitrobenzimidazoles, derivatives of 6-methoxy-4-nitrobenzimidazole and 5-methoxy-7-nitroquinoxaline were prepared for biological investigation.

A number of nitro and methoxy substituted benzotriazoles and benzimidazoles antagonize growth and/or development of certain biological species.[1,2] The systems investigated include *Tetrahymena geleii*[3] (a guanine-requiring protozoan), several strains of *Escherichia coli*,[3] and developing frog embryos (*Rana pipiens*).[4] The latter have been found to be sensitive to concentrations of these compounds below 0.002%. Accordingly, modifications of 4-methoxy-6-nitrobenzimidazole were prepared in which the structure of the benzene ring was maintained but substituents were placed on the 2-position (*i.e.* on the imidazole ring). Expanding the imidazole ring to a six-membered ring gave a series of quinoxalines and derivatives. Another group of compounds was prepared in which the methoxy and nitro groups were interchanged. Thus three general types of compounds are reported here: (A) 2-substituted 4-methoxy-6-nitrobenzimidazoles and derivatives, (B) 5-methoxy-7-nitroquinoxalines and derivatives and (C) derivatives of 6-methoxy-4-nitrobenzimidazole[5] and 7-methoxy-5-nitroquinoxaline.[6]

The 2-substituted 4-methoxy-6-nitrobenzimidazoles were prepared from 2,3-diamino-5-nitroanisole. Condensation of the diamine with acetic acid or urea yielded, respectively, the 2-methyl (I) and the 2-hydroxybenzimidazole (V). The 6-amino

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TABLE I

Compd.	Benzimidazoles Substituent 2	4	6	Formula	Carbon, % Calcd.	Found	Hydrogen, % Calcd.	Found	Nitrogen, % Calcd.	Found
I	CH_3	OCH_3	NO_2	$C_9H_9O_3N_3$	52.17	52.39	4.35	4.35	20.29	20.26
II	CH_3	OCH_3	NH_2	$C_9H_{11}ON_3$	61.02	60.76	6.21	6.44	23.73	23.97
III	CH_3	OH	NO_2	$C_8H_7O_3N_3$	49.74	50.00	3.63	3.57	21.76	21.59
IV	CH_3	OH	NH_2	$C_8H_9ON_3 \cdot H_2SO_4 \cdot {}^1/_2H_2O$	35.56	35.56	4.44	4.59	15.56	15.72
V	OH	OCH_3	NO_2	$C_8H_7O_4N_3$	45.93	45.50	3.35	3.45	20.09	19.89
VI	OH	OH	NO_2	$C_7H_5O_4N_3 \cdot H_2O$	39.40	39.64	3.29	3.62	19.71	19.75
VII	OH	OCH_3	NH_2	$C_8H_9O_2N_3$	53.63	53.84	5.03	4.91	23.46	23.62
VIII	H	NO_2	OH	$C_7H_5O_3N_3$	46.93	47.23	2.79	2.68	23.46	23.54
IX	H	NH_2	OH	$C_7H_7ON_3$	56.38	56.40	4.70	4.80	28.19	27.97
X	H	OCH_3	NO_2	$C_9H_7O_3N_3$	52.68	52.96	3.41	3.60	20.49	20.59
	Quinoxalines 2 and 3	Substituent 5	7							
X	H	OCH_3	NO_2	$C_9H_7O_3N_3$	52.68	52.96	3.41	3.60	20.49	20.59
XI	H	OCH_3	NH_2	$C_9H_9ON_3$	61.71	61.81	5.14	5.02	24.00	24.18
XII	CH_3	OCH_3	NO_2	$C_{11}H_{11}O_3N_3$	56.66	56.94	4.72	5.11	18.03	18.19
XIII	CH_3	OCH_3	NH_2	$C_{11}H_{13}ON_3$	65.02	65.10	6.40	6.67	20.64	21.04
XIV	CH_3	NO_2	OCH_3	$C_{11}H_{11}O_3N_3$	56.66	56.88	4.72	4.51	18.03	18.09

