The proportionality constant (κ) relating substituent constants (σ) and electron density changes was also taken from earlier work.[7b,25] The mutual atom polarizabilities ($\pi_{r,s}$)[24] for the compound $C_6H_5CH_2^-$, which were required for the evaluation of the coulomb integrals, are recorded in Table III.

The calculation of the localization energies was also based on standard perturbation methods.[3,24] The only comment required is the treatment of the inductive effect. As stated above, the inductive effect of the carbon atom at which localization occurs cannot be evaluated readily. Hence, the inductive effect of the nitrogen atom was treated by assigning to the α-carbon atoms coulomb integrals $\alpha_C = \alpha_N/8$.[4b] The approximation used in our earlier papers[6,7b,25] would not improve the agreement between calculated and observed reactivities. The molecular orbital energies and the electron densities used in the calculations are shown in Fig. 2. The coulomb integral $\alpha_N = 0.60$ for the nitrogen atom in pyridine was taken from work on substituent constants[7b] and the dipole moment[26] of pyridine.

(26) P.-O. Löwdin, *J. Chem. Phys.*, **19**, 1323 (1951).

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

Benzimidazoles and Benzotriazoles as Growth Antagonists

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

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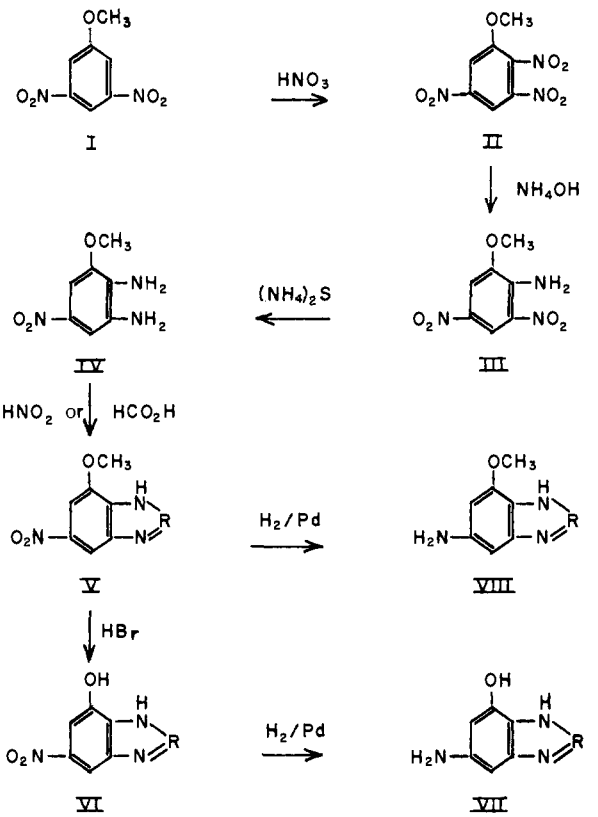
Analogs of the naturally existing purines are useful reagents for the study of metabolic processes. Several new benzimidazoles and benzotriazoles structurally related to guanine have been synthesized. Some of these compounds, among them 4-methoxy-6-nitrobenzimidazole and 4-methoxy-6-nitrobenzotriazole, were found to be effective growth inhibitors of *T. gelii*, a guanine requiring protozoan, and of developing embryos of *R. pipiens*. 6-Amino-4-hydroxybenzimidazole, a guanine analog, was a poor growth inhibitor, and 6-amino-4-hydroxybenzotriazole, an analog of 8-azaguanine, was not inhibitory in the systems tested. The growth of the transplanted mouse tumor, EO 755, was not affected by any of the compounds reported.

8-Azaguanine, an extremely effective antimetabolite to *T. gelii*[1] and also a carcinostatic agent for certain mouse tumors[2,3] differs from guanine only by N in place of CH in the 8-position. It seemed desirable, therefore, to simulate the guanine structure in another manner but without alteration of the ring substituents or the imidazole portion of the ring. 6-Amino-4-hydroxybenzimidazole therefore was prepared. This guanine analog, the synthetic intermediates, and the related benzotriazoles were all assayed biologically. The desired benzotriazoles and the benzimidazoles were prepared by ring closure with nitrous or formic acids from 2,3-diamino-5-nitroanisole which Borsche[4] reported merely as being formed "by reduction of 2-amino-3,5-dinitro anisole with ammonium sulfide." The product was described without other experimental details as dark red needles from water having m.p. 131–132°, which condensed with benzil in alcohol to give 2,3-diphenyl-7-nitro-5-methoxyquinoxaline (yellow needles from alcohol having m.p. 207–208°). Although the melting point of the quinoxaline derivative obtained in this Laboratory corresponded with the melting point reported by Borsche[4] the 2,3-diamino-5-nitroanisole obtained melted at 165–167° instead of 131–132°. The synthesis of 2-amino-3,5-dinitroanisole is so meagerly described by Blanksma[5] that a procedure for its preparation is included. The reactions utilized in the preparation of the compounds reported are indicated in the accompanying chart.



