of E_m at pH 7. Although this potential is greater than the potential of normal biological substrates, correction for the solvent system may, in fact, make the electrode potentials E_1, E_2, and E_m smaller by as much as 200–300 mV, resulting in a potential within the range of the isolated cytochromes, and considerably below the electrode potential of oxygen–water (0.810 V) at pH 7. One might speculate that the electron-transport particle is the site of action of these anthelmintics. Moreover, such a correction would result only in a bulk shift of the data as well as the curves as they appear in Figures 4 and 6, and would not affect any conclusions derived from these figures.

It should be emphasized that in the foregoing discussion the site and mechanism of action of the phenothiazine anthelmintics are hypothetical, and little is therefore known about the possible effects of other factors such as distributive and metabolic parameters. However, the observed correlation appears interesting and significant enough to encourage further investigation of systems in which semiquinone free radicals are suspected to be the biologically active species.

α,α,α-Trifluorotoluamides as Anticoccidial Agents

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The preparation and anticoccidial activity of a number of α,α,α-trifluorotoluamides and related compounds are reported. Several active compounds were obtained, but the most active were the amide, dimethylamide, ethylamide, and diethylamide in which the trifluoromethyl and a nitro group are in a 3,5 relationship. One other amide with 2-chloro-5-trifluoromethyl showed similar activity.

The use of nitrated and halogenated benzamides (**1–3**) as feed additives for the control of poultry coccidiosis has been known for several years.[1–4] In these compounds the nitro group is known to be essential for significant anticoccidial activity.[5] Certain aminobenzoic acids and related compounds are also known to have anticoccidial activity.[6] These compounds are believed to act as *p*-aminobenzoic acid (PABA) antagonists because simultaneous administration of PABA is reported to reduce their efficacy. Also, it has long been recognized that certain coccidia are sensitive to known PABA antagonists such as the sulfonamides and 4,4′-diaminophenyl sulfones.[7–11] In contrast there is no direct evidence that compounds such as **1–3** act as PABA antagonists.

During the past 20 years a substantial effort has been devoted to the replacement of hydrogen, nitro, halogen, or methyl by fluorine or trifluoromethyl in prototype molecules which are known to have chemotherapeutic activity.[12–14] This work has led to some compounds with interesting and often more powerful and varied biological activity.

As part of a continuing search for new and improved anticoccidial agents and prompted by previous work on organofluorine drugs, we became interested in trifluoromethylbenzamides similar to **1–3**. The object of the study was to determine if replacement of a nitro group by a trifluoromethyl group would give a compound with anticoccidial activity, and, if so, what structural requirements were necessary for this activity.

Chemistry.—The compounds initially prepared for testing are listed in Table I. Most of the amides were prepared from the acid chloride using commercially available α,α,α-trifluoro-*m*-toluic acid (**50**) as a starting point. However, several attempts to prepare the N-aminoethyl- and N-hydroxyethylamides by this route always gave the disubstituted derivatives **22** and **23**. Amides **31**, **34**, and **35** were obtained from hydrolysis of the appropriate nitriles.

The amino derivative **26** and the *o*-hydroxyamide (**27**) were prepared from the esters **46** and **48** and concentrated NH_4OH under pressure. A cursory attempt to prepare **26** from the *o*-amino ester **45** was not successful. The preparation of **24** was best accomplished by catalytic reduction of **7** rather than ammonolysis of the ester **49**. The other amides were prepared by the acid chloride–NH_3 route.

During the course of this investigation it was of interest to determine if a change in the amide portion of the molecule would give compounds with anticoccidial activity. Consequently the thioamide **53**, sulfonamide **55**, nitriles **51** and **56**, and amidine derivatives **52** and **54** were prepared as described in the Experimental Section.

