

4-Diethylamino-*trans*-2-butenylamine (VIII, **R** = **H**).—4-Diethylamino-2-butenylamine (VI, **R** = **H**) (20.0 g, 0.143 mol) was added dropwise to a stirred solution of Na (13.8 g, 0.60 g-atom) in 500 ml of liquid NH_3 in 0.5 hr. The reaction was allowed to stir for an additional period of 2 hr. NH_3 was allowed to evaporate off. Some H_2O was added to the residue and the mixture was extracted with Et_2O (three 100-ml portions). The Et_2O extracts were worked up in the usual manner to give an oil which distilled at 80° (10 mm), yield 11.0 g (55.0%). The amine was hygroscopic. It proved to be 99.0% *trans* with a trace of *cis* isomer by glpc on a 20% Carbowax-firebrick column at oven temperature of 160° . It was used as such for its reaction with 4,7-dichloroquinoline.

3-Bromo-1-butyne (III, **R** = **CH₃**) was prepared by the method of Rogers and Panish⁸ in 31.0% yield, bp $44\text{--}48^\circ$ (0.5 mm), n_{D}^{20} 1.4738.

1-Methyl-2-propynylamine-N-phthalimide (IV, **R** = **CH₃**).—3-Bromo-1-butyne (27.0 g, 0.15 mol) was slowly added to a mixture of potassium phthalimide (27.0 g, 0.15 mol) in 100 ml of dry DMF maintained at 70° . The mixture was stirred at this temperature for 12 hr and then cooled and poured into 5 vol of H_2O . The precipitated solid was filtered off, washed (H_2O), and crystallized ($\text{MeOH-H}_2\text{O}$) to give 20.0 g (65.0%) of product, mp $111\text{--}112.5^\circ$. *Anal.* ($\text{C}_{12}\text{H}_9\text{NO}_2$) C, H, N.

4-Diethylamino-1-methyl-2-butynylamine-N-phthalimide (V, **R** = **CH₃**).—A mixture of IV (**R** = **CH₃**) (60.0 g, 0.30 mol), paraformaldehyde (10.8 g, 0.36 mol), Et_2NH (24.0 g, 0.37 mol), and 50 ml of dioxane was refluxed for 4 hr. It was cooled and diluted with 7 vol of H_2O . The mixture was acidified to pH 4 and extracted with Et_2O (two 200-ml portions). The aqueous layer was neutralized with K_2CO_3 to pH 10–11 and extracted with CH_2Cl_2 (three 250-ml portions). The CH_2Cl_2 extracts were dried (CaCl_2) and concentrated to an oil which was distilled at $110\text{--}115^\circ$ (0.2 mm) to give 66.0 g (77.0%) of the product. *Anal.* ($\text{C}_{17}\text{H}_{20}\text{N}_2\text{O}_2$) C, H, N.

4-Diethylamino-1-methyl-2-butynylamine (VI, **R** = **CH₃**) **Dihydrochloride**.—A mixture of V (**R** = **CH₃**) (75.0 g, 0.264 mol), hydrazine hydrate (15.3 g, 0.27 mol), and 350 ml of EtOH was refluxed for 4 hr. It was cooled, acidified with concentrated HCl ,

and filtered and the solid was washed with 95.0% EtOH (three 100-ml portions). The filtrate was concentrated to a solid, dissolved in a minimum amount of H_2O , basified with 50% KOH solution with cooling, and extracted with Et_2O (three 100-ml portions). The combined Et_2O extracts were dried (K_2CO_3), concentrated, and distilled at $90\text{--}92^\circ$ (10 mm) to give 24.5 g (60.0%) of the amine. A portion was converted to a dihydrochloride salt which was crystallized from *i*- PrOH , mp $167\text{--}168.5^\circ$. *Anal.* ($\text{C}_9\text{H}_{20}\text{N}_2\text{Cl}_2$) N.

4-Diethylamino-1-methyl-*trans*-2-butenylamine (VIII, **R** = **CH₃**).—Compound VI (**R** = **CH₃**) (7.0 g, 0.045 mol) was reduced with *Na*-liquid NH_3 in 74.0% yield in the same manner as described for VIII (**R** = **H**); bp 74° (8 mm). Glpc on a 20% Carbowax-firebrick column showed it to be 100% pure *trans* isomer.