It has been reported that various benzimidazoles and benzotriazoles[6] are effective inhibitors of the growth of yeast,[7] lactobacilli,[8] vaccinia virus,[9] poliomyelitis virus[10] and influenza virus.[11] The

(1) G. W. Kidder and V. C. Dewey, *J. Biol. Chem.*, **179**, 181 (1949).
(2) G. W. Kidder, V. C. Dewey, R. E. Parks, Jr., and G. L. Woodside, *Science*, **109**, 511 (1949).
(3) A. Gellhorn, M. Engelman, D. Shapiro, S. Graff and H. B. Gillespie, *Cancer Research*, **10**, 170 (1950).
(4) W. Borsche, *Ber.*, **50**, 1348 (1917).
(5) J. J. Blanksma, *Rec. trav. chim.*, **23**, 113 (1904).
(6) F. R. Benson, L. W. Hartzel and W. L. Savell, THIS JOURNAL, **74**, 4917 (1952).
(7) D. W. Woolley, *J. Biol. Chem.*, **152**, 225 (1944).
(8) D. Hendlin and M. H. Soars, *J. Bact.*, **62**, 633 (1951).
(9) R. L. Thompson, *J. Immunol.*, **55**, 345 (1947).
(10) G. C. Brown, *ibid.*, **69**, 441 (1952).
(11) I. Tamm, K. Folkers, C. H. Shunk, D. Heyl and F. L. Horsfall, *J. Expt. Med.*, **98**, 245 (1953).

inhibitory action of benzimidazole and some of its derivatives has been ascribed to antagonism of vitamin B_{12} which contains a 5,6-dimethylbenzimidazole group[12] or by blocking of purine utilization.[10]

The compounds screened against the 8-azaguanine susceptible transplanted mouse adenocarcinoma 755, at sublethal doses, failed to inhibit or retard tumor proliferation. When these substances were tested against *T. gelii*,[13] a protozoan which requires guanine and is inhibited by 8-azaguanine, 6-amino-4-hydroxybenzimidazole exhibited at 10 to 200 μg./ml. (guanine concn. at 4 μg./ml.) a marked initial inhibition which was rapidly replaced by a steep growth curve, half optimum growth being attained in eight days for concentrations of 10 to 50 μg./ml. At 200 μg./ml., growth began after the eighth day and attained the control population on the fifteenth day. 4-Methoxy-6-nitrobenzimidazole exhibited a somewhat similar inhibitory pattern. 4-Methoxy-6-nitrobenzotriazole was, however, the most efficient growth antagonist of the compounds tested. After eight days at 4 μg./ml., (guanine at 4 μg./ml.) the population was only 4% of the control. Complete inhibition was obtained at levels between 10–20 μg./ml. up to the eighth day. The remaining compounds did not inhibit the growth of *T. gelii* at levels up to 200 μg./ml.

The amphibian embryo lends itself well to inhibition studies because it constitutes a closed system requiring only a balanced salt solution for a growth medium. Gross morphological and cytological changes are readily observed. Both 4-methoxy-6-nitrobenzotriazole and 4-methoxy-6-nitrobenzimidazole inhibited the growth and development of fertilized eggs of *R. pipiens*.[14] Eggs treated in the three-cell stage with 50 μg./ml. of 4-methoxy-6-nitrobenzimidazole survived for several hours; at 10 μg./ml., the eggs survived for one week. Many abnormalities were produced. Treatment of eggs at the blastula stage (Shumway's stage 8) with 10 μg./ml. permitted development to the tail bud stage (stage 17–18) accompanied by many abnormalities and lethals. Embryos treated at gastrulation (stage 11) succumbed at stage 20. Embryos treated at neurulation (stage 15) seemed to survive normally. The gross effect of 4-methoxy-6-nitrobenzotriazole was somewhat similar to the corresponding benzimidazole but at higher concentrations. 4-Hydroxy-6-nitrobenzotriazole produced similar effects at twice the concentration of the corresponding methoxy derivative.