(1) N. F. Morehouse and W. C. McGuire, *Poultry Sci.*, **36**, 1143 (1957); **37**, 1228 (1958); **38**, 410 (1959).
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(3) R. R. Baron, M. W. Moeller, and N. F. Morehouse, *ibid.*, **45**, 411 (1966).
(4) S. J. Ball and E. W. Parnell, *Nature*, **199**, 612 (1963).
(5) Salsbury Laboratories, unpublished results.
(6) E. F. Rogers, R. L. Clark, H. J. Becker, A. A. Pessolano, W. J. Leanza, E. C. McManus, F. J. Andriuli, and A. C. Cukler, *Proc. Soc. Exp. Biol. Med.*, **117**, 488 (1964).
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(10) E. H. Peterson, *ibid.*, **9**, 77 (1948).
(11) L. C. Grumbles, J. P. Delaplane, and T. C. Higgins, *Poultry Sci.*, **27**, 605 (1948).
(12) For a review of the trifluoromethyl group in medicinal chemistry and references to its inductive and hyperconjugative comparison to other groups, see H. L. Yale, *J. Med. Pharm. Chem.*, **1**, 121 (1959).
(13) A. Burger, "Medicinal Chemistry," Interscience Publishers, Inc., New York, N. Y., 1960, p 82.
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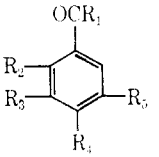
Table I

α,α,α-Trifluorotoluamides and Related Compounds



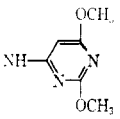
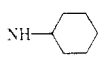
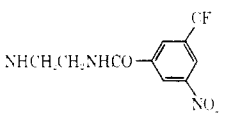
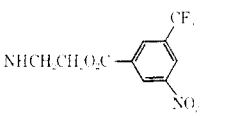
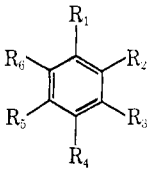
Compd	R_1	R_2	R_3	R_4	R_5	Mp, °C[a] (recrystn solvent)	% yield	Formula[b]	Min effect. dose, % in feed
4	NH_2	H	H	H	CF_3	121–123[n]	68		0.05
5	$NHNH_2$	H	H	H	CF_3	111–112 (*e*)	97	$C_8H_7F_3N_2O$	
6	NH_2	H	CF_3	H	CF_3	162–163 (*d*)	85	$C_9H_5F_6NO$[c]	
7	NH_2	H	NO_2	H	CF_3	139–140 (*d*)	94	$C_8H_5F_3N_2O_3$[c]	0.005
8	$NH(CH_3)$	H	NO_2	H	CF_3	107–108 (*d*)	95	$C_9H_7F_3N_2O_3$	0.0125
9	$N(CH_3)_2$	H	NO_2	H	CF_3	55–57 (*d*)	81	$C_{10}H_9F_3N_2O_3$	0.00625
10	$NH(CH_2CH_3)$	H	NO_2	H	CF_3	98–101 (*d*)	89	$C_{10}H_9F_3N_2O_3$	0.00625
11	$N(CH_2CH_3)_2$	H	NO_2	H	CF_3	140–145 (*m*)	89	$C_{12}H_{13}F_3N_2O_3$	0.00625
12	$NHNH_2$	H	NO_2	H	CF_3	115–117 (*e*)	71	$C_8H_6F_3N_3O_3$[c]	0.05
13	$NHCH_2CH{=}CH_2$	H	NO_2	H	CF_3	68–70 (*d*)	80	$C_{11}H_9F_3N_2O_3$	0.025
14	$NHCH_2CH_2Cl$	H	NO_2	H	CF_3	87–90 (*d*)	82	$C_{10}H_8ClF_3N_2O_3$	0.0125
15	$NHCH_2CH_2OCH_3$	H	NO_2	H	CF_3	76–78 (*d*)	58	$C_{11}H_{11}F_3N_2O_4$[c]	0.0125
16		H	NO_2	H	CF_3	167–169 (*d*)	38	$C_{14}H_{11}F_3N_4O_5$	
17	$NHCH_2CH_2N(CH_3)_2$	H	NO_2	H	CF_3	79–80 (*f*)	40	$C_{12}H_{14}F_3N_3O_3$	
18	$NHCH_2CH_2C_6H_5$	H	NO_2	H	CF_3	82–84 (*d*)	78	$C_{16}H_{13}F_3N_2O_3$	
19		H	NO_2	H	CF_3	129–130 (*d*)	49	$C_{12}H_{11}F_3N_2O_4$	
20		H	NO_2	H	CF_3	66–68 (*d*)	52	$C_{13}H_{13}F_3N_2O_3$[c]	0.05
21		H	NO_2	H	CF_3	151–153 (*g*)	73	$C_{14}H_{15}F_3N_2O_3$	0.05
22		H	NO_2	H	CF_3	255–257 (*l*)	83	$C_{18}H_{12}F_6N_4O_6$	
23		H	NO_2	H	CF_3	188–189 (*d*)	25	$C_{18}H_{11}F_6N_3O_7$	
24	NH_2	H	NH_2	H	CF_3	116–117.5 (*h*)	74	$C_8H_7F_3N_2O$	
25	NH_2	Cl	NO_2	H	CF_3	195–197 (*d*)	85	$C_8H_4ClF_3N_2O_3$	
26	NH_2	NH_2	NO_2	H	CF_3	227–228 (*d*)	47	$C_8H_6F_3N_3O_3$	0.05
27	NH_2	OH	NO_2	H	CF_3	210–211 (*d*)	65	$C_8H_5F_3N_2O_4$	
28	NH_2	OCH_3	NO_2	H	CF_3	160–161 (*d*)	91	$C_9H_7F_3N_2O_4$	
29	$NHNH_2$	OH	NO_2	H	CF_3	219–220 dec (yellow) (*i*)	80	$C_8H_6F_3N_3O_4$	
30	NH_2	Cl	H	H	CF_3	145–146 (*i*)	98	$C_8H_5F_3NO$	0.00625
31	NH_2	CF_3	H	H	H	160–162[n]	86		0.0125
32	NH_2	H	H	CF_3	H	182–183[o]	79		0.025
33	NH_2	CF_3	H	NO_2	H	190–192 (*i*)	84	$C_9H_5F_3N_2O_3$	0.0125
34	NH_2	NO_2	H	CF_3	H	167–169 (*i*)	34	$C_8H_5F_3N_2O_3$	0.0125
35	NH_2	H	H	NO_2	CF_3	136–138 (*j*)	76	$C_8H_5F_3N_2O_3$	0.025
36	OH	H	NO_2	H	CF_3	129–130[14]	89		
37	OH	H	CF_3	H	CF_3	135–138[15]	58		0.05
38	OH	H	NH_2	H	CF_3	138–140[16]	89		
39	OH	Cl	H	H	CF_3	92–94 (*k*)	70–99		
40	OH	Cl	NO_2	H	CF_3	175–177 (*j*)	83	$C_8H_3ClF_3NO_4$	
41	OH	NH_2	NO_2	H	CF_3	228–230 (*d*) (yellow)	88	$C_8H_5F_3N_2O_4$[c]	
42	OH	OCH_3	NO_2	H	CF_3	140–141 (*d*)	98	$C_9H_6F_3NO_5$	
43	OH	OH	NO_2	H	CF_3	168–170 (*k*)	72	$C_8H_4F_3NO_5$	
44	OCH_3	H	NO_2	H	CF_3	42–43 (*d*)	98	$C_9H_6F_3NO_4$[c]	
45	OCH_3	NH_2	NO_2	H	CF_3	86–87 (*d*)	59	$C_9H_7F_3N_2O_4$	
46	OCH_3	Cl	NO_2	H	CF_3	51–53 (*d*)	92	$C_9H_5ClF_3NO_4$	
47	OCH_3	OCH_3	NO_2	H	CF_3	45–46 (*d*)	100	$C_{10}H_8F_3NO_5$	

