vein, different sets of features will be tried. A completely different set of keys[18] designed for retrieval at the Walter Reed Army Institute of Research has the property that all the conditional probabilities are available from the generation of these keys in the form of a hierarchy according to information-theoretic principles.[19] It is also possible to generate features for the purpose of good biological discrimination, using the active and inactive training sets as guides.

The use of other feature systems must wait upon the full implementation of the new sets of features. Of course, to get a completely adequate set of features, we would need to include stereochemistry. One way to achieve three-dimensional features would be by autocorrelation on electron density maps. However, this is a fairly complex approach, which may be difficult to apply on a large scale.

The study of the feasibility of using this statistical-heuristic method for selecting drugs for testing in the P388 model is now under way and will be reported soon. Preliminary results show excellent separation of actives from inactives by means of the activity score alone. In the P388 data, it seems that including the inactivity score produces a small, perhaps marginal, improvement, and eliminating redundancy as by the elimination of keys produces a bigger, but still marginal, improvement. If further work on the P388 model bears out the early results, then it is possible that very sophisticated structure features may not be necessary, or merely marginal.

References and Notes

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(9) The weights cited appear in the summary of ref 7 in G. Redl, R. D. Cramer III, and C. E. Berkoff, *Chem. Soc. Rev.*, 4, 273 (1974). The probabilities, however, are not calculated from the entire file but from the tested compounds. Their formula has the general effect of giving each feature a weight proportional to its incidence, for the same relative activity. The weights used in ref 7 are indeed different and are simply the number active divided by the number tested for each feature. This can give low incidence features an exaggerated effect. Our method falls between these two extremes. Statistical considerations yield weights that vary with the square root of the incidence, for the same relative activity.
(10) Among those structure features getting negative weight should be those which do not appear at all in the training set. If they are among the large number of low incidence keys then their expected number of occurrences would be very close to zero, anyway, so their weight, through negative, would be extremely small. We have, however, ignored all keys which did not occur in the training set. In a future version of the program weights will be computed for keys which do not occur if they have some significant expected number of occurrences, say 0.5.
(11) A reviewer has pointed out that, from Figure 1, it would be just as well to use the number of standard deviations as a weight, instead of the two-tiered system. This turns out to be true, even in a test on the P388 data. One can simply use the number of standard deviations as a weight and consider additivity as an heuristic. However, the theoretical rationale lies with the log $1/P$ weight. The heuristic of the number of standard deviations was forced on the principle author because keys 20–40 SD's from their expected value would get extremely large weights. When characteristics are repeated in several keys, those with large weights (in terms of standard deviations) increase their effect on the score toward the log $1/P$ direction.
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(13) R. I. Geran et al., *Cancer Chemother. Rep., Part 2*, 4, 53 (1974).
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Anticoccidial Derivatives of 6-Azauracil. 1. Enhancement of Activity by Benzylation of Nitrogen-1. Observations on the Design of Nucleotide Analogues in Chemotherapy

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Benzylation of 6-azauracil at N-1 (which corresponds to the point of attachment of the ribose phosphate unit in pyrimidine nucleotides) has been found to augment its anticoccidial activity fourfold. The high potency of 1-benzyl-6-azauracil is ascribed to a combination of intrinsic activity, efficient oral absorption, and a moderate rate of excretion. Metabolism experiments using 1-benzyl-6-azauracil labeled with ^{14}C in the heterocycle and (separately) in the side chain showed that, in the drug accounted for, no cleavage had occurred. Additional activity increases were achieved by introducing small, electron-withdrawing substituents in the meta and/or para position(s) of the benzyl group. One of the most active derivatives, 1-(3-cyanobenzyl)-6-azauracil, is about 16 times as potent as 6-azauracil.

The exigencies of the world food supply have led to ever more intensive agriculture and animal husbandry. Large-scale enclosed poultry rearing has been made possible during the last 25 years by the discovery of feed-incorporated anticoccidials to control the most troublesome social disease of fowl. Coccidiosis is caused

by several species of *Eimeria*, an obligately parasitic protozoon. The intracellular location and complex life cycle of the parasite make the chemotherapeutic problem a difficult one, resembling somewhat those of malaria and viral diseases. Coccidia have shown a remarkable ability to develop resistance to chemotherapeutic agents, so that there is a continuing demand for new and effective drugs.

The synthesis of 6-azauracil was reported in 1947,[1] but another decade was required for general recognition of its antimetabolite character and its medicinal potential.[2-9] In one or another of its forms it has been used experimentally or clinically in the chemotherapy of cancer,[10-12] the inhibition of viruses,[13-15] as a fungicide,[16] and as an oral chemotherapeutic agent for psoriasis,[17] for polyarthritis,[18] and for polycythemia vera.[19] In our laboratories it was found to have a low degree of anticoccidial[20] and plant growth retardant[21] activity. In many biological systems, it has been shown to be converted to 6-azauridine[22-29] and to 6-azauridylic acid.[24,25,29] In tumor-bearing mice 6-azauracil ribonucleoside, administered subcutaneously, was six times more active as a chemotherapeutic agent than 6-azauracil itself by the same route.[30] While 6-azauridylic acid inhibits the enzyme orotidylate decarboxylase (OMP decarboxylase), 6-azauracil does not,[25] and it is believed that many of the biological effects of 6-azauracil are due to its conversion in vivo to 6-azauridylic acid.[31]

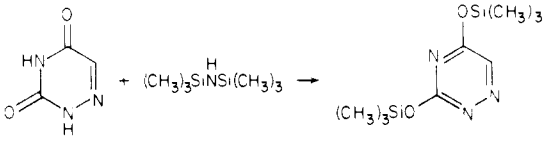
As OMP decarboxylase accomplishes a key step in the biosynthesis of uridylic acid and so of nucleic acids, it occurs widely in living organisms, and in a number of these it has been shown to be inhibited by 6-aza-UMP. The *Eimeria* genus of protozoa consists of intracellular obligate parasites, and their nutrition is not well understood. The discovery that 6-azauracil inhibited this genus in the natural host indicated the possibility that coccidia also contain the enzyme. Evolutionary differentiation of the apoprotein structure between parasite and host enzymes, together with distributive factors, seemed to offer the possibility that proper substitution of 6-azauracil would improve its selectivity for parasite over host, its pharmacokinetics, and its general performance as a chemotherapeutic agent for *Eimeria* species infections. This and several ensuing papers describe the elaboration of the 6-azauracil structure in a manner which has resulted in potent new drug candidates for the prevention or cure of coccidiosis.