4-Hydroxy-6-nitro- and 4-methoxy-6-nitrobenzotriazole produced abnormally large cells in *E. coli* cultures.[15] These effects were obtained at a rather high concentration but did not follow the concentration proportionately.

From the preliminary results of the assays one may tentatively conclude that for systems which are inhibited by these compounds, the metabolic antagonism is enhanced by the presence of a nitro and methoxy group and diminished by an amino or hydroxy group. The effect of the position of substituents on the benzene ring, substituents in the 2-position, and alterations in the imidazole ring are under study. The detailed results of the biological assays will be published elsewhere.

(12) N. G. Brink and K. Folkers, THIS JOURNAL, **72**, 4442 (1950).

(13) Private communication from Dr. F. P. Ryan and Sheldon Greer.

(14) Private communication from Dr. Kathe Liedke.

(15) Private communication from Dr. L. E. Loveless.

Experimental

2-Amino-3,5-dinitroanisole (III).—A solution of 10 g. of 2,3,5-trinitroanisole (II) (prepared according to Blanksma[5] by nitration of 3,5-dinitroanisole (I)) in 540 ml. of absolute ethanol, was cooled to 20° and diluted with 60 ml. of 28% aqueous ammonium hydroxide. After refluxing for one hour, the red-orange solution was allowed to cool slowly to room temperature. The product crystallized as long red needles, was filtered and washed with cold ethanol; yield 7.8 g. (88%), m.p. 177–180°[16] (lit. 181°[17]).

2,3-Diamino-5-nitroanisole Hydrochloride.—Hydrogen sulfide was passed through 850 ml. of 3.3% ammonium hydroxide until the solution had gained 13 g. in weight. 2-Amino-3,5-dinitroanisole, 10.7 g., was added to the ammonium sulfide solution. The solution was stirred mechanically and heated on a steam-bath for 2 hours. The solid gradually dissolved to form a deep red solution while sulfur separated out. The mixture was brought to a boil with a free flame and then filtered. The filtrate was refrigerated overnight. The dark red solid product containing some sulfur was collected on the filter and washed with a small volume of cold water. The product, 8 g., was extracted with 600 ml. of hot 3 *N* hydrochloric acid. On cooling the filtrate, 6.8 g. of crude 2,3-diamino-5-nitroanisole hydrochloride, dark brown needles, was obtained. This was dissolved in 400 ml. of 3 *N* hydrochloric acid, clarified with Darco, filtered and the residue washed with 50 ml. of 3 *N* hydrochloric acid. The hydrochloride, 5.8 g., separated as bright yellow needles; m.p. 230° with previous darkening at 200°.

Anal. Calcd. for $C_7H_9O_3N_3 \cdot HCl \cdot H_2O$: N, 17.68; Cl, 15.08. Found: N, 17.83; Cl, 14.80.

2,3-Diamino-5-nitroanisole (IV).—A hot concentrated aqueous solution of the hydrochloride was made slightly basic to litmus with concentrated ammonium hydroxide solution. The free base separated as an orange powder; m.p. 165–167° (lit.[4] 131–132°).

Anal. Calcd. for $C_7H_9O_3N_3$: C, 45.90; H, 4.92; N, 22.95. Found: C, 46.23; H, 5.04; N, 22.92.

4-Methoxy-6-nitrobenzotriazole (Va).—A suspension of 11.9 g. (0.05 mole) of 2,3-diamino-5-nitroanisole hydrochloride in 500 ml. of 0.5 *N* hydrochloric acid solution was treated with 4.3 g. (0.065 mole) of sodium nitrite in 50 ml. of water dropwise. The mixture was stirred mechanically at room temperature. The yellow suspension gradually changed to a creamy white solid. After the addition was completed, the suspension was stirred for one hr. at room temperature and then for 45 min. on the steam-bath. The reaction mixture was refrigerated overnight, filtered, and the amorphous powder was washed with water and dried; crude yield 10.4 g. (83%). The product was recrystallized from 50% ethanol (Darco) and dried at 140° under reduced pressure; m.p. 258–260°.

Anal. Calcd. for $C_7H_6O_3N$: C, 43.29; H, 3.09; N, 28.87. Found: C, 43.48; H, 3.33; N, 29.03.

