HOAc, the solution was cooled to 10°, and 2.5 ml of 4 *N* HBr–HOAc was added. The mixture was allowed to stand for 20 min at 10–15°. Excess HBr was removed *in vacuo* and the product was pptd by addition of 100 ml of dry Et_2O. The crude product was filtered, washed with Et_2O, and crystallized from MeOH–Et_2O; yield 250 mg (60%), mp 184–5°, $[\alpha]^{23}D$ +45.3° (*c*, 1 in MeOH). *Anal.* ($C_{10}H_{13}BrN_2O_2$) C, H, N, Br.

***N*-Benzyloxycarbonyl-2-[4-chlorobutyl]-D-cycloserine.**—To a solution of 1.2 g (5 mmoles) of *N*-benzyloxycarbonyl-D-cycloserine in 20 ml of $CHCl_3$ was added 1.41 ml of Et_3N (10 mmoles) and 1.96 g (10 mmoles) of 1-bromo-4-chlorobutane. After stirring overnight at room temp the mixture was evapd to dryness. The residue was dissolved in EtOAc and washed successively with 5% K_2CO_3 solution and H_2O, then dried, and evapd. The residue was crystallized from Et_2O giving 1 g (60%); mp 74–75°, $[\alpha]^{23}D$ +38.3° (*c*, 2 in MeOH). *Anal.* ($C_{15}H_{19}ClN_2O_4$) C, H, N, Cl.

***N*-Benzyloxycarbonyl-2-benzhydryl-D-cycloserine** was prepared in 75% yield by the procedure described above; mp 118–19°, $[\alpha]^{23}D$ +50.1° (*c*, 2 in MeOH). *Anal.* ($C_{23}H_{22}N_2O_4$) C, H, N.

Nitroheterocyclic Antimicrobial Agents. II. 5-Nitro-1,3,4-thiadiazole-2-carboxaldehyde Derivatives

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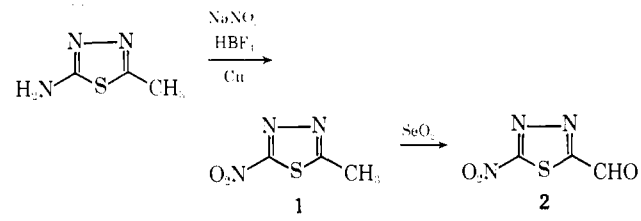
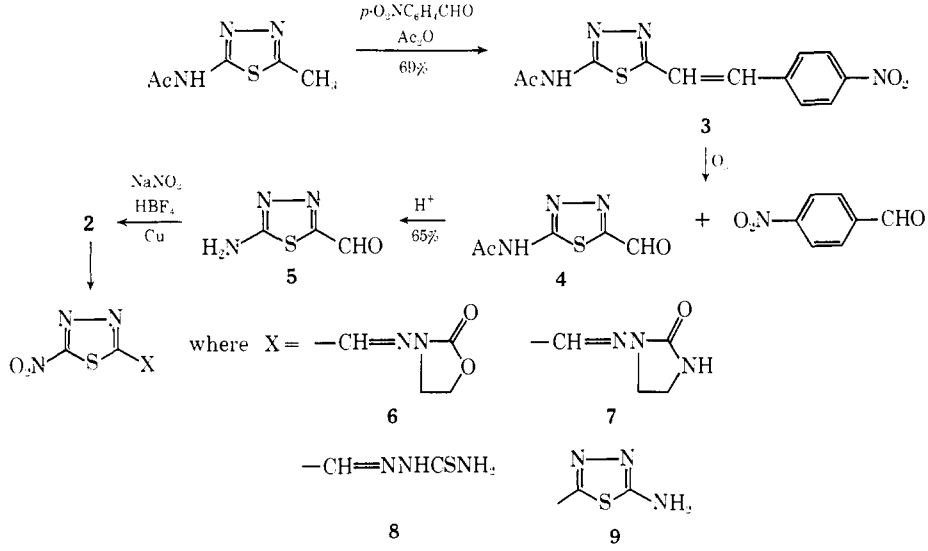
We recently reported the synthesis of 2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole,[1] a broad-spectrum antimicrobial agent. Preparation of this compound and related nitroimidazoles arose from a program based on the replacement of the nitrofuryl moiety of antimicrobially active nitrofurans by isosteric nitroheteroaromatic groups. The first series investigated, derivatives of nitrothiazolecarboxaldehydes,[2] exhibited *in vitro* antibacterial and antifungal activity, and several members showed *in vivo* antimicrobial activity. This report deals with the second group of nitroheterocyclic compounds we examined, namely derivatives of 5-nitro-1,3,4-thiadiazole-2-carboxaldehyde.