Rationale for Drug Design. Early substitutions of 6-azauracil were primarily at the 3-N atom or at the 5-C atom (Table I). Such substitutions in our experience did not increase anticoccidial activity. Furthermore (in Table II), **110**, **112**, **115**, and **118** with benzyl groups at the 3-N or 5-C positions were inactive when tested at 250 ppm. The 5-bromo derivatives **108** and **109** present an inexplicable inconsistency. While **109** has the same minimum effective concentration (MEC) as the unbrominated **42**, **108** retains only about 10% of the activity of the unbrominated **39**. Yet, **117** is slightly more active than unbrominated **116**. Possibly the 5-brominated 6-azauracils are acting by different or by mixed mechanisms. They could, e.g., have some inhibitory effect on thymidylate synthetase. Many other 5-substituted 6-azauracils prepared for another purpose but tested at 250 ppm in our screen were inactive.[32]

In general, alkyl substitutions at 5-C or elsewhere on the 6-azauracil ring reduced potency. Thus, **106** is less active than **28** and **107** less than **39**. Table I shows a number of aliphatic substitutions, all inactive at 250 ppm, although **17**, **18**, and **23** were slightly more active than 6-azauracil itself. It is noteworthy that while 5-halogenated uracils and 5-fluoroorotic acid effectively interfere with the biosynthesis of the pyrimidine nucleic acids in certain systems, the corresponding 5-alkyl derivatives are ineffective.[33]

The 1-N atom of 6-azauracil corresponds to the 1-N atom of uracil, which is the site of ribose attachment in the nucleoside. Thus, the 1-N position in 6-azauracil seemed to be the best location in which to place a substituent in order to mimic the size and shape of the natural nucleoside. In some heterocycles structurally related to the nucleic acid purines and pyrimidines, binding to certain enzymes has been improved by N-aralkylation.[34-44] Baker and Sachdev[34] placed carboxyl groups at the meta or para position of a benzyl ring linked to the 9 position of adenine, the rationale being that such a side chain would approximate not only the size but also the polarity of a ribose phosphate moiety. These same side chains attached to the 1-N atom of 6-azauracil yielded compounds inactive either as plant growth retardants or as anticoccidials. An *unsubstituted* benzyl group at this position, however, caused a fourfold enhancement of anticoccidial activity and aroused the hope that a suitably substituted benzyl side chain might not only potentiate 6-azauracil but that it would also confer upon it the other qualities of a useful drug.

Chemistry. Convenient to our design a recent method for the 1-N-benzylation of 6-azauracil has been published.[45] Improved yields were obtained by silylation of the 2,4-oxo functions of 6-azauracil prior to benzylation as has been done in the synthesis of nucleoside analogues.[46]



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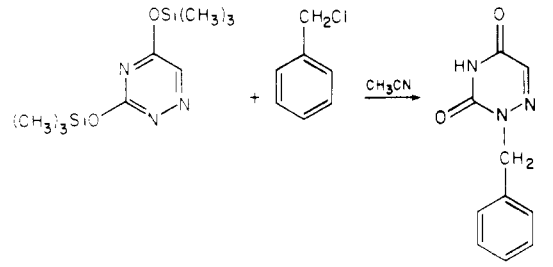

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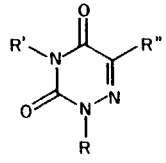
Structure-Activity Relationships and Biological Results. The 1-benzyl-6-azauracils are listed in Table II, and their minimum effective concentrations (MEC) compared with that of unsubstituted 6-azauracil. These MEC's (parts per million by weight in feed) were determined by a modification of the screening method published by Lynch[47] (as modified by Chappel et al.),[48] using *Eimeria tenella* infections in Leghorn cockerels.

Compact electronegative substituents on the benzyl side chain, especially halogens or the cyano group alone or in combinations, produced the most active derivatives.[49] Attempted correlation of the relative significance of electronic and hydrophobic characteristics by the Hansch linear regression method was inconclusive. Small ortho substituents such as fluoro were tolerated while large ones reduced potency. The meta position was favored somewhat over the para.

The most active derivative of the series was 1-(3-cyanobenzyl)-6-azauracil **39**, with an MEC of about 60 ppm. It is compared in Table III with the principal control drug, amprolium, and with several other experimental or

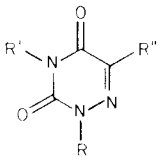
Table I. Early Substituted 6-Azauracils and Their Anticoccidial Activities



No.	R	R'	R''	Mol formula[a]	Mp, °C	Crystn solvent	Yield, %[b]	Preparative method[c]	MEC, ppm[d]
1	CH_3	H	H	$C_4H_5N_3O_2$	154-156	H_2O	52	Known[e]	>250
2	H	C_2H_5	H	$C_5H_7N_3O_2$	157	H_2O	47	Known[e]	>250
3	CH_3	CH_3	Br	$C_5H_6BrN_3O_2$	100-101	EtOH-H_2O	75	Known[f]	>250
4	C_2H_5	C_2H_5	H	$C_7H_{11}N_3O_2$	81-84	Distilled	15	Known[e]	>250
5	$(Me)_2C{=}CHCH_2$-	CH_3	H	$C_9H_{13}N_3O_2$	42-43	Distilled	58	III	>250
6	H	CH_3	Br	$C_4H_4BrN_3O_2$	183-185	H_2O	85	Known[f]	>250
7	$(Me)_2C{=}CHCH_2$-	$(Me)_2C{=}CHCH_2$-	Br	$C_{13}H_{18}BrN_3O_2$	56-57	EtOH-H_2O	14	II	>250
8	H	$(Me)_2C{=}CHCH_2$-	Br	$C_8H_{10}BrN_3O_2$	125-126	EtOH-H_2O	35	II	>250
9	H	$MeCH_2CH_2CH_2$-	H	$C_7H_{11}N_3O_2$	135-136	EtOH-H_2O	31	II	>250
10	H	$(Me)_2C{=}CHCH_2$-	H	$C_8H_{11}N_3O_2$	109-110	EtOH-H_2O	34	II	>250
11	$NC(CH_2)_4$-	H	H	$C_8H_{10}N_4O_2$	91-92	Benzene	14	Ia	>250
12	$HOOC(CH_2)_4$-	H	H	$C_8H_{11}N_3O_4$	125-127	EtOAc	37	Special[g]	>250
13	H	$NCCH_2$-	H	$C_5H_4N_4O_2$	182-184	2-Propanol	25	II	>250
14	$MeSCH_2CH_2$-	H	H	$C_6H_9N_3O_2S$	78-80	H_2O	21	Ib	>250
15	$MeSO_2CH_2CH_2$-	H	H	$C_6H_9N_3O_4S$	180-181	2-Propanol	80	IVb	>250
16	$MeSOCH_2CH_2$-	H	H	$C_6H_9N_3O_3S$	176-178	2-Propanol	80	IVa	>250
17	H_2NCOCH_2-	H	H	$C_5H_6N_4O_3$	241-242	Chromatographed	7	Id	500
18	$(Me)_2NCOCH_2$-	H	H	$C_7H_{10}N_4O_3$	193-194	Chromatographed	10	Ib	750
19	$C_6H_5SCH_2CH_2$-	H	H	$C_{11}H_{11}N_3O_2S$	121-123	Toluene	30	Ic/Ib	>250
20	$C_6H_5SO_2CH_2CH_2$-	H	H	$C_{11}H_{11}N_3O_4S$	180-181	2-Propanol	72	IVb	>250
21	$MeCH_2SCH_2CH_2$-	H	H	$C_7H_{11}N_3O_2S$	56-57	Isopropyl ether	9	Ib	>250
22	$MeCH_2SO_2CH_2CH_2$-	H	H	$C_7H_{11}N_3O_4S$	128-129	2-Propanol	5	IVb	>250
23	$(i\text{-}Pr)_2NCH_2$-	H	H	$C_{11}H_{20}N_4O_2$	125-126.5	MeOH-H_2O	9	Ic	500
24	H	H	CH_3S-	$C_4H_5N_3O_2S$	220-223	H_2O	40	*h*	>1000
25	H	H	CH_3CH_2S-	$C_5H_7N_3O_2S$	257-260	2-Propanol	42	*h*	>1000
26	H	H	$CH_3(CH_2)_2S$-	$C_6H_9N_3O_2S$	225-228	2-Propanol	62	Special[g]	>1000