Reaction of the Unsaturated Amines with 4,7-Dichloroquinoline (1–4, 6).—The same general procedure was followed for this reaction which consisted of heating a mixture of 4,7-dichloroquinoline (0.04 mol) and the unsaturated amine (0.05 mol) in 40 ml of phenol at $140\text{--}145^\circ$ for about 4 hr. The mixture was cooled, poured into 15% NaOH solution, and extracted (CH_2Cl_2 or Et_2O), and the extracts were dried (K_2CO_3) and evaporated to give an oil which usually solidified on cooling, standing, or scratching. The solid was then crystallized from a suitable solvent (see Table I). In one case (6), the oil was distilled at $155\text{--}160^\circ$ (0.2 mm) in a Kugelrohr apparatus. The distillate solidified on standing.

7-Chloro-4-(4-diethylamino-1-methyl-*cis*-2-butenylamino)-quinoline (5).—A mixture of 7-chloro-4-(4-diethylamino-1-methyl-2-butynylamino)quinoline (4) (3.3 g, 0.0105 mol), Lindlar catalyst (0.3 g), and 75 ml of EtOAc was hydrogenated at room temperature and atmospheric pressure. H_2 uptake was complete in 3 hr. The catalyst was filtered through Celite and the filtrate was evaporated to dryness to give a white solid which was crystallized from cyclohexane (see Table I).

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(8) M. T. Rogers and M. B. Panish. *J. Amer. Chem. Soc.*, **77**, 3684 (1955).

3,5-Dinitrosalicylic Acid (5-Nitrofurfurylidene)hydrazide, a Potent New Preventive of Histomoniasis in Turkeys

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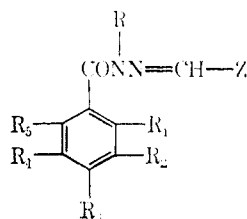
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A series of substituted benzoic acid (5-nitrofurfurylidene)hydrazides has been prepared and screened extensively for antibacterial and antiprotozoal activity. One of these compounds, 3,5-dinitrosalicylic acid (5-nitrofurfurylidene)hydrazide (I), has shown outstanding antihistomonial activity in poultry. The related compounds that have been synthesized and their test results in preventing blackhead disease in turkeys are presented. Possible reasons for the high degree of activity and specificity of I are discussed.

In recent years, a considerable number of reports have been published on the biological properties of nitrofurans, especially their antibacterial activity. During a search for novel nitrofurans which might have antibacterial or antiprotozoal properties, it was noted that few benzoic acid (5-nitrofurfurylidene)hydrazide derivatives had been reported. Numerous nitrofurans of this type were subsequently prepared in our laboratory and were screened against the blackhead parasite *Histomonas meleagridis* as well as other organisms. One of these compounds, 3,5-dinitrosalicylic acid (5-nitrofurfurylidene)hydrazide (I) was found to be exceptionally effective in preventing blackhead disease in poultry.

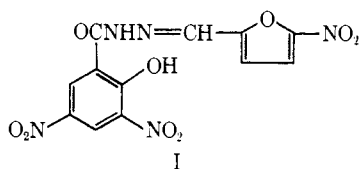
All of the related compounds in Table I (II–XXI) that were prepared had little or no activity against blackhead. Each of these 21 compounds was screened by administering the compound in the feed to turkeys at 0.05% or down to the least effective level. These compounds were prepared, with the exception of XVI, by one of three general methods: A, treatment of the substituted benzoic acid hydrazide with 5-nitro-2-furanmethanediol diacetate and mineral acid catalyst; B, treatment of the substituted benzoic acid hydrazide with 5-nitro-2-furaldehyde; C, treatment of the substituted benzoyl chloride with the appropriate hydrazone.