Chemistry.—Initially, 2-methyl-5-nitro-1,3,4-thiadiazole (**1**) was selected as the primary precursor, and it was prepared from 2-amino-5-methyl-1,3,4-thiadiazole by diazotization and reaction of the diazonium salt with NO_2^- in the presence of Cu. Compound **1**, being unstable under the reaction conditions, did not condense with pyridinecarboxaldehyde in the presence of $ZnCl_2$, Ac_2O, or piperidine. It could be oxidized with SeO_2 in the absence of solvent to afford *ca.* 5% of **2**; however, this method was impractical for our purposes and an alternate route was developed.



The *p*-nitrobenzylidene derivative **3** was prepared and was ozonized in MeOH to afford **4**, which was hydrolyzed with acid to the aminoaldehyde **5**. The thiadiazolecarboxaldehyde **5** was separated from *p*-nitrobenzaldehyde by acid extraction and converted into **2** by diazotization and displacement with NO_2^- in the presence of Cu. The crude nitroaldehyde was used without purification and overall yields of 6–34% (based on aminoaldehyde **5**) of azomethine derivatives **6–8** were obtained. Ferric ammonium sulfate oxidative cyclization of **8** afforded **9**.



Compound **5** was difficult to purify, and microanalyses were unsatisfactory. However, ir and nmr [Me_2CO-d_6; τ 1.83 (s, 2 H, NH_2), −0.04 (s, 1 H, CHO)] supported its structure. The aldehyde **4** was also separable from *p*-nitrobenzaldehyde but it contained some starting material (2-acetamido-5-methylthiadiazole), which persisted as a contaminant even after repeated recrystallizations, and thus the microanalysis

(1) G. Berkelhammer and G. Asato, *Science*, **162**, 1146 (1968).

(2) G. Asato, G. Berkelhammer, and E. L. Moon, *J. Med. Chem.*, **12**, 374 (1969).

was unsatisfactory. Nonetheless, the structure assigned to **4** was unequivocally supported by ir, nmr, and mass spectral data.

Biological Results.—The nitroaldehyde derivatives **6**–**9** were assayed *in vitro* against selected microorganisms, as reported earlier.[2] Only **7** exhibited fairly good growth inhibitory effect (161–250 μg/ml) against Gram-positive and Gram-negative bacteria, while **9** showed interesting broad-spectrum antifungal activity (15–125 μg/ml) (Tables I, II). None of these compounds was active *in vivo* when administered orally against *Salmonella gallinarum* in chicks or *Staphylococcus aureus* (Smith) or *Escherichia coli* in mice.

TABLE I

In Vitro ANTIBACTERIAL ACTIVITY[a,b]

	Compounds			
Microorganisms	**6**	**7**	**8**	**9**
Bacillus cereus ATCC 10702	250	125		250[c]
B. subtilis ATCC 6633	125	125		250[c]
B. thuringiensis		250		
Micrococcus	250[c]	62		
Staphylococcus aureus ATCC 6538		250		
Streptococcus agalactiae		125		
Streptococcus faecalis ATCC 8043		250		
Aerobacter aerogenes	250[c]	125		
Alcaligenes faecalis ATCC 8750	125	125	250[c]	250[c]
Bordetella bronchiseptica	250	62	250[c]	250[c]
Escherichia coli 2	250[c]	125		
Pasteurella multocida RC 315	125	16	250	250
Salmonella choleraesuis—var. *kunzendorf*	250[c]	125		
S. dublin	250[c]	125		
S. gallinarum 605	250	125		
S. typhimurium	250[c]	125	250[c]	250[c]
S. typhosa ATCC 6539	250	125	250[c]	250[c]

[a] Agar dilution tests, minimum inhibitory concn, μg/ml. [b] Where no value is given the compound was inactive at highest test level, 250 μg/ml. [c] Slight activity at this concn.