[a] All compounds have analyses for C, H, and N within ±0.4% of calculated values except as noted. [b] Yields were not optimized. [c] The general preparative methods are described in the Experimental Section. [d] Minimum effective concentration, parts per million by weight in feed. [e] See ref 47. [f] See ref 48. [g] See Experimental Section. [h] See ref 26.

Table II. 2-Benzyl-*as*-triazine-3,5(2*H*,4*H*)-diones (1-Benzyl-6-azauracils), Related Structures, and Their Coccidiostat Activities



No.	R	R'	R''	Mol formula[a]	Mp, °C	Crystn solvent	Yield, %[b]	Preparative method[c]	MEC, ppm
27	H-	H	H	$C_3H_3N_3O_2$				Known[d]	1000
28	$C_6H_5CH_2$-	H	H	$C_{10}H_9N_3O_2$		EtOH-H_2O		Known[e]	250
29	$(C_6H_5)_2CH$-	H	H	$C_{16}H_{13}N_3O_2$	169-170.5	MeOH	9	Ic	>250
30	2-(NO_2)-$C_6H_4CH_2$-	H	H	$C_{10}H_8N_4O_4$	224-226	MeCN	28	Id	500
31	2-Me-$C_6H_4CH_2$-	H	H	$C_{11}H_{11}N_3O_2$	171-172	MeOH	4	Ia	250
32	2-CN-$C_6H_4CH_2$-	H	H	$C_{11}H_8N_4O_2$	208-210	MeCN	31	Id	500
33	2-Cl-$C_6H_4CH_2$-	H	H	$C_{10}H_8ClN_3O_2$	172-173	EtOH-H_2O	10	Ic	250
34	3-MeO-$C_6H_4CH_2$-	H	H	$C_{11}H_{11}N_3O_3$	171-172	MeOH	40	Id	>500
35	3-F-$C_6H_4CH_2$-	H	H	$C_{10}H_8FN_3O_2$	195-196	MeCN	23	Id	60
36	3-Cl-$C_6H_4CH_2$-	H	H	$C_{10}H_8ClN_3O_2$	179-181	MeOH	16	Id	60
37	3-CF_3-$C_6H_4CH_2$-	H	H	$C_{11}H_8F_3N_3O_2$[f]	158-160	EtOH	19	Id	125
38	3-(MeOOC)-$C_6H_4CH_2$-	H	H	$C_{12}H_{11}N_3O_4$	206-208	MeOH	50	Id	>250
39	3-CN-$C_6H_4CH_2$-	H	H	$C_{11}H_8N_4O_2$	179-181	MeOH	30	Id	60
40	3-I-$C_6H_4CH_2$-	H	H	$C_{10}H_8IN_3O_2$	206-208	MeOH	42	Id	250
41	3-(EtOOC)-$C_6H_4CH_2$-	H	H	$C_{13}H_{13}N_3O_4$	162-164	EtOH	18	Ic	60
42	3-NO_2-$C_6H_4CH_2$-	H	H	$C_{10}H_8N_4O_4$[g]	175.5-176	MeCN	21	Id	500
43	3-(HOOC)-$C_6H_5CH_2$-	H	H	$C_{11}H_9N_3O_4$	270-272	DMF-H_2O	16	Ia	>250
44	3-(CF_3CH_2OOC)-$C_6H_4CH_2$-	H	H	$C_{13}H_{10}F_3N_3O_4$	143-145	Sublimed	10	Ic	>500
45	3-Cl-C_6H_4-NHCO-3-$C_6H_4CH_2$-	H	H	$C_{17}H_{13}ClN_4O_3$	256-258	DMF-H_2O	64	Id	>500
46	4-F-$C_6H_4CH_2$-	H	H	$C_{10}H_8FN_3O_2$	199-200	MeOH	15	Id	60
47	4-($MeSO_2$)-$C_6H_4CH_2$-	H	H	$C_{11}H_{11}N_3O_4S$	233-234	DMF-H_2O	17	Id	250
48	4-CF_3-$C_6H_4CH_2$-	H	H	$C_{11}H_8F_3N_3O_2$	202-203	MeOH	33	Id	250
49	4-(Me_2NSO_2)-$C_6H_4CH_2$-	H	H	$C_{12}H_{14}N_4O_4S$	215-216	MeOH	20	Id	>250
50	4-CN-$C_6H_4CH_2$-	H	H	$C_{11}H_8N_4O_2$	208-209	EtOH-H_2O	18	Ic	250
51	4-NO_2-$C_6H_4CH_2$-	H	H	$C_{10}H_8N_4O_4$	209-212	MeCN	10	Ic	250
52	4-MeO-$C_6H_4CH_2$-	H	H	$C_{11}H_{11}N_3O_3$	168-169.5	MeCN	7	Ic	500
53	$C_6H_5COC_6H_4CH_2$-	H	H	$C_{17}H_{13}N_3O_3$	242-243	MeCN	41	Id	>1000
54	4-(H_2NSO_2)-$C_6H_4CH_2$-	H	H	$C_{10}H_{10}N_4O_4S$[h]	220-223	MeOH	10	Ic	>100
55	2,3-Cl_2-$C_6H_3CH_2$-	H	H	$C_{10}H_7Cl_2N_3O_2$	227-228	Acetone	40	Id	1000
56	2-Cl-3-(MeO)-$C_6H_3CH_2$-	H	H	$C_{11}H_{10}ClN_3O_3$	162-163	EtOH-H_2O	10	Ia	>500
57	2,3-$(MeO)_2$-$C_6H_3CH_2$-	H	H	$C_{12}H_{13}N_4O_3$	137-138	MeOH-H_2O	2	Ia	125
58	2-Cl-4-NO_2-$C_6H_3CH_2$-	H	H	$C_{10}H_7ClN_4O_4$	199-200	MeOH	15	Id	250
59	2-F-4-Cl-$C_6H_3CH_2$-	H	H	$C_{10}H_7FClN_3O_2$[i]	215-216	MeOH	18	Id	125
60	2,4-Cl_2-$C_6H_3CH_2$-	H	H	$C_{10}H_7Cl_2N_3O_2$	208-209	MeCN	5	Ia	125
61	2-F-5-Cl-$C_6H_3CH_2$-	H	H	$C_{10}H_7ClFN_3O_2$	203-204	EtOH	14	Id	60
62	2-F-5-NO_2-$C_6H_3CH_2$-	H	H	$C_{10}H_7FN_4O_4$[j]	217-218	MeCN	12	Id	>500
63	2,5-Cl_2-$C_6H_3CH_2$-	H	H	$C_{10}H_7Cl_2N_3O_2$	215-216.5	Acetone	32	Id	125
64	2,6-Cl_2-$C_6H_3CH_2$-	H	H	$C_{10}H_7Cl_2N_3O_2$	218-219	MeOH	13	Ic	>500
65	3,4-$(CF_3)_2$-$C_6H_3CH_2$-	H	H	$C_{12}H_7F_6N_3O_2$	130-131.5	Sublimed	32	Id	500
66	4-Cl-3-F-$C_6H_3CH_2$-	H	H	$C_{10}H_7ClFN_3O_2$	238-239	MeOH	35	Id	60
67	3-Br-4-CN-$C_6H_3CH_2$-	H	H	$C_{11}H_7BrN_4O_2$[k]	225-226	MeCN	36	Id	125
68	3-Cl-4-CN-$C_6H_3CH_2$-	H	H	$C_{11}H_7ClN_4O_2$	205-206.5	MeOH	44	Id	60
69	3,4-(COOCO)-$C_6H_3CH_2$-	H	H	$C_{12}H_7N_3O_5$	228-230	MeCN	27	Id	>500
70	3-F-4-NO_2-$C_6H_3CH_2$-	H	H	$C_{10}H_7FN_4O_4$	220-221	MeOH	15	Id	>500