TABLE I (*Continued*)

Compd	R1	R2	R3	R4	R5	Mp, °C[a] (recrystn solvent)	% yield	Formula[b]	Min effect. dose, % in feed
48	OCH_3	OH	NO_2	H	CF_3	87–89 (*d*)	56	$C_9H_6F_3NO_5$	
49	OCH_3	H	NH_2	H	CF_3	77–79 (*d*)	83	$C_9H_8F_3NO_2$	

[a] Melting points are uncorrected and were taken in open capillaries using a Thomas–Hoover apparatus. [b] All compounds except those for which no formula is listed were analyzed for C, H, N using an F & M Model 185 analyzer; analytical results obtained for those elements were with ±0.4% of the theoretical values. [c] Also analyzed for F by Schwarzkopf Microanalytical Laboratories, Woodside, N. Y.; results obtained were within ±0.4% of the theoretical values. [d] H_2O–EtOH. [e] C_6H_6. [f] Hexane. [g] MeCN. [h] $CHCl_3$. [i] MeOH. [j] H_2O. [k] Hexane–C_6H_6. [l] EtOH. [m] Boiling point at 15 mm; purified by glpc on a 183 × 0.48 cm stainless column packed with 10% Qf-1 on 30–60 mesh Chromosorb W. [n] L. M. Yagupol'skii and N. I. Man'ko, *Zh. Obshch. Khim.*, **23**, 988 (1953), reported mp 161°. [o] P. Buu-Hoi, N. D. Xuong, and N. V. Bac, *Compt. Rend.*, **257**, 3182 (1963), reported mp 123°. [p] J. Lichtenberger and F. Weiss, *Bull. Soc. Chim. France*, 915 (1962), reported mp 180–181°.