TABLE I
 SUBSTITUTED BENZOIC ACID HYDRAZIDES


Compd	R ₁	R ₂	R ₃	R ₄	R ₅	R	Z ⁱ	Method ^h	Yield, %	Crystic solvent ^k	Mp, °C ^c	Formula	Analyses	Act/black-head act. in turkeys ^d
I	OH	NO ₂	H	NO ₂	H	H	NF	A	88.7	A	227-229	C ₁₂ H ₇ N ₃ O ₃	C, H, N	0.003
II	NH ₂	NO ₂	H	NO ₂	H	H	NF	B	83	B	282-284	C ₁₂ H ₈ N ₃ O ₃	C, H, N	NE
III	OCH ₃	NO ₂	H	NO ₂	H	H	NF	C	35	A	223-226.5	C ₁₃ H ₉ N ₃ O ₃	C, H, N	NE
IV	N(CH ₃) ₂	NO ₂	H	NO ₂	H	H	NF	C	56.5	B	253-255	C ₁₄ H ₁₂ N ₃ O ₃	C, H, N	NE
V	Cl	NO ₂	H	NO ₂	H	H	NF	C	38.2	A	240-243	C ₁₃ H ₇ ClN ₃ O ₃	C, H, N	0.05 ^b
VI	H	NO ₂	H	NO ₂	H	H	NF	A	79	A	267-269	C ₁₂ H ₇ N ₃ O ₃	C, H, N	NE
VII	CH ₃	NO ₂	H	NO ₂	H	H	NF	A	96.2	C	204.5-265	C ₁₃ H ₉ N ₃ O ₃	C, H, N	NE
VIII	OH	H	H	H	H	H	F	B	96.5	C	227-229 ^e	C ₁₂ H ₉ N ₃ O ₂	-	NE
IX	OH	H	H	H	H	H	NF	A	92.8	C	266 dec ^f	C ₁₂ H ₉ N ₃ O ₂	-	NE
X	OH	NO ₂	H	NO ₂	H	H	F	B	45.4	D	201-201.5	C ₁₂ H ₈ N ₃ O ₂	C, H, N	NE
XI	OH	NO ₂	H	H	H	H	NF	A	79	E	217-219.5	C ₁₂ H ₈ N ₃ O ₂	C, H, N	0.05
XII	OH	H	H	NO ₂	H	H	NF	A	24.5	E	258-259	C ₁₂ H ₈ N ₃ O ₂	C, H, N	0.05 ^b
XIII	OH	H	NO ₂	H	H	H	NF	A	85	A	245	C ₁₂ H ₈ N ₃ O ₂	C, H, N	NE
XIV	OH	NO ₂	H	NO ₂	H	CH ₃	NF	C	30	A	187-189	C ₁₃ H ₉ N ₃ O ₂	C, H, N	NE
XV	OH	NO ₂	H	NO ₂	H	CH ₂ OH	NF	C	44.7	C	150-152	C ₁₃ H ₉ N ₃ O ₃	C, H, N	NE ^g
XVI	OCOCH ₃	NO ₂	H	NO ₂	H	COCH ₃	NF	D	48.8	E	161-163	C ₁₄ H ₁₁ N ₃ O ₆	C, H, N	NE
XVII	H	NO ₂	OH	NO ₂	H	H	NF	A	63	D	246-247	C ₁₃ H ₉ ClN ₃ O ₂	C, H, N	NE
XVIII	OH	NO ₂	H	NO ₂	OH	H	NF	B	99	F	257-259 dec	C ₁₃ H ₉ N ₃ O ₃	C, H, N	0.05 ^b
XIX	OH	NO ₂	OH	NO ₂	H	H	NF	B	87	F	231-232.5 dec	C ₁₃ H ₉ N ₃ O ₄	C, H, N	0.05 ^b
XX	OH	NO ₂	H	Cl	H	H	NF	A	88	D	202-204	C ₁₂ H ₈ ClN ₃ O ₂	C, H, N	NE
XXI	OH	NO ₂	H	NO ₂	H	H	NT	C	18	B	217-219	C ₁₂ H ₇ N ₃ O ₂ S	C, H, N	NE

^a See Experimental Section. ^b A, AcOH; B, AcOH-MeCN; C, not crystallized; D, MeCN; E, Me₂CO-H₂O; F, AcOH-H₂O. ^c Melting points were determined on a Mel-Temp apparatus or on a Kofler apparatus and are uncorrected. ^d NE means not effective at highest dosage (0.05%) used in preventing 100% mortality in a two-bird test. The figures given are the lowest per cent of the compound effective in completely preventing mortality unless otherwise noted. ^e J. Klossa [Arch. Pharm., **288**, 49 (1955)]; Chem. Abstr., **51**, 15512h (1957)] reported mp 232°. ^f C. Ivanov, L. Jelyaskov, M. Dodova, and M. Agova [Compt. Rend. Acad. Bulgare Sci., **10**, 313 (1957); Chem. Abstr., **53**, 15038i (1959)] reported mp 246-250°. ^g Includes a molecule of AcOH not removed upon heating *in vacuo* at 100° for several hours. ^h Partly effective at 0.05%. ⁱ Not effective at 0.03%. ^j NF = 5-nitro-2-furyl, F = 2-furyl, NT = 5-nitro-2-thienyl.