71	4-F-3-NO_2-$C_6H_3CH_2$-	H	H	$C_{10}H_7FN_4O_4$[l]	198-200	MeOH	6	Ia	>125
72	3,4-$(NO_2)_2$-$C_6H_3CH_2$-	H	H	$C_{10}H_7N_5O_6$	201-203	MeCN	8	Ia	>500
73	4-Cl-3-MeO-$C_6H_3CH_2$-	H	H	$C_{11}H_{10}ClN_3O_3 \cdot H_2O$[m]	191-193	MeOH	12	Id	250
74	3,4-(OCH_2O)-$C_6H_3CH_2$-	H	H	$C_{11}H_9N_3O_4$	182-183	MeCN	16	Id	250
75	3,4-F_2-$C_6H_3CH_2$-	H	H	$C_{10}H_7F_2N_3O_3$	207-208	MeOH	13	Id	30
76	3,4-Me_2-$C_6H_3CH_2$-	H	H	$C_{12}H_{13}N_3O_2$	166-167	Sublimed	5	Ia	>500
77	3,4-Cl_2-$C_6H_3CH_2$-	H	H	$C_{10}H_7Cl_2N_3O_2$[n]	178-178.5	MeCN	22	Id	60
78	4-Cl-3-NO_2-$C_6H_3CH_2$-	H	H	$C_{10}H_7ClN_4O_4$	183-185	MeOH	10	Id	250
79	3,5-Cl_2-$C_6H_3CH_2$-	H	H	$C_{10}H_7Cl_2N_3O_2$	184.5-185.5	EtOH	32	Id	60
80	3,5-$(CN)_2$-$C_6H_3CH_2$-	H	H	$C_{12}H_7N_3O_2$[o]	281-284	MeOH	11	Ic	>60
81	3,5-$(CF_3)_2$-$C_6H_3CH_2$-	H	H	$C_{12}H_7F_6N_3O_2$[p]	144-147	Chromatographed	54	Id	500
82	2-Cl-4,5-(CH_2OCH_2)-$C_6H_2CH_2$-	H	H	$C_{11}H_8ClN_3O_4$	218-220	MeCN	24	Id	>500
83	2,3,6-Cl_3-$C_6H_2CH_2$-	H	H	$C_{10}H_6Cl_3N_3O_2$	226-228	MeOH	6	Ia	>250
84	2,3,6-Cl_3-5-NO_2-C_6HCH_2-	H	H	$C_{10}H_5Cl_3N_4O_4$	198-199	EtOH	48	Id	>500
85	$C_6F_5CH_2$-	H	H	$C_{10}H_4F_5N_3O_2$	159-160	MeOH	25	Id	60
86	$C_6Cl_5CH_2$-	H	H	$C_{10}H_4Cl_5N_3O_2$[q]	291-291.5	MeCN	16	Ic	>500
87	4-Br-$C_6H_4OCH_2CH_2$-	H	H	$C_{11}H_{10}BrN_3O_3$	149-150	MeOH	8	Ic	500
88	$C_6H_5OCH_2CH_2$-	H	H	$C_{11}H_{11}N_3O_3$	177-178.5	MeOH	4	Ic	>250
89	$C_6H_5CH{=}CHCH_2$-	H	H	$C_{12}H_{11}N_3O_4$	185-186	MeOH	24	Id	>500
90	C_6H_4-2,3-(CONCO)-$CH_2CH_2CH_2$-	H	H	$C_{14}H_{12}N_4O_4$[r]	286-290	MeOH	43	Id	>250
91	$C_6H_5CH_2CH_2$-	H	H	$C_{11}H_{11}N_3O_2$	199-200.5	MeOH	18	Id	1000
92	$C_6H_5COCH_2$-	H	H	$C_{11}H_9N_3O_3$[s]	300 dec	MeCN	52	Id	>1000
93	4-Cl-$C_6H_4COCH_2$-	H	H	$C_{11}H_8ClN_3O_3$	263-265	DMF-H_2O	46	Id	1000
94	4-F-$C_6H_4COCH_2$-	H	H	$C_{11}H_8FN_3O_3$	229-230	EtOH	9	Ia	500
95	3-$CF_3C_6H_4COCH_2$-	H	H	$C_{12}H_8F_3N_3O_3$	222-224	MeOH	31	Id	500
96	$C_6H_5SCH_2CH_2$-	H	H	$C_{11}H_{11}N_3O_2S$	121-123	Toluene	30	Id	>250
97	2-Naphthyl-CH_2-	H	H	$C_{14}H_{11}N_3O_2$	221-223	MeOH	14	Ia	>500
98	1-Naphthyl-CH_2-	H	H	$C_{14}H_{11}N_3O_2$	228-229.5	MeOH	6	Ia	1000
99	c-C_6H_{11}-CH_2-	H	H	$C_{10}H_{15}N_3O_2$	145-146	MeOH-H_2O	37	Id	1000
100	1-(4-Me-naphthyl)-CH_2-	H	H	$C_{15}H_{13}N_3O_2$	216-217	EtOH	2	Ic	125
101	2-SPh-CH_2-	H	H	$C_9H_7N_3O_2S$	163-165	Sublimed	10	Ia	>1000
102	2-Pyridyl-CH_2-	H	H	$C_9H_8N_4O_2$	225-230	Sublimed	5	Ic	>500
103	2-Quinolyl-CH_2-	H	H	$C_{13}H_{10}N_4O_2$	221-223	EtOH	10	Ic	>100
104	3-SPh-CH_2-	H	H	$C_8H_7N_3O_2S$	179-180	MeOH	24	Id	1000
105	4-(5-Me-3-Ph)isoxazolyl-CH_2-	H	H	$C_{14}H_{12}N_4O_3$	178-181	MeOH	53	Id	1000
106	$C_6H_5CH_2$-	H	CH_3	$C_{11}H_{11}N_3O_2$[t]	148-149	MeOH	40	Id	>250
107	3-CN-$C_6H_4CH_2$-	H	CH_3	$C_{12}H_{10}N_4O_2$[t]	194-196	MeOH	36	Id	>500
108	3-CN-$C_6H_4CH_2$-	H	Br	$C_{11}H_7BrN_4O_2$	205-206	MeOH	21	Id	500
109	3-NO_2-$C_6H_4CH_2$-	H	Br	$C_{10}H_7BrN_4O_4$	210-212	MeOH	37	Id	500
110	H	3-CF_3-$C_6H_4CH_2$	H	$C_{11}H_8F_3N_3O_2$[u]	134-136	Chromatographed	6	Ia	>250
111	$C_6H_5CH_2$-	CH_3	H	$C_{11}H_{11}N_3O_2$	77-78	EtOH-H_2O	55	Id	1000
112	2-Cl-$C_6H_4CH_2$-	2-Cl-$C_6H_4CH_2$	H	$C_{17}H_{13}Cl_2N_3O_2$	96-97	EtOH-H_2O	19	Id	>250
113	H	$C_6H_5CH_2$	H	$C_{10}H_9N_3O_2$	121-123	EtOH-H_2O	45	Id	>250
114	H	2-Cl-$C_6H_4CH_2$	H	$C_{10}H_8ClN_3O_2$	108-110	EtOH-H_2O	22	Id	≥1000
115	H	3,4-Cl_2-$C_6H_3CH_2$	H	$C_{10}H_7Cl_2N_3O_2$	172-174	EtOH-H_2O	12	Ic	>250
116	$C_6H_5CH_2$-	$C_6H_5CH_2$	H	$C_{17}H_{15}N_3O_2$	96-98	EtOH-H_2O	11	Known[e]	500
117	$C_6H_5CH_2$-	$C_6H_5CH_2$	Br	$C_{17}H_{14}BrN_3O_2$	128-129	EtOH-H_2O	19	Ic	250
118	H	3,4-Cl_2-$C_6H_3CH_2$	3,4-Cl_2-$C_6H_3CH_2$	$C_{17}H_{11}Cl_4N_3O_2$	127-128	EtOH-H_2O	20	Id	>250
119	H	H	Br	$C_{10}H_8BrN_3O_2$	147-148.5	EtOH-H_2O	28	Id	>250