	R1	R2	R3	R4	R5	R6
1	$CONH_2$	H	NO_2	H	NO_2	H
2	$CONH_2$	Cl	H	NO_2	H	H
3	$CONH_2$	CH_3	NO_2	H	NO_2	H
50	CO_2H	H	CF_3	H	H	H
51	CN	H	CF_3	H	NO_2	H
52	C(NH)OEt	H	CF_3	H	NO_2	H
53	$CSNH_2$	H	CF_3	H	NO_2	H
54	$C(NH)NH_2$	H	CF_3	H	NO_2	H
55	SO_2NH_2	H	CF_3	H	NO_2	H
56	CN	CF_3	H	NO_2	H	NO_2
57	CO_2H	H	CO_2H	H	H	Cl
58	CO_2H	OH	H	H	CF_3	H
59	CN	Cl	H	H	CF_3	H
60	CO_2H	H	CO_2H	H	NO_2	OH

Some of the carboxylic acids (**36–38**) shown in Table I were synthesized by reported procedures.[15–17] The *ortho*-substituted acids (**39–43**) were prepared as outlined in the Experimental Section. Several attempts to convert 6-chloro-α,α,α-trifluoro-*m*-tolunitrile (**59**) to 6-hydroxy-α,α,α-trifluoro-*m*-toluic acid (**58**) with 10 or 33% NaOH gave the 6-chloro acid **39**. Treatment of **59** with hot 75% H_2SO_4 gave 6-chloroisophthalic acid (**57**) (94% yield), but refluxing with 60–63% acid gave **39** in 50–70% yield. Treatment of **40** with 5, 10, or 20% NaOH at reflux did not give the salicylic derivative (**43**), but gave 6-hydroxy-5-nitroisophthalic acid (**60**) in high yield.

Biological Results.—From the biological data in Table I, it is apparent that optimum anticoccidial activity is obtained when R_3 and R_5 are trifluoromethyl or nitro and R_1 is amino, dimethylamino, ethylamino, or diethylamino (**7**, **9–11**). One other compound (**30**) showed similar activity.

Removal of the nitro group at R_3 resulted in lowering of activity (**4**) and its reduction to amino (**24**) gave no activity.[18]

Only one acid (**37**) displayed activity and none of the esters were active. It is also of interest that although the thioamide **53** and the sulfonamide **55** displayed a minimum effective dose of 0.00625%, the nitriles **51** and **56** and amidine derivatives **52** and **54** showed no activity.

Experimental Section[19]

α,α,α-Trifluoro-*m*-toluic Acid Methyl Ester and Hydrazide (5).—The methyl ester of α,α,α-trifluoro-*m*-tolic acid (**50**)[20] was prepared in 86% yield by a normal Fischer procedure to give a colorless liquid, bp 44° (1.0 mm), lit.[21] bp 207° (757 mm). The ester was refluxed for 7 hr in EtOH with a twofold excess of hydrazine hydrate[22] to give the hydrazide **5**.

5-Nitro-α,α,α-trifluoro-*m*-toluic Acid (36), the Methyl Ester (44), and Hydrazide (12).—Nitration of **50** according to the method of Hauptschein[15] gave **36** in 85–90% yield from several runs; lit.[14] mp 128–129°. The methyl ester (**44**) was prepared by refluxing **36** in anhydrous MeOH with H_2SO_4 catalyst for 48 hr. It was converted to the hydrazide **12** by refluxing for 4 hr in EtOH with excess hydrazine hydrate.

Preparation of Amides 4, 6–11, 13–18, 19–21, 22, 23, 25, 28, 30, 32, and 33.—The acid chloride of **36** or other appropriate acid was prepared by heating at reflux for 3–4 hr in excess $SOCl_2$.[23] The $SOCl_2$ was then removed under vacuum and the residue was added slowly to chilled concentrated NH_4OH or a mixture of the appropriate amine and $NaHCO_3$ in H_2O. The suspension was then heated at 35–50° for 0.5 hr, cooled, and filtered or, in the case of the liquid product **11**, extracted with $CHCl_3$ or CH_2Cl_2.