derivatives (II and VII) were obtained by catalytic reduction of the corresponding nitro compounds. The 4-hydroxybenzimidazoles (III and VI) resulted from the hydrolysis of the methoxy compounds by 48% hydrobromic acid. Treatment of 2,3-diamino-5-nitroanisole in alcoholic solution with glyoxal produced 5-methoxy-7-nitroquinoxaline (X). The 2,3-dimethyl derivative (XII) of this quinoxaline was readily synthesized by reaction of the diamine with diacetyl. For comparison of biological behavior, 6-methoxy-4-nitrobenzimidazole[5] and 7-methoxy-5-nitroquinoxaline[6] were prepared. 2,3-Dimethyl-7-methoxy-5-nitroquinoxaline (XIV) resulted from the action of diacetyl on 3,4-diamino-5-nitroanisole in acetic acid solution. 6-Hydroxy-4-nitrobenzimidazole (VIII) was formed when the corresponding methoxy compound was refluxed with 48% hydrobromic acid.

Some of the compounds reported here arrested the development of frog embryos[4] at concentrations below 20 mg. %. A detailed study[7] of the behavior of frog embryos in the presence of 5-methoxy-7-nitroquinoxaline or 4-methoxy-6-nitrobenzotriazole indicated a stage-specific response. In the early developmental stages of highest mitotic rate, the sensitivity of the embryo was greater to 5-methoxy-7-nitroquinoxaline. The embryo, on the other hand, was not actually affected by 4-methoxy-6-nitrobenzotriazole[2] in early cleavage. As the embryos became more differentiated, the susceptibility to this compound at the same concentration increased and arrested development was then observed in the tailbud stage.

Strains of *E. coli* mutated in the presence of 6-hydroxy-4-nitrobenzimidazole.[8,9] The compound induced high frequency of mutation at concentrations that were only slightly toxic. The effect was observed only on growing cells. Most known mutagens affect both resting and growing cells.

Experimental

The compounds prepared are listed in Table I.

4-Methoxy-2-methyl-6-nitrobenzimidazole (I).—A solution of 2.4 g. (10 mmoles) of 2,3-diamino-5-nitroanisole hydrochloride in 50 ml. of glacial acetic acid containing 820 mg. (10 mmoles) of anhydrous sodium acetate was refluxed for two hours. After the solvent was removed *in vacuo* at 60°, the residue was washed with water and dried. The crude 4-methoxy-2-methyl-6-nitrobenzimidazole (1.98 g., 96%) was crystallized from ethanol (100 ml.), m.p. 257–261°.[10]

6-Amino-4-methoxy-2-methylbenzimidazole (II).—Ninety mg. of 10% palladium-on-carbon was added to a solution of 414 mg. (2 mmoles) of 4-methoxy-2-methyl-6-nitrobenzimidazole in 75 ml. of ethanol. The mixture was stirred overnight in the presence of hydrogen at atmospheric pressure. The catalyst was removed by filtration. When the filtrate was concentrated to dryness under reduced pressure with exclusion of air, a residue of 350 mg. of crude 6-amino-4-methoxy-2-methylbenzimidazole was left which was crystallized from water and dried in vacuum oven at 70°, m.p. 121–127°.

4-Hydroxy-2-methyl-6-nitrobenzimidazole (III).—A solution of 715 mg. (3.45 mmoles) of 4-methoxy-2-methyl-6-nitrobenzimidazole (I) in 7 ml. of hydrobromic acid (48%) was refluxed for 6 hours. The solution was diluted with 50 ml. of water, boiled briefly with Darco and filtered. Cautious addition of concentrated aqueous ammonia (28%) to the hot filtrate until the *p*H was 5–6 resulted in the precipitation of 4-hydroxy-2-methyl-6-nitrobenzimidazole (308 mg.). The crude product was crystallized from 25% ethanol, m.p. 305° dec. The compound sublimes at 230° (8 mm.).