[a] All compounds have analyses for C, H, and N within ±0.4% of calculated values except as noted. [b] Yields were not optimized. [c] The general preparative methods are described in the Experimental Section. [d] Known compound. See ref 1. [e] Known compound. See ref 43. [f] N: calcd, 15.49; found, 14.98. [g] C: calcd, 48.93; found, 48.28. [h] N: calcd, 19.84; found, 21.18. [i] C: calcd, 46.98; found, 46.31. [j] N: calcd, 21.05; found, 21.46. [k] N: calcd, 18.24; found, 17.75. [l] C: calcd, 45.12; found, 44.64. [m] C: calcd, 49.36; found, 49.85. N: calcd, 15.70; found, 14.57. [n] N: calcd, 15.44; found, 16.01. [o] C: calcd, 56.91; found, 56.33. N: calcd, 27.65; found, 27.04. [p] C: calcd, 42.49; found, 43.11. [q] N: calcd, 11.19; found, 11.60. [r] C: calcd, 56.00; found, 55.54. [s] C: calcd, 57.14; found, 56.65. N: calcd, 18.17; found, 18.97. [t] C: calcd, 59.50; found, 59.09. [u] C: calcd, 48.71; found, 48.28.

Table III. Minimum Effective Concentrations[a] of Some Anticoccidials vs. *Eimeria tenella*

Anticoccidial nonproprietary name	MEC, ppm by wt in feed
Amprolium	60
Monensin	125
Robenidine	15
Meticlorpindol	125
Buquinolate	125
Decoquinate	10
Nequinate	2
6-Azauracil	1000
39	60

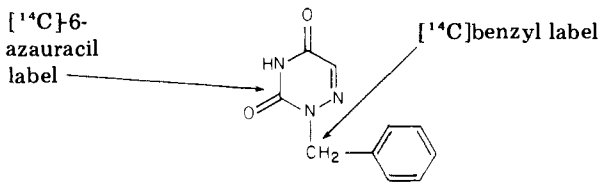
[a] Also sometimes called the minimum inhibitory concentration or MIC. Use levels may be higher in order to ensure adequate medication during periods of reduced feed intake and to prevent resistance development. Also, commercial formulations may contain additives or combinations to broaden the spectrum so that all of the commonly occurring *Eimeria* species will be controlled.

commercial anticoccidials.[50] It was effective against the major species of pathogenic poultry coccidia: *E. tenella*, *E. maxima*, *E. acervulina*, *E. brunetti*, and *E. necatrix*. The MEC of **39** for *E. acervulina* infections was about double that for the other species. The maximum tolerated dose was not established, but it exceeded 1000 ppm.

The ability of **28** and **39** to inhibit in vitro the decarboxylation by yeast OMP decarboxylase of orotidine-*carboxyl*-^{14}C 5′-monophosphate was tested. It was found that 29 μmol of **28** gave 99% inhibition while 30 μmol of **39** gave 100% inhibition. The control inhibitor, 6-azauridine 5′-monophosphate, gave 93–100% inhibition at 1–4 μmol.[51] Our assumption that the 1-benzyl-6-azauracils are effective oral anticoccidials by virtue of their inhibition of OMP decarboxylase in the parasite must remain speculative. There is, as mentioned above, ample analogy for this mode of action of 6-azauracil or its ribonucleotide in various other biological systems.

The thymidylate synthetase inhibitor, 5-fluorouracil, displayed a degree of coccidiostat activity similar to that of 6-azauracil. It is noteworthy that 1-benzyl-5-fluorouracil (prepared by the method of Baker and Jackson)[52] showed no anticoccidial activity at the levels of our test, and it was evident that in this instance 1-N-benzylation had *reduced* potency.

Radiotracer Studies. To elucidate the metabolism and disposition of this class of compounds as well as to determine the persistence of substituted 6-azauracils in chicken tissues, the prototype 1-benzyl-6-azauracil **28** was labeled with carbon-14. Since N-dealkylation is a potential metabolic pathway resulting in the scission of **28** into two separate ring systems, it was decided to label both halves of the molecule. Thus, **28** labeled with carbon-14 at C-2 of the 6-azauracil moiety or C-7 of the benzyl ring system was prepared. The radioactive residues found in chicken tissues after a single oral dose of 17.5 mg/kg of [^{14}C]-1-benzyl-6-azauracil (**28**) to broiler chickens are listed in Table IV.