Preparation of Amides 31, 34, and 35.—The nitrile[24] (0.05 mol) was dissolved in 25 ml of EtOH and 2 ml of 6 *N* NaOH. H_2O_2 (30%, 20 ml) was then added dropwise at 35–45°. The mixture was then heated at 50–55° for 3 hr. H_2O (30 ml) and $CHCl_3$ (5 ml) were then added and the mixture was filtered to give the amide as a residue.

5-Nitro-α,α,α-trifluoro-*m*-tolunitrile (51).—A mixture of **7** (60.0 g, 0.256 mol) and 90.0 g of P_2O_5 was carefully heated at reflux for 10 min with a bunsen flame. The mixture was then distilled at 0.2 mm to give 45.0 g (82%) of distillate which solidified to a white, crystalline solid, mp 78–81°. *Anal.* ($C_8H_3F_3N_2O_3$) C, H, N.

5-Nitro-α,α,α-trifluoro-*m*-toluamidic Acid Ethyl Ester Hydrochloride (52).—A solution of 66.0 g (0.306 mol) of **51**, 18.2 g (0.396 mol) of EtOH, and 400 ml of dry Et_2O was cooled to 0° and saturated with dry HCl. The mixture was left to stand overnight and then filtered. The residue was washed with Et_2O and dried to give 80.0 g (87.5%) of white solid, mp 120–121°. *Anal.* ($C_{10}H_{16}ClF_3N_2O_3$) C, H, N.

(15) M. Hauptschein, E. A. Nodiff, and A. J. Saggiomo, *J. Amer. Chem. Soc.*, **76**, 1051 (1954).

(16) J. Lichtenberger and F. Weiss, *Bull. Soc. Chim. France*, 587 (1962).

(17) M. Hauptschein, U. S. Patent 3,052,603 (1962).

(18) From the results of previous testing, it would appear that the lowest effective level of 3,5-dinitrobenzamide is 0.0125% and that *m*-nitrobenzamide is not effective.

(19) Ir spectra of all compounds listed here and in Table I were consistent with the structure and were determined in KBr or $CHCl_3$ with a Beckman IR 4 spectrophotometer. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

(20) Pierce Chemical Co., Rockford, Ill.

(21) S. DeBrouwer, *Bull. Soc. Chim. Belges*, **39**, 298 (1930).

(22) Olin Mathieson.

(23) Matheson Coleman & Bell.

(24) α,α,α-Trifluoro-*o*-tolunitrile was purchased from Pierce Chemical Co.; 2-nitro-α,α,α-trifluoro-*p*-tolunitrile,[15] and 4-nitro-α,α,α-trifluoro-*m*-tolunitrile [W. T. Caldwell and A. N. Sayin, *J. Amer. Chem. Soc.*, **73**, 5125 (1951)] were prepared by the reported procedures.

5-Nitro-α,α,α-trifluoro-*m*-toluamidine Hydrochloride Hydrate (54).—A slurry of 32 g (0.107 mol) of **52** in 320 ml of EtOH was cooled to 5° and saturated with anhydrous NH_3. The mixture was stirred overnight during which time it slowly became homogeneous and turned light yellow. The solution was then treated with Norit and filtered. Evaporation of the filtrate to dryness in air gave 25.0 g (81.5%) of a white solid, mp 80–85° dec. *Anal.* ($C_8H_9ClF_3N_3O_3$) C, H, N.

5-Nitro-α,α,α-trifluoro-*m*-thiotoluamide (53).—A suspension of 75.0 g (0.32 mol) of **7** in 400 ml of xylene was heated to near reflux and treated with 35.5 g (0.16 mol) of P_2S_5 in small portions. The mixture was then heated at reflux for 2.5 hr and filtered hot. The filtrate was chilled and filtered to give 57 g (71%) of pale yellow solid, mp 131–132.5°. *Anal.* ($C_8H_5F_3N_2O_2S$) C, H, N, F, S.