6-Amino-4-hydroxy-2-methylbenzimidazole Sulfate (IV).—Reduction of 386 mg. (2 mmoles) of 4-hydroxy-2-methyl-6-nitrobenzimidazole (III) in 60 ml. of ethanol as described for II gave 324 mg. of the crude amino compound. This was dissolved in 15 ml. of hot 0.1 *N* sulfuric acid, treated with Darco and filtered. From the filtrate on cooling, there separated 320 mg. of 6-amino-4-hydroxy-2-methylbenzimidazole sulfate. The salt, which has the composition $C_8H_9ON_3 \cdot H_2SO_4 \cdot {}^1/_2H_2O$, decomposes at about 270°.

2-Hydroxy-4-methoxy-6-nitrobenzimidazole (V).—An intimate mixture of 1 g. of 2,3-diamino-5-nitroanisole hydrochloride and 1 g. of urea, in a test-tube, was placed in an oil-bath and temperature gradually raised. The mixture melted at 120–130° and solidified at 140–150°. After being kept at 180° for 30 min., the product was cooled and dissolved in 25 ml. of 10% sodium hydroxide. The deep red solution was filtered and acidified with acetic acid. An almost quantitative precipitate of 2-hydroxy-4-methoxy-6-nitrobenzimidazole as a light yellow amorphous powder was collected, washed with water and dried. When crystallized from 50% ethanol (150 ml.) the yield was 716 mg. (88%), m.p. 318–324°.

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(9) S. B. Greer, personal communication.

(10) All melting points are corrected. The analytical results are listed in Table I.

2,4-Dihydroxy-6-nitrobenzimidazole (VI).—Two millimoles (417 mg.) of 2-hydroxy-4-methoxy-6-nitrobenzimidazole (V) did not dissolve in 6 ml. of hydrobromic acid (48%) after two hours of refluxing. Solution was effected by adding 5 ml. of glacial acetic acid. The solution was refluxed for 4 hours, cooled and poured into 100 ml. of water. The precipitate of 2,4-dihydroxy-6-nitrobenzimidazole (364 mg., 93%), was collected, washed with water and dried. It crystallized from 10% ethanol solution, m.p. 338° dec.

6-Amino-2-hydroxy-4-methoxybenzimidazole (VII).—Reduction of 386 mg. (2 mmoles) of 2-hydroxy-4-methoxy-6-nitrobenzimidazole (V) in 100 ml. of ethanol was accomplished as described for II. The 6-amino-2-hydroxy-4-methoxybenzimidazole was recrystallized from a minimum volume of hot water and dried at 110°, m.p. 176–178°.

6-Hydroxy-4-nitrobenzimidazole (VIII).—A solution of 2.39 g. (12.4 mmoles) of 6-methoxy-4-nitrobenzimidazole[2] in 15 ml. of hydrobromic acid (48%) was refluxed for 5 hours, diluted with 140 ml. of hot water and clarified with Darco. The hot filtrate was adjusted to *p*H 5 by addition of 28% ammonium hydroxide solution and cooled. The precipitated 6-hydroxy-4-nitrobenzimidazole was collected, washed with water and dried in a vacuum oven at 70° overnight. The yield was 1.56 g. (70%; crystallized from 25% ethanol (125 ml.), m.p. 288°.

4-Amino-6-hydroxybenzimidazole (IX).—The reduction of 300 mg. of 6-hydroxy-4-nitrobenzimidazole in 100 ml. of ethanol was conducted in the same manner as II. The crude 4-amino-6-hydroxybenzimidazole was crystallized from a small volume (6–10 ml.) of water, m.p. 250° dec.

5-Methoxy-7-nitroquinoxaline (X).—A solution of 1.04 g. (6 mmoles) of 2,3-diamino-5-nitroanisole in 75 ml. of ethanol was treated with 4 ml. of a 30% aqueous solution of glyoxal. After refluxing for 2 hours, the solution was cooled and 672 mg. (58%) of 5-methoxy-7-nitroquinoxaline separated. It was recrystallized from ethanol (50 ml.) with the aid of Nuchar, m.p. 177–179°.

This compound was also obtained when an aqueous solution of equimolar quantities of the base and glyoxal bisulfite was heated on the steam-bath for 30 min. The hot reaction mixture was filtered by gravity through coarse filter paper to remove a resinous by-product. The filtrate was made slightly basic by addition of 28% ammonium hydroxide solution in order to precipitate the quinoxaline.