Drug does not concentrate in any specific tissue but is rather widely and uniformly distributed. However, kidney, skin, heart, and liver consistently retain more radioactivity than the remaining tissues. Tissue radioactivity declines

Table IV. Radioactive Tissue Residues (μg/g) after [^{14}C]-1-Benzyl-6-azauracil[a]

Tissue	Study 1 (Triazine Labeled) Day 1	2	5	7	10
Lung	1.54	1.27	<0.01	<0.01	<0.01
Kidney	12.54	2.48	2.94	0.14	0.08
Skin	9.05	1.71	0.76	0.03	0.03
Light muscle	3.90	1.09	0.32	<0.01	<0.01
Heart	9.71	2.47	0.46	<0.01	<0.01
Stomach	4.84	1.31	0.27	<0.01	<0.01
Liver	8.58	2.29	0.96	0.02	0.02
Dark muscle	6.75	1.46	0.32	<0.01	<0.01

Tissue	Study 2 (Benzyl Labeled) Day 1	3	5	7	10
Lung	1.3	0.58	0.01	0.01	
Kidney	13.2	1.33	0.45	0.07	
Skin	13.4	1.14	0.20	0.03	
Light muscle	6.7	0.75	0.10	0.02	
Heart	15.9	1.56	0.23	0.02	
Stomach	5.1	0.66	0.08	0.01	
Liver	11.8	1.68	0.21	0.06	
Dark muscle	7.0	0.84	0.10	0.02	

[a] Single dose of 17.5 mg/kg to broiler chickens. Calculated as unchanged drug, micrograms of drug per gram of tissue (ppm).

Table V. Excretion of Radioactivity after 17.5 mg/kg of [^{14}C]-1-Benzyl-6-azauracil

Bird	Percent excreted/day 1	2	3	4	Total
	Study 1 (Triazine Labeled)				
1	27.0				
2	33.9	17.39	11.43		
3	31.9	17.84	7.90	2.13	59.77
4	34.9	20.47	7.40	1.78	64.55
5	34.4	23.10	9.90	3.83	71.23
Av	32.22	19.70	9.16	2.58	65.18
	Study 2 (Benzyl Labeled)				
1	46.3				
2	36.6	22.2	12.3		
3	30.4	26.4	15.5	9.0	81.2
4	31.3	22.2	13.3	7.6	74.5
5	23.9	23.3	13.4	5.3	65.9
Av	33.7	23.5	13.6	7.3	73.9

with an average half-life of approximately 0.5 day, and all tissues are cleared (less than 0.1 μg/g) by the seventh day.

The overall similarity of radioactive residues obtained from the azauracil- or benzyl-labeled studies suggests that the prototype **28** was not extensively cleaved in the chicken. This was confirmed by reverse isotope dilution procedures designed to achieve chemical identification of the radioactivity found in chicken excreta. Thus, in the case of 6-azauracil-labeled **28**, 23% of the administered radioactivity was identified as unchanged **28**, and if any cleaved 6-azauracil was present in chicken excreta, it constituted less than 1% of the administered dose. Similarly, 16% of administered benzyl-labeled **28** was recovered unchanged from the excreta, and if cleavage to the benzyl moiety occurred (as benzoic acid), it accounted for less than 6% of the dose.

The recovery of excreted radioactivity from chickens dosed with [^{14}C]-1-benzyl-6-azauracil is shown in Table V. Similar results were obtained with either label. The drug does not appear to be very rapidly excreted but rather slowly over a period of several days. By comparison,

6-azauracil is reported to be excreted by the chicken in a matter of hours.[53]

Discussion and Conclusions

For many years the "lethal synthesis" concept has dominated the design of nucleotide antimetabolites although some investigators, notably Baker, early outgrew its constraints at least with in vitro enzyme inhibition studies. This concept embodied the idea that pyrimidine and purine antimetabolites in order to be effective must be capable of enzymic ribosidation and phosphorylation in order to form false nucleotides. Thus it has been shown that 5-fluorouracil, 6-azauracil, and various other effective antimetabolites can be converted by enzymes or by certain microorganisms to the corresponding nucleotides.