5-Nitro-α,α,α-trifluoro-*m*-toluenesulfonamide (55).—A mixture of 58.0 g (0.304 mol) of *m*-nitrobenzotrifluoride[20] and 98 ml of freshly distilled $ClSO_3H$[25] was heated at reflux for 8 hr. The volatiles were then distilled under vacuum, and the dark syrupy residue was slowly poured into 300 ml of chilled concentrated NH_4OH with stirring. The suspension was filtered and the residue was recrystallized (H_2O–EtOH) to give 13 g (16%) of tan solid, mp 140–142°, lit.[26] mp 140.5–141°.

4,6-Dinitro-α,α,α-trifluoro-*o*-tolunitrile (56).—A solution of 59.0 g (0.235 mol) of 4,6-dinitro-α,α,α-trifluoro-*o*-toluidine[27] in 450 ml of AcOH was added dropwise at 10–20° to a solution of 19.3 g (0.28 mol) of $NaNO_2$ in 125 ml of concentrated H_2SO_4. The mixture was stirred for a few minutes and then added slowly with vigorous stirring to a chilled KCN–$Ni(CN)_2$ solution previously prepared by adding 98.3 g (1.51 mol) of KCN in 590 ml of H_2O to a solution of 82.0 g (0.31 mol) of $NiSO_4 \cdot 6H_2O$ and 413 g of Na_2CO_3 in 730 ml of H_2O. The temperature was allowed to rise to 30–35° during the addition. The mixture was then heated to 90° for 0.5 hr, cooled, and extracted with Et_2O. The Et_2O was washed (H_2O) and dried ($MgSO_4$). Filtration and vacuum distillation of the filtrate gave **56** as a yellow oil, 17.4 g (27%), bp 130–150° (1.5 mm), which solidified on standing, mp 92–94°. *Anal.* ($C_8H_2F_3N_3O_4$) C, H, N.

6-Chloro-α,α,α-trifluoro-*m*-toluic Acid (39).—A mixture of 100 g (0.487 mol) of 6-chloro-α,α,α-trifluoro-*m*-tolunitrile[28] (**59**) and 600 ml of 33% NaOH was heated at reflux for 4 hr. The mixture was then cooled and filtered. The residue and filtrate were washed with Et_2O, recombined, and acidified to give a white solid (97.0 g, 89%). Recrystallization (C_6H_6–hexane) gave mp 91–93°, lit.[28] mp 91–94°.

Hydrolysis of **59** with 60–63% H_2SO_4 at reflux gave **39** in 50–70% yield.

6-Chloroisophthalic Acid (57).—Compound **59** (30.0 g, 0.146 mol) and 150 ml of 75% H_2SO_4 was heated at 180–190° for 1 hr. The solid which precipitated after cooling was recovered by filtration, washed (H_2O), and dried to give 27.5 g (94%) of white solid, mp 293–295°, lit.[29] mp 294.5°.

6-Chloro-5-nitro-α,α,α-trifluoro-*m*-toluic Acid (40) and the Methyl Ester (46).—To 308 ml of fuming H_2SO_4 below 25° was added 102.5 g (0.50 mol) of **59.** The solution was then treated dropwise below 70° with 98 ml of fuming HNO_3. The mixture started to foam during the latter part of the addition. The temperature was then slowly raised to 95° where the mixture exothermed rapidly to *ca.* 160°. After the exotherm had subsided the mixture was heated at 120–130° for 45 min. The solution was then cooled and the resulting thick paste was quenched on crushed ice. The white solid suspension was stirred for 0.5 hr, removed by filtration, and washed (cold H_2O). Drying gave mp 172–173°. Recrystallization (H_2O) gave 112 g (83%) of **40** as white needles, mp 175–177°.

The methyl ester (**46**) was prepared as a white solid by the Fischer method (8 hr of reflux in MeOH–H_2SO_4).

6-Amino-5-nitro-α,α,α-trifluoro-*m*-toluic Acid (41), the Ester (45), and Amide (26).—A mixture of 15 g (55.8 mmol) of **40** and 150 ml of concentrated NH_4OH was heated at 90–100° in a Parr pressure apparatus for 2 hr. The mixture was then cooled, transferred to an evaporating dish, and left to stand overnight. The resulting crystalline suspension was dissolved in warm H_2O, treated with Norit, and filtered. Acidification of the chilled filtrate with concentrated HCl gave 12.2 g (88%) of **41** as a yellow precipitate.