The completely acetylated derivative of I (XVI) was prepared by treatment of I with Ac₂O. Attempts to prepare a monoacetyl derivative were unsuccessful.



Discussion

The first seven compounds in Table I are nitrofurans in which R₁ was varied. None of the compounds II-VII approached the activity of I (R₁ = OH) in preventing mortality in turkeys from blackhead. Even where R₁ could possibly be converted to hydroxy *in vivo* (III, R₁ = OCH₃; V, R₁ = Cl), only the chloro derivative (V) had partial activity at the highest test level (0.05%) used.

The second group of compounds (VIII-XIII) is those derivatives where R₁ = OH but the number of nitro groups and their position is varied. The biological test results indicate that the 5-nitrofuranyl moiety is essential for minimum blackhead activity. This is confirmed where 5-nitrothienyl (XXI) is substituted for 5-nitrofuranyl in I since all activity is lost. Another requirement

for biological activity is a nitro group in the aromatic ring that is *ortho* or *para* to OH. Although the activity of the 3-nitro derivative (XI) was greater than that of the 5-nitro derivative (XII), it was still much less active than I.

Replacement of the hydrazide hydrogen (XIV and XV) of I by methyl and 2-hydroxyethyl gave inactive compounds as did acetylation of I (XVI).

Loss of all activity resulted in the three compounds (XVII-XIX) where only the position or number of OH groups in I was changed. This was also the case when an aromatic nitro group in I was replaced by another deactivating group (XX, R₁ = Cl). Apparently I is highly specific for the prevention of blackhead disease in poultry since a screen of I against several species of coccidia, bacteria, and fungi failed to reveal any additional activity.¹ However, preliminary test data in different animals suggest that I has a beneficial effect upon growth in some cases.

Table II gives comparative efficacy data of I, *p*-ureidobenzencarsonic acid,² and 1,2-dimethyl-5-nitroimidazole³ against blackhead in turkeys. Although levels of I at 0.003% in feed usually prevent all turkey mortality, about twice this level (0.006%) is required to

(1) *In vivo* as well as *in vitro* screens were carried out. The *in vivo* tests were primarily in poultry.

(2) Carbasone[®].

(3) Entyl[™].

TABLE II
COMPARISON OF COMPOUND I WITH ESTABLISHED HISTOMONASTATS

No.	Compound	Concn used	Efficacy ^a	
			% without blackhead	% survival
1	3,5-Dinitrosalicylic acid (5-nitrofurfurylidene)-hydrazide (I)	0.0075	100	100
		0.005	83	100
		0.0025	50	83
2	1,2-Dimethyl-5-nitroimidazole	0.02	100	100
		0.015	67	83
3	p-Ureidobenzencarsonic acid	0.0375	17	50
4	Infected controls		All died of blackhead	
5	Uninfected controls		No blackhead infection	

^a Six birds in each test group were used. Medication was administered in the feed the day of infection and continued for 21 days. The test was completed after a total of 28 days following infection.

eliminate all clinical signs⁴ of the disease when a severe challenge is given. Compound I also has given comparable results against the same organism in chickens, and appears to be quite safe as indicated by toxicity data obtained in several animals. Indeed, it has been difficult to establish an LD₅₀ in animals. No turkey mortality was caused by an oral dose of 5 g/kg although some mortality (less than that required for an LD₅₀) was observed in the chicken and rat at 10 g/kg. Possibly the safety of I is due to its insolubility in most solvents and the fact that the hydrazide precursor of I is even more insoluble than the nitrofurans. Hydrolysis of the hydrazide gives 3,5-dinitrosalicylic acid, which is very water soluble.

Compound I has the properties of a typical nitrofurans since it is light sensitive and rapidly decomposes in strong bases. As expected for a nitrophenol derivative, I is quite acidic and has a pK_a of 1.12 (2,4-dinitrophenol, pK_a = 3.96; picric acid, pK_a = 0.38⁵). Alkali metal salts of I, such as potassium, sodium, and ammonium, have been prepared by dissolving I in DMSO and H₂O and then adding an equivalent of base. All of the salts prepared are exceedingly insoluble in water.