TABLE II

In Vitro ANTIFUNGAL ACTIVITY[a,b]

	Compounds			
Organisms	**6**	**7**	**8**	**9**
Candida albicans—Bergen Strain, E-3	250			125
C. myoderma—ATCC 9888	250			62
Saccharomyces cerevisiae—ATCC 4100				62
Mucor ramannianus—M-143				62
Fusarium episphaeria—F-105	250			31
Hormodendrum cladosporoides—Z-516				62
Trichophyton mentagrophytes—E-11	62	250	250	15
Microsporum gypseum—E-28	31		250	31
Penicillium digitatum—P-308B	250			62
Memnoniella echinata—Z-583	250			125
Chaetomium globosum—H-71, QM 6694	31		125	125
Aspergillus fumigatus—S-246				125

[a,b] See corresponding footnotes in Table I.

Experimental Section[3]

2-Methyl-5-nitro-1,3,4-thiadiazole (1).—A solution of 2.78 g (0.024 mole) of 2-amino-5-methylthiadiazole in 17.5 ml of 48–50% HBF_4 was stirred at 0° and 1.67 g (0.024 mole) of $NaNO_2$ was added over a period of 30 min. After 20 min of additional stirring, the mixture was added dropwise to a vigorously stirred suspension of 4.9 g of Cu powder and 24.7 g of $NaNO_2$ in 50 ml of H_2O at 25°. The mixture foamed and became dark green. After an additional 30 min of stirring, the mixture was filtered, the filter cake was washed well with H_2O, and the combined filtrate and wash solution was extracted (C_6H_6, 3 × 150 ml). The combined extracts were dried ($MgSO_4$) and evaporated to dryness *in vacuo* to afford 1.75 g of yellow syrup which crystallized on standing. This material melted at 54–55° and exhibited a strong 1700-cm^{-1} band which could be removed by recrystallization from 50% aq Me_2CO: mp 65–66° for the analytical sample. *Anal.* ($C_3H_3N_3O_2S$) C, H, N, S.

2-Acetamido-5-(*p*-nitrostyryl)-1,3,4-thiadiazole (3).—A mixture of 157 g (1 mole) of 2-acetamido-5-methylthiadiazole and 151 g (1 mole) of *p*-nitrobenzaldehyde in 1500 ml of hot Ac_2O was heated at reflux temperature for 17 hr and cooled and the yellow product collected. The product was washed throughly with Me_2CO and dried *in vacuo* to afford 198.7 g (69%), mp >310°, nmr (DMSO-d_6, 100°, integration unattainable owing to noise level): τ 7.73 (s, CH_3CO), 2.30 (broad m, aryl H), 1.75 and 1.90 (db, J = 19 Hz, $-HC{=}CH-$). The analytical sample was recrystallized from DMF–Me_2CO. *Anal.* ($C_{12}H_{10}N_4O_3S$) C, H, N, S.