4-Hydroxy-6-nitrobenzotriazole (VIa).—4-Methoxy-6-nitrobenzotriazole, 3.9 g. (0.02 mole), was dissolved in 40 ml. of hot 48% hydrobromic acid solution. The solution was refluxed for six hours, was cooled and added dropwise to 250 ml. of water. The product, a yellow powder, was removed by filtration and washed with water. After recrystallizing from 25% ethanol, 3.4 g. (95%) of 4-hydroxy-6-nitrobenzotriazole, m.p. 197–199° was obtained.

Anal. Calcd. for $C_6H_4O_3N_4$: C, 40.00; H, 2.22; N, 31.11. Found: C, 40.15; H, 2.31; N, 31.23.

6-Amino-4-hydroxybenzotriazole Hydrochloride (VIIa).—A solution of 1.09 g. (0.006 mole) of 4-hydroxy-6-nitrobenzotriazole in 90 ml. of 95% ethanol was shaken in an hydrogen atmosphere (45 lb. pressure) with palladium catalyst for 5 hours. Ten ml. of 3 *N* hydrochloric acid solution was added, the catalyst was removed by centrifugation, and the

(16) All melting points are corrected.

(17) R. Medola and J. G. Hay, *J. Chem. Soc.*, **91**, 1477 (1907).

solution was evaporated to dryness. The residue was dissolved in 50 ml. of hot abs. alcohol, cooled and diluted with 3 volumes of anhyd. ether. The hydrochloride precipitated as a white powder which became slightly pink during filtration and drying; yield 915 mg., dec. at 220° after subliming at 210–215°.

Anal. Calcd. for $C_6H_6ON_4 \cdot 2HCl$: N, 25.12. Found: N, 25.53.

6-Acetamido-4-hydroxybenzotriazole.—A solution of 335 mg. (1.5 m*M*) of 6-amino-4-hydroxybenzotriazole dihydrochloride and 300 mg. of anhydrous sodium acetate in 15 ml. of water was treated with 0.5 ml. of acetic anhydride. A precipitate began to form in the yellow solution almost immediately. After refrigerating overnight, the crude product (230 mg.) was collected and washed with water. It was recrystallized from 75% acetic acid; m.p. 284–289° dec.

Anal. Calcd. for $C_8H_8O_2N_4$: C, 50.00; H, 4.17; N, 29.17. Found: C, 50.19; H, 4.41; N, 28.82.

6-Amino-4-methoxybenzotriazole (VIIIa).—This compound was prepared by catalytic reduction of Va as described for VIIa but without the addition of hydrochloric acid. From 390 mg. (0.02 mole) of 4-methoxy-6-nitrobenzotriazole there was obtained, after recrystallization from water (Darco), 207 mg. (63%) of 6-amino-4-methoxybenzotriazole, pink crystals; m.p. 196–198°.

Anal. Calcd. for $C_7H_8ON_4$: C, 51.22; H, 4.88; N, 34.15. Found: C, 51.10; H, 4.86; N, 33.93.

4-Methoxy-6-nitrobenzimidazole (Vb).—2,3-Diamino-5-nitroanisole hydrochloride, 7.7 g. (0.033 mole) and 2.4 g. (0.035 mole) of sodium formate were dissolved in 150 ml. of hot 3 *N* hydrochloric acid solution. After refluxing for two hours, the dark brown solution was clarified with Darco and made basic with 28% ammonium hydroxide solution while still hot. After cooling, a light yellow amorphous powder was collected by filtration, washed with water and dried in air. The crude product (5.8 g.) was recrystallized from 400 ml. of 50% ethanol (Nuchar) yielding 4.9 g. (78%) of colorless 4-methoxy-6-nitrobenzimidazole. This was recrystallized to constant m.p. 263–265°.

Anal. Calcd. for $C_8H_7O_3N_3$: C, 49.74; H, 3.63; N, 21.76. Found: C, 49.98; H, 3.72; N, 21.59.

4-Hydroxy-6-nitrobenzimidazole (VIb).—A solution of 3.9 g. (0.02 mole) of 4-methoxy-6-nitrobenzimidazole in 35 ml. of hot 48% hydrobromic acid was refluxed for 6 hours. After diluting the solution with 150 ml. of hot water, it was treated with Darco and filtered. The hot filtrate was brought to *p*H 5.0 by addition of 28% ammonium hydroxide solution. 4-Hydroxy-6-nitrobenzimidazole precipitated as a yellow powder. It was collected, after cooling, and recrystallized from 50% ethanol (250 ml.). The yield was 3.0 g. (83%) having m.p. 286–289°. A sample for analysis was sublimed at 210–215° under reduced pressure.