Several plausible explanations for the remarkable specificity and high activity of I against blackhead disease can be advanced. From the data presented in Table I and the knowledge that some simple nitrofurans derivatives such as 5-nitro-2-furaldehyde hydrazone⁶ and 5-nitro-2-furaldehyde 2-ethylsemicarbazone⁷ have some blackhead activity, whereas derivatives of 3,5-dinitrosalicylic acid, such as the hydrazide, are inactive, it seems likely that the activity of I resides primarily in the nitrofuryl portion of the molecule. However, the 3,5-dinitrosalicyloyl group greatly enhances the activity of 5-nitro-2-furaldehyde hydrazone in structure I and a comparison of the blackhead activity of the 4-nitro (XIII), 5-nitro (XII), and 3-nitro (XI) derivatives with I suggests that the activity is directly related to the acidity of the phenolic hydrogen. Furthermore, since the 4-hydroxy isomer (XVII) of I is inactive, possibly interaction of the phenolic hydrogen of I with the hydrazide group is important for biological activity. It has been suggested by Foye and Turcotte⁸ that some, if not all, of the biological effects of salicylates may be due to complexation of metalloenzymes since the avidity

of salicylic acid to complex with iron, copper, and other transition metals is well known. Such a complex might be stabilized by resonance as described recently⁹ for 2-hydroxynicotinic acid. This explanation alone, however, is probably incomplete. Two compounds (XVIII and XIX) that have the structure of I with an additional OH group were inactive, suggesting that an additional electron-releasing group would decrease their resonance stability. Undoubtedly the molecular geometry and the solubility of I are also important for activity. The acidity of I may also serve to bind it to protein sites where the nitrofurans could disrupt the metabolism of *H. meleagridis*.

Additional work is planned for the preparation of transition metal salts of I and 5-nitro-2-furyl ketone analogs of I to study their effect upon blackhead activity.

Experimental Section¹⁰

3,5-Dinitrosalicylic Acid (5-Nitrofurfurylidene)hydrazide (I). **Method A.**—To a suspension of 17 g (0.07 mole) of 3,5-dinitrosalicylic acid hydrazide¹¹ in 200 ml of SD3A EtOH, 100 ml of H₂O, and 10 ml of concentrated H₂SO₄ was added 17 g (0.07 mole) of 5-nitro-2-furanmethanediol diacetate and the mixture was heated just below the boiling point for 0.5 hr. The mixture was cooled, filtered, then washed with SD3A EtOH and finally H₂O. Drying at 110° gave 23.5 g (88.7%) of yellow solid, mp 212–219°. An analytical sample was prepared by crystallizing it twice from AcOH (see Table I).

The following nitrofurans were prepared similarly: VI, VII, IX, XI, XII, XIII, XVII, and XX. MeOH was used instead of EtOH in the preparation of XI and XII.

3,5-Dinitroanthranilic Acid Hydrazide.—A mixture of 24.8 g (0.1 mole) of methyl 3,5-dinitroanthranilate,¹² 330 ml of dry MeOH, and 20.5 ml (0.4 mole) of hydrazine hydrate was refluxed for 5 hr. The mixture was cooled, filtered, washed (MeOH), and air dried. We obtained 22.8 g (92%), mp 205–207°, and purified a sample for analysis by crystallizing from AcOH–MeCN (charcoal) and then from SD3A EtOH; yellow solid, mp 208–210°. *Anal.* (C₇H₇N₅O₆) C, H, N.

The hydrazides corresponding to the following nitrofurans were prepared similarly: XI, mp 209–209.5°; XII, mp 320–326° dec.;¹³ XVII (monohydrate), mp 200–201.5° dec; XVIII, mp 237–239° dec; and XIX, mp 242–243° dec. The first two hydrazide derivatives were warmed with AcOH and the last two were treated with HCl followed by AcOH to remove the excess hydrazine. Acceptable values for C, H, and N were obtained for the five hydrazides except in the last case for N: calcd, 21.70; found, 21.15.

3,5-Dinitroanthranilic Acid (5-Nitrofurfurylidene)hydrazide (II). **Method B.**—The above hydrazide (10 g, 0.041 mole) and 700 ml of SD3A EtOH were heated near boiling and a solution of 5.8 g (0.041 mole) of 5-nitro-2-furaldehyde in EtOH was added with stirring. The mixture was cooled after boiling for 1.5 hr, then collected on a filter and washed (EtOH). The yield of air-dried yellow solid was 18 g (83%), mp 282–284°, from which a sample was purified for analysis (see Table I).