2-Amino-1,3,4-thiadiazole-2-carboxaldehyde (5).—A suspension of 50 g (0.17 mole) of **3** in 500 ml of 99% aq MeOH at 0° was stirred and O_3 (0.081 mole/hr in O_2, generated from a Welsbach Corp. ozonator) was introduced through a capillary tube for 3 hr. The reaction mixture was purged with N_2 for 30 min and the mixture was reduced with 100 g of NaI in 500 ml of H_2O and 100 ml of HOAc below 25°. After 35 min of additional stirring, the I_2 was titrated with saturated $Na_2S_2O_3$ solution. The mixture was extracted twice with EtOAc (2 l. and 1 l.), the extracts were dried ($MgSO_4$), and the EtOAc removed *in vacuo*. The residue was heated on a steam bath for 90 min with 100 ml of HOAc and 100 ml of concentrated HCl and the mixture was evaporated *in vacuo* to give a dark sludge. To this was added 400 ml of 10% HCl and 1 l. of EtOAc. The aq layer was removed after shaking and further extracted with 300 ml of EtOAc to remove the last traces of *p*-nitrobenzaldehyde. The aq layer was neutralized with solid $NaHCO_3$ and it was extracted with EtOAc (5 × 600 ml). These extracts were dried ($MgSO_4$) and evaporated to dryness *in vacuo* to yield 14.4 g (65%) of aminoaldehyde **5**, mp 155–157°. A sample recrystallized from Me_2CO–hexane melted at 166–168° dec. *Anal.* ($C_3H_3N_3OS$) H, N, S; C: calcd, 27.91; found, 30.01.

A sample of 2-acetamido-1,3,4-thiadiazole-5-carboxaldehyde (**4**) was obtained in the following manner: the reduced mixture from the ozonolysis was evaporated to dryness, the *p*-nitrobenzaldehyde was removed by washing with Et_2O and the inorganic solids were washed away with H_2O to leave about a 3% yield of **4**. An analytical sample, mp 231° dec, was obtained from MeOH recrystallizations. *Anal.* ($C_5H_5N_3SO_2$) H, N, S; C: calcd, 35.09; found, 35.74.

The nmr spectrum of another sample of recrystallized **4** (F_3CCO_2H) showed bands at τ −0.2 (s, 1 H, CHO), 7.38 (s, 3 H, CH_3CO), 6.92 (s, CH_3) and 7.42 (s, CH_3CO). The latter two peaks could be intensified with added 2-acetamido-5-methyl-1,3,4-thiadiazole and the presence of the latter compound was confirmed by the mass spectral analysis which gave *m/e* 171 and 157 as two parent peaks.

5-Nitro-1,3,4-thiadiazole-2-carboxaldehyde (2).—A solution of 4.0 g (0.031 mole) of 2-amino-1,3,4-thiadiazole-2-carboxaldehyde (**5**) in 8 ml of 48–50% HBF_4 and 20 ml of H_2O was added slowly (*ca.* 75 min) to a vigorously stirred mixture of 2 g of Cu powder and 8.0 g of $NaNO_2$ in 40 ml of H_2O at 25°. During the addition bright yellow solids were deposited in the reaction mixture. After stirring an additional 2 hr, the mixture was filtered and the filtrate extracted with $CHCl_3$ (2 × 150 ml). The aq layer was then acidified to pH 2 and extracted with Et_2O (4 × 200 ml). The combined extract was dried ($MgSO_4$) and evaporated to dryness *in vacuo* to afford 1.8 g of brown syrup, which was used unpurified in subsequent reactions with derivatizing reagents. The ir spectrum of the syrup exhibited bands at 3400 (m), 1700 (w), and 1165–1195 (broad) cm^{-1}, which suggested the aldehyde readily formed a hemihydrate.

The aldehyde **2** was also obtained by mixing 1.45 g (10 mmoles) of 2-methyl-5-nitrothiadiazole with 1.10 g (5 mmoles) of pulverized SeO_2 and heating on a hot plate. At *ca.* 110° an exothermic reaction was observed and the temperature rose to 170°. This mixture was cooled and extracted with 30 ml of CH_2Cl_2. The extract was filtered and evaporated to dryness to give a yellow–orange

(3) Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Ir spectra were taken on a Perkin-Elmer Model 137 spectrophotometer; nmr spectra were taken on a Varian A-60 instrument (Me$_4$Si). Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Where analyses are indicated only by symbols of the elements, analytical results obtained for the elements were within ±0.4% of the theoretical values.

liquid, which was further dissolved in Et_2O and filtered to remove an insoluble material. Removal of Et_2O from the filtrate afforded 0.65 g of liquid [ν max (neat) 1700 (broad), 1565, and 1355 cm^{-1}].