On the other hand, such molecules blocked with, e.g., a methyl group to prevent enzymic ribosidation, are ineffective antimetabolites. It has even been assumed that incorporation of such "lethally synthesized" false nucleotides into nucleic acids was a requisite for efficacy. Some incorporation of this sort, e.g., of 5-fluorouracil into microbial ribonucleic acids, has been demonstrated.[54] In the case of 6-azauracil, however, such incorporation does not seem to be extensive, perhaps only 2–3% of the uracil content of the RNA of certain bacteria.[25,55]

It is known that 6-azauridylic acid is an excellent inhibitor of OMP decarboxylase, and this characteristic probably accounts for most of its activity.[56] As a drug, however, 6-azauridylic acid leaves much to be desired. Orally administered it is not well absorbed, its competitive inhibition may be overcome by accumulation of orotidylic acid, and because of its polarity, it is rapidly eliminated. In the treatment of severe psoriasis, the absorption problem was solved by use of the triacetate, but rather heroic doses were required to counter the other difficulties mentioned. As 6-azauracil probably is converted to 6-azauridylic acid in vivo, it is subject to many of the same problems. Similarly, the false sugar approach to antimetabolite design in which sugars other than ribose are attached to biologically important pyrimidines or purines usually leads to drugs which cannot be administered orally. The use of more lipophilic, nonsugar side chains as described here overcomes these difficulties and may represent a promising approach to the design of nucleotide analogues.

Our data indicate that the 1-benzyl side chains did not simply deliver 6-azauracil to an active site and then undergo removal. We believe that 1-benzyl substitution of 6-azauracil increases its anticoccidial efficacy by slowing excretion and, possibly, by eliminating the need for activation via in vivo ribosidation. We speculate that efficacy can be attributed to competitive inhibition of OMP decarboxylase. Efficacy is maximized in 1-benzyl-6-azauracils with 3′- or 4′-fluorine, -chlorine, or -nitrile substituents. These compact electron-withdrawing substituents, in contrast to the many closely related inactive structures, may increase potency by influencing drug kinetics, affinity for the enzyme, or both. The discovery of even more highly potentiating side chains will be discussed in future publications.

Experimental Section

In addition to the C, H, and N analyses cited in Table II, most product structures were checked by mass spectrum and some by ^{1}H NMR, UV, or IR spectra. The mass spectrograph used was the RMU 6E Hitachi, the NMR was a Varian A60, the UV a Model 11 or 13 Cary, and the IR a Perkin-Elmer Model 21. Melting points were determined on the Thomas-Hoover Uni-Melt apparatus or on a Kofler Micro Hot-Stage. Both devices had calibrated thermometers, but melting points were not rigorously corrected.

Procedures for the Preparation of 2-Substituted *as*-Triazine-3,5(2*H*,4*H*)-diones. Method Ia. This was a modification of an earlier procedure,[45] which in our hands gave mixtures. A solution of 6-azauracil (0.1 mol) and NaOH (0.3–0.5 mol) in water (300–400 mL) was treated with the aralkyl or alkyl halide (0.2–0.3 mol). The reaction mixture was heated at 100 °C for 3 h. The resulting basic solution was washed with Et_2O or $CHCl_3$; then the pH was adjusted to 4.5–6.0 with HCl. The product was collected by filtration and/or extraction and recrystallized from aqueous EtOH or CH_3CN. Yields were in the 10–35% range.

Method Ib. A mixture of 2–3 equiv of KOH in water and 1 equiv of 6-azauracil was treated with 1.2 equiv of alkyl halide in a cosolvent, either Me_2SO or EtOH. The work-up was the same as in method Ia except that the solvent was removed at reduced pressure. The crude product was purified by column chromatography and/or recrystallization.

Method Ic. To a solution of 6-azauracil (0.1 mol) and KOH (0.2 mol) in ethylene glycol (200 mL) maintained at 60 °C were added simultaneously the benzyl or alkyl halide (0.2 mol) and more KOH (0.1 mol) in the same solvent. Heating at 60 °C was continued for 5–15 min to complete reaction. Solvent was removed at reduced pressure, and the residue was dissolved in water (250 mL). The resulting solution was treated with decolorizing charcoal and filtered. The filtrate was adjusted to pH 8.5–9.5 and washed with Et_2O. After acidification to pH 4.5–6.0, the product was collected by filtration and/or extraction with CH_2Cl_2. The product was recrystallized from aqueous EtOH or CH_3CN, affording a 2–20% yield.

Method Id. A solution of 3,5-bis(trimethylsilyloxy)-*as*-triazine[46] (0.017 mol) in CH_3CN was treated with a benzyl or alkyl halide (0.013 mol) and the mixture refluxed overnight. The precipitate from the cooled reaction was collected by filtration. A second crop was obtained by concentration of the filtrate and trituration of the residue with EtOH. The crops were combined and recrystallized to give a 3–40% yield.

3-[3,5(2*H*,4*H*)-Dioxo-*as*-triazin-2-yl]methylbenzoic Acid Ethyl Ester. To 3-[3,5(2*H*,4*H*)-dioxo-*as*-triazin-2-yl]methylbenzoic acid (15 g), prepared by method Ia, in EtOH (60 mL) was added $SOCl_2$ (3 mL). The reaction mixture was heated to reflux for 30 min and then filtered hot. Crystallization from the filtrate afforded 0.85 g of product, mp 162–164 °C.

Method II. 4-Substituted and Symmetrically 2,4-Disubstituted *as*-Triazine-3,5(2*H*,4*H*)-diones. A solution of 6-azauracil (0.1 mol) and 2,6-dimethylpiperidine (0.11 mol) in DMF at 100–130 °C under nitrogen was treated over a 2–3-h period with a benzyl or alkyl halide (0.11 mol). The reaction mixture was heated at 120–130 °C for 3–4 h while maintaining the pH of the reaction mixture at 7.0–7.5 by the addition of more dimethylpiperidine. The mixture was cooled to room temperature, filtered, and evaporated to dryness. The residue was dissolved in Et_2O, washed with 1% NaOH, treated with HCl gas, filtered, and dried ($MgSO_4$). The 2,4-disubstituted *as*-triazine-3,5(2*H*,4*H*)-dione was collected by filtration and/or concentration of the Et_2O and recrystallized from aqueous EtOH, affording about a 20% yield. All the basic aqueous portions were combined and the pH was adjusted to 7.0. The neutral aqueous was concentrated, and the 4-substituted *as*-triazine-3,5(2*H*,4*H*)-dione was collected and recrystallized from aqueous EtOH, affording a 10–30% yield.