The following compounds were prepared similarly: VIII, X, XVIII, and XIX. The solvents employed for the reactions were THF–H₂O (2:1) for VIII, AcOH for X, and AcOH–H₂O (9:1) for XVIII and XIX.

2-Methoxy-3,5-dinitrobenzoic Acid (5-Nitrofurfurylidene)hydrazide (III). **Method C.**—A solution of 22.8 g (0.1 mole) of 3,5-dinitro-2-methoxybenzoic acid¹⁴ in 60 g (0.5 mole) of SOCl₂ was refluxed for 4 hr and then the excess SOCl₂ was distilled *in vacuo*. CH₂Cl₂ (50 ml) was added and then distilled from the

(9) W. O. Foye, M. D. Baum, and D. A. Williams, *ibid.*, **56**, 332 (1967).

(10) Melting points were obtained on a Mel-Temp block or Kofler apparatus and are uncorrected.

(11) C. N. Haksar and R. D. Wankhade, *Vikram J. Vikram Univ.*, **4**, 133 (1960), *Chem. Abstr.*, **58**, 6741a (1963).

(12) G. C. A. Van Dorp, *Rec. Trav. Chim.*, **23**, 319 (1904).

(13) Previously reported containing 1 mole of MeOH, mp 154° dec. by W. Baker, C. N. Haksar, and J. F. W. McOmie, *J. Chem. Soc.*, **170** (1950).

(14) F. Ullmann, *Ann. Chem.*, **366**, 85 (1909).

(4) Clinical signs of blackhead disease in turkeys include yellow watery droppings, a listless appearance of the bird, and weight loss.

(5) C. D. Hodgeman, "Handbook of Chemistry and Physics," 44th ed. Chemical Rubber Publishing Co., Cleveland, Ohio, 1962, pp 1754–1756.

(6) Partly effective in preventing turkey mortality at 0.025%.

(7) C. A. Johnson, U. S. Patent 3,253,987 (May 31, 1966).

(8) W. O. Foye and J. G. Turcotte, *J. Pharm. Sci.*, **51**, 329 (1962).

acid chloride which was again diluted (CH_2Cl_2) and added dropwise to a stirred mixture of 15.7 g (0.1 mole) of 5-nitro-2-furaldehyde hydrazone¹⁵ in 160 ml of dry pyridine cooled below 15°. After completing the addition, stirring was continued 1.5 hr and then the brown solid was collected on a filter and washed (dilute AcOH). The crude product was crystallized from AcOH (see Table I).

The following compounds were prepared similarly: IV, V, XIV, XV, and XXI except that bis(2-methoxyethyl) ether replaced CH_2Cl_2 in preparing IV. The hydrazones used for the last three nitrofurans were 5-nitro-2-furaldehyde methyl hydrazone, 5-nitro-2-furaldehyde hydroxyethyl hydrazone,¹⁶ and 5-nitro-2-thiophenecarboxaldehyde hydrazone, respectively. The preparation of the latter derivative and others not previously described in the literature is presented as follows.

2,6-Dihydroxy-3,5-dinitrobenzoic Acid.—To a mixture of 15.4 g (0.1 mole) of finely divided 2,6-dihydroxybenzoic acid and 40 ml of concentrated H_2SO_4 cooled below 10° was added dropwise with stirring at -5 to 5° about three-fifths of a cold mixture containing 19 g (0.21 mole) of HNO_3 (70%) and 10 ml of concentrated H_2SO_4 . The remainder of the mixed acid was added at 5-10°. The mixture was poured on ice after stirring at room temperature 1 hr. The solid was filtered and sucked as free of liquid as possible before drying *in vacuo* at 60° to constant weight. The yield of crude pink acid was 21 g (86%), mp 145-174° dec. The acid was exceedingly water soluble and an analytical sample was prepared by crystallizing twice from AcOH and drying *in vacuo* at 100°; pale yellow solid, mp 186.5-188°. *Anal.* ($\text{C}_7\text{H}_3\text{N}_2\text{O}_8$) C, H, N.

Methyl 2,6-Dihydroxy-3,5-dinitrobenzoate.—A solution of 21 g (ca. 0.086 mole) of the crude acid (mp 145-174° dec) was refluxed with 100 ml of SOCl_2 for 4.5 hr and then most of the excess SOCl_2 was distilled *in vacuo*. MeOH (250 ml) was added to the acid chloride, and the solution was refluxed for 1 hr and then concentrated until the ester started to precipitate (final volume ~125 ml). The solution was cooled near 0° overnight, and the crystals were collected by filtration and dried at 85°. Glistening white plates were obtained that weighed 14 g (ca. 63%), mp 137-139°. A purified sample was prepared by crystallizing twice (MeOH): pale cream needles, mp 139-140.5°. *Anal.* ($\text{C}_8\text{H}_6\text{N}_2\text{O}_8$) C, H, N.