5-Nitro-2-thiadiazole Derivatives.—Standard techniques or methods[2] were used for the preparation of the compounds described below and the yields are based on the amount of **5** used.

3-{[(5-Nitro-1,3,4-thiadiazol-2-yl)methylene]amino}-2-oxazolidinone (**6**) was obtained in 6–15% yield and recrystallized from Me_2CO–EtOH as yellow crystals, mp 250–255°. *Anal.* ($C_6H_5N_5O_4S$) C, H, N, S.

1-{[(5-Nitro-1,3,4-thiadiazol-2-yl)methylene]amino}-2-imidazolidinone (**7**) was obtained in 13–23% yield and recrystallized from 50% aq EtOH as yellow crystals, mp 230–233°; nmr (DMSO-d_6): τ 2.1 (s, 1 H, CH=N), 2.3 (s, 1 H, NH), 5.8–6.7 (m, 4 H, CH_2CH_2). *Anal.* ($C_6H_6N_6O_3S$) C, H, N, S.

5-Nitro-1,3,4-thiadiazole-2-carboxaldehyde thiosemicarbazone (**8**) was obtained in 22% yield as a red solid; no suitable solvent for recrystallization was found, mp > 290°. *Anal.* ($C_4H_4N_6O_2S_2$) C, N, S, H: calcd 1.73; found 2.70.

2-Amino-5-(5-nitro-1,3,4-thiadiazol-2-yl)-1,3,4-thiadiazole (**9**). —The method reported previously[2] was used; 46% yield from **8**, recrystallized from EtOH–DMF, yellow crystals, mp 240° dec. *Anal.* ($C_4H_2N_6O_2S_2$) C, H, N, S.

Acknowledgment.—We wish to thank Dr. T. L. Chang (Stamford Laboratories, American Cyanamid Co.) for the mass spectral data and interpretation, Dr. G. A. Kemp and staff for *in vitro* and *in vivo* antibacterial assays, Mr. A. C. Dornbush and staff (Lederle Laboratories) for the *in vitro* antifungal assays, and Mr. G. S. Redin and staff (Lederle Laboratories) for their *in vivo* antibacterial assays.

New Compounds

Some Indole Derivatives[1]

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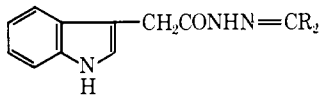
In connection with other work in progress in this laboratory it was necessary to prepare the compounds described in Tables I and II for screening purposes,

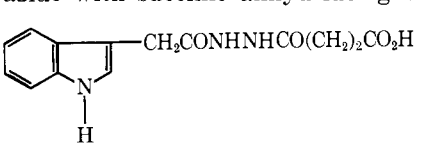
TABLE I



R_2CO	Mp, °C[a]	Yield, %	Formula	Analyses[b,c]
Androstanolone benzoate	161–162	90	$C_{36}H_{43}N_3O_3$	H, N[d]
Estrone 3-methyl ether	225–227	10[e]	$C_{29}H_{33}N_3O_2$	H, N[f]
Pregnenolone	252–254[g]	66[h]	$C_{31}H_{41}N_3O_2$	C, H
Testosterone benzoate	165–167	67[i]	$C_{36}H_{41}N_3O_3$	N
2-Nitrobenzaldehyde	174–175[j]	85	$C_{17}H_{14}N_4O_3$	C, H, N
4-Chloro-2-nitrobenzaldehyde	203–204	83	$C_{17}H_{13}ClN_4O_3$	C, H
Indole-3-carboxaldehyde	228–230[k]	77	$C_{19}H_{16}N_4O$	C, H, N

[a] Recrystallized from EtOH unless otherwise noted. [b] Analyses indicated within 0.3%. [c] All compounds exhibited expected spectra. [d] Calcd: C, 76.43. Found: C, 75.72. [e] Yield (93%) based on recovered steroid. [f] Calcd: C, 76.45. Found: C, 75.70. [g] Not recrystallized. [h] Yield (94%) based on recovered steroid. [i] Reaction time increased to 5 hr. [j] Reaction product is $C_{17}H_{14}N_4O_3 \cdot C_2H_5OH$, (mp 111–112°, analyses: C, H); the product was heated to 130° to give the product in the Table. [k] A. Alemany, M. Bernabe, C. Elorriaga, E. F. Alvarez, M. Lora-Tamayo, and O. Nieto [*Bull. Soc. Chim. Fr.*, 2486 (1966)] report mp 193°.