Anal. Calcd. for $C_7H_5O_3N_3$: C, 46.93; H, 2.79; N, 23.46. Found: C, 46.57; H, 2.91; N, 23.26.

6-Amino-4-hydroxybenzimidazole Sulfate (VIIb).—A solution of 1.07 g. (6 m*M*) of 4-hydroxy-6-nitrobenzimidazole in 90 ml. of 95% ethanol was shaken in an hydrogen atmosphere (45 lb. pressure) with palladium catalyst for 3 hours. The catalyst was removed by centrifugation. Evaporation of the alcoholic solution left a dark red residue which was taken up in 30 ml. of hot 2 *N* sulfuric acid and the solution was filtered. On adding 75 ml. of ethanol to the filtrate and cooling 1.24 g. of crude 6-amino-4-hydroxybenzimidazole sulfate precipitated. This was collected on a filter and washed with ethanol and ether. The crude sulfate was redissolved in hot 2 *N* sulfuric acid, the solution clarified with Darco, and the sulfate reprecipitated by addition of ethanol. The yield was 622 mg. having m.p. 248–252°.

Anal. Calcd. for $C_7H_7ON_3 \cdot H_2SO_4 \cdot H_2O$: C, 31.70; H, 4.15; N, 15.85. Found: C, 32.01; H, 4.21; N, 15.64.

6-Amino-4-methoxybenzimidazole (VIIIb).—Reduction of 580 mg. (0.003 mole) of 4-methoxy-6-nitrobenzimidazole in 90 ml. of 95% ethanol by the procedure described for VIIb yielded 301 mg. (61%) of 6-amino-4-methoxybenzimidazole after recrystallization (Darco) from 25 ml. of water. A second recrystallization gave 258 mg. of the compound having m.p. 216–218°.

Anal. Calcd. for $C_8H_9ON_3$: C, 58.90; H, 5.52; N, 25.77. Found: C, 58.89; H, 5.47; N, 25.47.

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NEW YORK, NEW YORK

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, OREGON STATE COLLEGE]

Quinazoline. XIV. Preparations and Structure of Diquinazolyl Ethers[1]

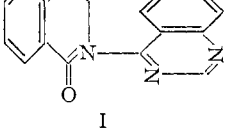
BY H. CULBERTSON, CHARLES WILLITS AND BERT E. CHRISTENSEN

RECEIVED FEBRUARY 10, 1954

In an attempt to prove the N or O ether structure, the ether was warmed with dilute hydrochloric acid; this yielded a product ($C_{15}H_{13}N_3O$) which appeared to be a compound containing a quinazolone unit bound to a degraded quinazoline nucleus. The failure to obtain 4-quinazolone, which should have resulted if it were an O-ether, supported the concept of an N-ether linkage. The proof of structure by the above method is only indicative and not certain since the hydrolysis product was not identified. For this reason it was decided to extend the study to include the synthesis of other diquinazolyl ethers and to study the infrared spectra of these compounds with a view of possibly establishing their structures. A series of three reactions were run using mixtures of 2-chloroquinazoline and potassium 2-quinazolinate, 2-chloroquinazoline and potassium 4-quinazolinate, and 4-chloroquinazoline and potassium 2-quinazolinate using essentially the technique of Tomisek and Christensen.[2] If O-ethers resulted from the reactions, two new diquinazolyl ethers would be formed; on the other hand, if N-ethers resulted, there would be three new isomeric ethers produced.

The synthesis of a diquinazolyl ether whose structure was reported as either I or II has recently been described by Tomisek and Christensen[2]; this ether was obtained by refluxing potassium 4-quinazolinate with 4-chloroquinazoline in dioxane.

2-Chloroquinazoline and potassium 4-quinazolinate gave a white fibrous compound (m.p. 240°);



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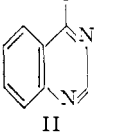

II

in both reactions involving potassium 2-quinazolinate; however, the starting materials were re-

(1) The work described in this paper was made possible by a grant from the Research Corporation. Published with the approval of the Monograph Publications Committee, Oregon State College, as Research Paper No. 244, School of Science, Department of Chemistry.

(2) A. J. Tomisek and B. E. Christensen, THIS JOURNAL, **70**, 874 (1948).