The ester (**45**) was prepared from **41** by the Fischer method using MeOH–dry HCl and a 3-hr reflux.

The amide (**26**) was best prepared from **46** and excess concentrated NH_4OH in a Parr pressure apparatus at 90–100° for 2 hr. The mixture was then cooled and filtered, and the residue was washed with H_2O to give **26** as a yellow solid. Acidification of the filtrate gave **41** as a yellow precipitate which was recovered and identified by mixture melting point and comparison of infrared spectra with previously identified material. A preliminary attempt to prepare **26** from the amino ester (**45**) and concentrated NH_4OH in a pressure bottle at 80–100° for 2 hr was not successful. Only **45** was recovered.

6-Methoxy-5-nitro-α,α,α-trifluoro-*m*-toluic Acid (42) and the Methyl Ester (47).—To a mixture of 26.9 g (0.10 mol) of **40** and 100 ml of anhydrous MeOH was added 16.2 g (0.30 mol) of NaOMe.[23] After the exotherm had subsided, the mixture was heated at reflux for 7 hr. The MeOH was then removed under vacuum, and the residue was dissolved in 100 ml of H_2O, treated with Norit, and filtered. Acidification of the filtrate with concentrated HCl gave 26 g (98%) of a light tan solid.

The methyl ester (**47**) was prepared by the Fischer procedure (H_2SO_4–MeOH, 24-hr reflux).

6-Hydroxy-5-nitro-α,α,α-trifluoro-*m*-toluic Acid (43), the Ester (48), Amide (27), and Hydrazide (29).—A mixture of 10.0 g (37.8 mmol) of **42** and 125 ml of 48% HBr was heated at 120–140° for 4 hr and then cooled. The solid was removed by filtration, washed (H_2O), dried, and recrystallized to give 6.8 g of **43** (72%) as white plates.

The methyl ester (**48**) was prepared from **43** by the Fischer procedure (MeOH–H_2SO_4, 24-hr reflux).

The amide (**27**) was prepared from **48** by stirring in concentrated NH_4OH for 24 hr in a sealed flask. The solution was then chilled, acidified with 20% HCl, and **27** was recovered by filtration.

The hydrazide (**29**) was prepared from **48** by refluxing for 4 hr with a sixfold excess of hydrazine hydrate in MeOH. The MeOH was then removed under vacuum and the dark, syrupy residue was dissolved in warm AcOH and poured into ice–H_2O. The yellow solid (**29**) was recovered by filtration, washed (H_2O), and dried.

6-Hydroxy-5-nitroisophthalic Acid (60).—A solution of 45.5 g (0.169 mol) of **40** and 175 ml of NaOH (5–20%) was heated at reflux for 2 hr. The resulting red suspension was then cooled and quenched in 200 ml of concentrated HCl–200 ml of crushed ice. The solid was recovered by filtration and recrystallized (H_2O) to give 37.0 g (96%) as a white solid, mp 232–234°. *Anal.* ($C_8H_4F_3NO_5$) C, H, N.

The dimethyl ester of **60** was prepared in 93% yield using MeOH–H_2SO_4 and a 6-hr reflux to give a white solid, mp 101–102°. *Anal.* ($C_{10}H_9N_7$) C, H, N.

5-Amino-α,α,α-trifluoro-*m*-toluic Acid Methyl Ester (49) and Amide (24).—The acid **38** was prepared by the method of Hauptschein[15] and esterified in 83% yield by heating at reflux for 4 hr with MeOH–H_2SO_4. The MeOH was then removed under vacuum and the residue was quenched with ice–H_2O. Neutralization with $NaHCO_3$, filtration, and drying the residue gave **49** as a white solid.

Several attempts to convert **49** to the amide (**24**) with concentrated NH_4OH met with limited success, and it was subsequently found that the most convenient route to **24** was by catalytic reduction of the nitroamide **7**. In a typical experiment 19.5 g (83 mmol) of **7**, 0.35 g of 10% Pd–C, and 100 ml of 95% EtOH was stirred at room temperature for 1.5 hr under 3.5 kg/cm² of H_2 in a Parr pressure apparatus. The mixture was then filtered and the filtrate was concentrated to an oil under vacuum. Petroleum ether (bp 30–60°) was added and then removed under vacuum during which the oil crystallized. The solid was slurried in 10% HCl and filtered. Neutralization of the filtrate at 5° with $NaHCO_3$ gave **24** as a white precipitate which was recovered by filtration, washed with cold H_2O and dried.