7-Amino-5-methoxyquinoxaline (XI).—This compound was obtained by reduction of 410 mg. (2 mmoles) of 5-methoxy-7-nitroquinoxaline (X) as described for II. It was recrystallized from a minimum volume of hot water (Darco), m.p. 199–201°.

2,3-Dimethyl-5-methoxy-7-nitroquinoxaline (XII).—A mixture of 1.83 g. (10 mmoles) of 2,3-diamino-5-nitroanisole and 3.4 g. (40 mmoles) of diacetyl in 175 ml. of ethanol was refluxed for one hour. The dimethylquinoxaline separated as light brown needles on cooling; crystallized from ethanol (Nuchar), m.p. 218–220°.

7-Amino-2,3-dimethyl-5-methoxyquinoxaline (XIII).—By reduction of 466 mg. (2 mmoles) of 2,3-dimethyl-5-methoxy-7-nitroquinoxaline in 80 ml. of ethanol in the manner described for II, 421 mg. of 7-amino-2,3-dimethyl-5-methoxyquinoxaline was obtained. This was recrystallized from 10% ethanol solution, m.p. 226–229°.

2,3-Dimethyl-7-methoxy-5-nitroquinoxaline (XIV).—A suspension of 549 mg. (3 mmoles) of 3,4-diamino-5-nitroanisole[6] in 50 ml. of 10% acetic acid was stirred and heated on the steam-bath. A solution of 260 mg. of diacetyl in 2 ml. of 10% acetic acid was added dropwise. Heating and stirring were continued for 30 min. after all of the diacetyl had been added. The resulting solution was cooled. The precipitate (652 mg., 93%) of 2,3-dimethyl-7-methoxy-5-nitroquinoxaline was collected, washed with water and dried. It was recrystallized from 50% ethanol, m.p. 155–156°.

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NEW YORK 32, N. Y.

[CONTRIBUTION FROM THE OHIO STATE UNIVERSITY RESEARCH FOUNDATION]

Triazines. XIV. The Extension of the Pinner Synthesis of Monohydroxy-*s*-triazines to the Aliphatic Series. 2,4-Dimethyl-*s*-triazine[1-3]

BY HANSJUERGEN SCHROEDER AND CHRISTOPH GRUNDMANN

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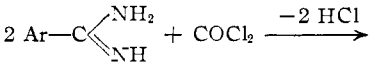
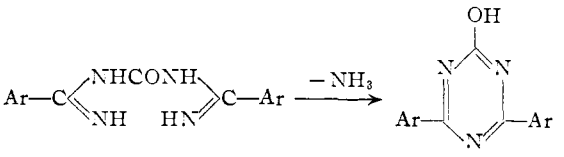
Hitherto the Pinner synthesis of monohydroxy-*s*-triazines has been limited to the preparation of 2,4-diaryl-6-hydroxy-triazines. This reaction has now been extended to the aliphatic series using α-chlorinated amidines. By hydrogenolysis dialkyl-hydroxy-triazines are obtained which are converted into a series of derivatives. From 2-chloro-4,6-dimethyl-*s*-triazine 2,4-dimethyl-*s*-triazine, the first known dialkyl-triazine, is obtained.

The synthesis of 2,4-diaryl-6-hydroxy-*s*-triazines from two moles of an arylamidine and one mole of phosgene has been described by Pinner and coworkers.[4-6] In the first step of this reaction an N,N'-bisimidylurea is formed which when heated above its m. p. undergoes ring closure to the desired hydroxy-*s*-triazine with elimination of ammonia.





It has now been found in a more detailed study of the reaction of benzamidine that the intermediate urea is obtained exclusively only when working with ice–salt cooling. However, if the reaction is carried out without cooling, the reaction product contains, beside the intermediate urea, some 2,4-diphenyl-6-hydroxy-*s*-triazine, the amount of the latter increasing with the reaction temperature. In the case of *p*-chlorobenzamidine only the hydroxy-*s*-triazine has been obtained even at a temperature of −10°.[7]

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(2) Presented before the Organic Division of The American Chemical Society at the 128th Meeting at Minneapolis, Minn., Sept. 12, 1955.

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