2,4-Dihydroxy-3,5-dinitrobenzoic Acid.—The nitration of β -resorcylic acid to prepare the dinitro acid was unsuccessful in our

hands and the following procedure was found to be more expedient than reported methods.¹⁷ A mixture of 50 g (0.178 mole) of 2,4-dichloro-3,5-dinitrobenzoic acid¹⁸ and 1000 ml of 10% NaOH was heated on a steam bath for 7 hr and then cooled near 0°. The Na salt was collected on a filter, dissolved in water, and acidified with HCl. On recrystallizing (charcoal) the solid from H_2O and drying at 115° there was obtained 28 g (64.4%) of white acid, mp 200-203°.

Methyl 2,4-Dihydroxy-3,5-dinitrobenzoate.—The methyl ester was prepared from 22 g (0.09 mole) of the acid *via* the acid chloride as described above for methyl 2,6-dihydroxy-3,5-dinitrobenzoate. The yield of white ester was 15 g (60%), mp 196-200°. A purified sample was obtained by crystallizing (MeOH): pale yellow crystals, mp 197-200°. *Anal.* ($\text{C}_9\text{H}_6\text{N}_2\text{O}_8$) C, H, N.

Acetyl-3,5-dinitrosalicylic Acid (5-Nitrofurfurylidene)-N-acetylhydrazide (XVI).—A solution of 5.0 g (0.014 mole) of I in 25 ml of Ac_2O -AcOH (4:1) was obtained by warming and after 15 min it was cooled to 25° and an equal volume of H_2O was slowly added with swirling. Then the diacetyl derivative was crystallized by chilling near 0°. The pale yellow solid was filtered and dried at 85° to give 3.0 g (40%), mp 159-163°. A purified sample of pale yellow square tablets (see Table I) was prepared by crystallization (charcoal) twice (Me_2CO - H_2O).

5-Nitro-2-thiophenecarboxaldehyde Hydrazone.—5-Nitro-2-thiophenemethanediol diacetate⁶ (10 g, 0.0386 mole) was dissolved in 225 ml of dry MeOH and cooled to 5°. A solution of 5.8 g (0.116 mole) of hydrazine hydrate and 15 ml of MeOH was slowly added to this solution. A red solid precipitated in a few minutes. The MeOH was allowed to evaporate at room temperature after stirring for 2 hr below 5°. The solids were crystallized (charcoal) from EtOH- H_2O to yield 4.0 g (61%) of red solid, mp 140-142°. An analytical sample was prepared by crystallization from this solvent pair: red solid, mp 142-144°. *Anal.* ($\text{C}_8\text{H}_7\text{N}_3\text{O}_8\text{S}$) C, H, N.

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Nitroheterocyclic Antimicrobial Agents. I. Nitrothiazolecarboxaldehyde Derivatives

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A series of derivatives of 2-thiazolecarboxaldehyde, 2-nitro-5-thiazolecarboxaldehyde, 5-nitro-2-thiazolecarboxaldehyde, and 4-nitro-2-thiazolecarboxaldehyde was synthesized and assayed for antimicrobial activity. Only 2,5-disubstituted thiazoles in which one substituent is a nitro group were active against microorganisms *in vitro*. 1-[5-Nitro-2-thiazolyl)methylene]amino]-2-imidazolidinone exhibited activity against *Staphylococcus aureus* and *Escherichia coli* infections in mice.

The disclosure of the antibacterial activity of 5-nitro-furfural derivatives by Dodd and Stillman² has spurred the syntheses of a large number of new nitrofuryl compounds.^{3,4} In the majority of these compounds a nitro group in position 5 is necessary and a conjugated $-\text{C}=\text{N}$ moiety in position 2 is desirable for antimicrobial

activity. The search for new antimicrobial agents with the nitrofuran moiety replaced by nitropyrrole^{5,6} and nitrothiophene⁷⁻⁹ has been pursued, and, although interesting antimicrobial activities were reported in some cases, no clinically useful drug has yet emerged.

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