Biological Methods.—Chicks used in the coccidiosis efficacy trials were either broiler-type heavy-breed or hybrid Leghorn-type birds raised in batteries during the growing period using special precautions to ensure freedom from coccidiosis infection.

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At 3–5 weeks of age, the chicks were transferred to individual cages with hardware cloth floors where the efficacy experiments were conducted.

The *Eimeria tenella* cultures used in these experiments were serially propagated in our laboratory over a period of several years. These cultures were isolated by single oocyst inoculation of coccidiosis-free birds to ensure the purity of the cultures. Infection was accomplished by depositing a predetermined volume of calibrated oocyst suspension directly into the crop of each chick.

The compounds tested in these trials were incorporated into a standard ration and fed to the birds for 2 days prior to infection, and continued for the duration of the test.

The anticoccidial efficacy in these experiments was based on three factors: (1) mortality, (2) weight gain or loss, and (3) droppings scores. The primary criterion of efficacy was the mortality produced in the medicated–infected chicks as compared to the nonmedicated–infected chicks. Droppings scores and ratios of mean weight gains, medicated–infected *vs.* nonmedicated–noninfected, were used as indicators of morbidity.[3]

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Chemotherapeutic Nitroheterocycles. Derivatives of 5-Nitrothiazole-2-carboxaldehyde and 5-Nitrothiazole-2-carboxylic Acid[1]

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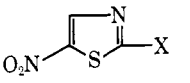
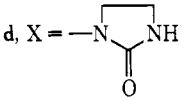
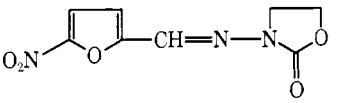
A series of new 5-nitrothiazoles bearing carbon substituents in the 2 position has been prepared. Treatment of 2-bromo-5-nitrothiazole with CuCN provided 5-nitrothiazole-2-carbonitrile, a key intermediate for subsequent conversion to other derivatives of 5-nitrothiazole-2-carboxylic acid. The corresponding aldehyde was obtained by condensing 2-methyl-5-nitrothiazole with benzaldehyde and oxidatively cleaving the resulting styryl intermediate. The compounds prepared in this study were evaluated *in vivo* for antimalarial and antischistosomal activity and *in vitro* for activity against bacteria, yeast, and a fungus. Little activity was noted in the malaria and schistosomiasis tests, but broad-spectrum inhibitory effects were widely evident in the *in vitro* assays. The most potent compound, 5-nitrothiazole-2-carboxaldehyde acethydrazone, was inhibitory at 1 μg/ml or less in all but one of the latter tests.

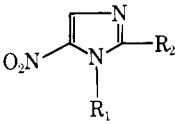
Among the several classes of nitroheterocyclic drugs possessing useful properties in clinical or veterinary medicine,[2] the 5-nitrothiazoles are of special recent interest. In addition to the well-established use of 2-amino-5-nitrothiazole, and simple derivatives thereof (**1a–c**), for the treatment of histomoniasis in turkeys,[3] another closely related nitrothiazole, niridazole (**1d**), has been found highly effective in human schistosomiasis[4–6] and amebiasis.[4,5,7] Favorable preliminary results against two other parasitic diseases, dracunculosis[8–10] and strongyloidiasis,[8,11] have also been reported for this drug. In a recent paper in which Avramoff, *et al.*,[12] revealed a group of bis-5-nitrothiazoles with marked *in vitro* antiprotozoal activity,



1a, X = NH_2
b, X = $NHCOCH_3$
c, X = NHCONHEt
d, X = 



2



3a, $R_1 = CH_2CH_2OH$; $R_2 = CH_3$
b, $R_1 = R_2 = CH_3$
c, $R_1 = CH_3$; $R_2 = p\text{-}C_6H_4F$

they provided a brief survey of current developments in the nitrothiazole field.

A characteristic feature of essentially all reported chemotherapeutic nitrothiazoles is the presence of a free or substituted amino group in the 2 position. In contrast, the antiprotozoal nitrofurans[13,14] (*e.g.*, furazol-

(1) This work was supported by the U. S. Army Medical Research and Development Command under Contract No. DA-49-193-MD-2750. This is Contribution Number 420 from the Army Research Program on Malaria.

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