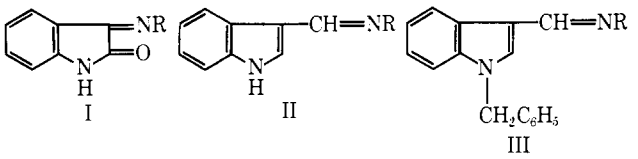
by condensing indole-3-acetic acid hydrazide with carbonyl compounds and by condensing isatin, indole-3-carboxaldehyde, and 1-benzylindole-3-carboxaldehyde with various amines. Reaction of indole-3-acetic acid hydrazide with succinic anhydride[2] gave I while



I

reaction of 3-aminocarbazole with 4-[bis(2-chloroethyl)-amino]-*o*-tolualdehyde gave the expected imine.[3]

TABLE II



I II III

Type	RNH_2	Mp, °C[a]	Yield, %	Formula	Analyses[b,c]
I	Sulfanilamide[l]	276–278	83	$C_{14}H_{11}N_3O_3S$	C, H
I	Sulfisoxazole[l]	237–239[d]	60	$C_{19}H_{16}N_4O_4S$	C, H
I	Sulfathiazole[l]	335–336	93	$C_{17}H_{12}N_4O_3S_2$	C, H
I	Sulfaguanidine[l]	296–297	44	$C_{15}H_{13}N_5O_3S$	H, N[e]
I	*p*-Sulfobenzoic acid *p*-hydrazide[l]	254–256	74	$C_{15}H_{11}N_3O_5S$	C, H
I	*p*-Toluenesulfon-*p*-hydrazide	207–209[f]	90	$C_{15}H_{13}N_3O_3S$	C, H
I	3-(2-Aminoethyl)-indole	174–176	66	$C_{18}H_{15}N_3O$	H[g]
II	*p*-Sulfobenzoic acid hydrazide	240–241	88	$C_{16}H_{13}N_3O_4S$	C, H
II	2-Nitrophenyl-hydrazine	224–226[d]	93	$C_{15}H_{12}NO_2$	H[h]
II	D-Cycloserine	223–224[j]	56	$C_{12}H_{11}N_3O_2$	H, N[i]
III	Sulfathiazole	192–193[k]	51	$C_{25}H_{20}N_4O_3S_2$	C, H
III	Sulfanilamide	156–158	53	$C_{22}H_{19}N_3O_2S$	C, H

[a] Recrystallized from EtOH unless otherwise noted. [b] Analyses indicated within 0.3%. [c] All compounds exhibited expected spectra. [d] Not recrystallized. [e] Calcd: C, 52.47. Found: C, 51.99. [f] M. P. Cava, R. O. Little, and D. R. Napier [*J. Amer. Chem. Soc.*, **80**, 2257 (1958)] report mp 190–200°. [g] Calcd: C, 74.72. Found: C, 74.11. [h] Calcd: C, 64.28. Found: C, 63.78. [i] Calcd: C, 62.87. Found: C, 62.18. [j] Triturated with hot EtOH–EtOAc. [k] From EtOAc. [l] Inactive (T/C = 83 – 102%) at 400 mg/kg against L-1210 lymphoid leukemia.

(1) This work was supported by a research grant (CA 10345) from the National Cancer Institute, U. S. Public Health Service.

(2) F. W. Short and L. M. Long, *J. Heterocycl. Chem.*, **6**, 707 (1969).

(3) F. D. Popp, *J. Med. Chem.*, **7**, 210 (1964).