Method III. Unsymmetrically 2,4-Disubstituted *as*-Triazine-3,5(2*H*,4*H*)-diones. The sodium salt of the appropriate 4-substituted *as*-triazine-3,5(2*H*,4*H*)-dione was prepared by adding the triazine (0.0625 mol) to $NaOCH_3$ (0.065 mol) in MeOH (200 mL) and evaporating the resulting solution to dryness. The salt was then dissolved in a minimum amount of DMF and treated with an alkyl halide (0.065 mol) at 100 °C. Heating was continued for 2 h, during which time the pH fell from 10 to 6. The reaction mixture was taken to dryness, and to the residue were added 10% Na_2CO_3 and Et_2O. The Et_2O was separated, washed with 10% Na_2CO_3, dried ($MgSO_4$), and filtered. The filtrate was concentrated to give a 20–60% yield of product.

4-[3,5(2*H*,4*H*)-Dioxo-*as*-triazin-2-yl]valeric Acid (12). The nitrile 11 (4.5 g, 0.023 mol) in concentrated HCl (30 mL) was heated under reflux for 18 h. The reaction mixture was stirred

for 30 min with activated charcoal and then filtered hot. Evaporation of the filtrate to 0.5 vol under reduced pressure gave a solution from which the product crystallized on cooling. Recrystallization from EtOAc yielded 1.86 g (37%), mp 125–127 °C.

IV. Preparation of Sulfoxides and Sulfones. 2-(2-Methylthioethyl)-*as*-3,5(2*H*,4*H*)-dione, 2-(2-phenylthioethyl)-*as*-triazine-3,5(2*H*,4*H*)-dione, and 2-(2-ethylthioethyl)-*as*-triazine-3,5(2*H*,4*H*)-dione were prepared by method Ib.

Method IVa. Oxidation to Sulfoxides. A solution of the appropriate sulfide (0.01 mol) in HOAc (10–20 mL) was treated at room temperature over a 10-min period with 30% H_2O_2 (0.012 mol) and allowed to stir overnight.

(1) In the case of 2-(2-methylthioethyl)-*as*-triazine-3,5(2*H*,4*H*)-dione, the reaction gave a positive starch–KI test at this point. More starting triazine was added to consume remaining peroxide. 2-Propanol was then added to precipitate the product which was collected in 60% yield.

(2) In the case of 2-(2-phenylthioethyl)-*as*-triazine-3,5(2*H*,4*H*)-dione, the mixture was treated with sodium thiosulfate until a negative starch–KI test was obtained. The reaction mixture was evaporated and the residue was dissolved in H_2O. The product was extracted with CH_2Cl_2 and recrystallized from 2-propanol, giving a 40% yield.

Method IVb. Oxidation to Sulfones. To a solution of the appropriate 2-thio-substituted *as*-triazine-3,5(2*H*,4*H*)-dione (0.01 mol) in HOAc (20–40 mL) at 90 °C, 30% H_2O_2 (0.03 mol) was added dropwise. Heating was continued until the mixture gave a negative starch–KI test (5–6 h). The reaction mixture was cooled and the product was collected. If no solid formed, 2-propanol was added, and the mixture was cooled and filtered to give a 60–80% yield.

6-(*n*-Propylthio)-*as*-triazine-3,5(2*H*,4*H*)-dione. To 6-bromo-*as*-triazine-3,5(2*H*,4*H*)-dione (50 g, 0.26 mol), prepared according to the procedure of Cristescu and Marcus,[57] in 1 N NaOH (500 mL) was added 1-propanethiol (24 mL, 0.26 mol). The reaction mixture was stirred under reflux for 5 h and then at room temperature overnight. It was acidified with concentrated HCl, and the precipitate was collected, washed with H_2O, and recrystallized from IPO to give 36 g (74% yield), mp 225–228 °C.

Radiochemical Methods. Carbon-14 labeled 1-benzyl-6-azauracil (**28**) was synthesized from [2-^{14}C]-6-azauracil (Tracerlabs, Waltham, Mass.) or [7-^{14}C]benzyl chloride (ICN, Irvine, Calif.) by the procedures described above. Azauracil-labeled **28** had a specific activity of 2.34 μCi/mg, and the benzyl-labeled **28** had a specific activity of 1.92 μCi/mg.

Radioactive samples were measured in a Nuclear Chicago Model 6860 (Mark I) liquid scintillation counter. Samples were corrected for quench by the use of a ^{133}Barium External Standard which is an integral part of this counter. The method has been described by Figdor.[58] Occasionally, to verify the method, some samples were assayed by the conventional internal standard procedure, in which a known amount of [^{14}C]toluene is added to the sample in order to determine counting efficiency.

Samples were assayed in a scintillator consisting of 0.3% 2,5-diphenyloxazole (PPO) and 0.01% *p*-bis[2-(5-phenyloxazolyl)]benzene (POPOP) in toluene or in a mixture of 30% absolute ethanol and 70% toluene (30/70 scintillator).

Tissue Assay. Tissue samples were assayed by homogenizing a mixture of 3 mL of Hyamine (Packard Instrument Co.) per gram of fresh wet tissue in a Potter–Elvehjem tissue grinder. The homogenate was allowed to stand at room temperature for several hours to complete digestion. One milliliter of the clear digested homogenate (equivalent to 250 mg of fresh wet tissue) was pipetted into a scintillation vial and 15 mL of the 30/70 scintillator added. Samples were generally permitted to remain in the liquid scintillation spectrometer for 24–48 h to achieve equilibrium before any counting was attempted.

Excreta. Fresh excreta was homogenized in a Waring Blendor with the addition of water and the slurry lyophilized. Approximately 50–75 mg of the dry lyophilate was combusted in an atmosphere of oxygen in a modified Schoniger flask. The resulting combustion products were trapped in 10 mL of ethanolamine–ethyl cellosolve (1:2 v/v). An aliquot was counted in ethyl cellosolve–toluene (1:2), containing 5.5 g of PPO/L.

Broiler chickens weighing 1–1.2 kg each, and not stressed by any previous procedure, received a single oral dose of uracil or [^{14}C]benzyl labeled **28** as an aqueous suspension at a dose of 17.5 mg/kg. Birds were housed in wire screened metabolism cages that permitted the daily collection of excreta. One bird from each group was sacrificed on days 1, 2, 5, 7, and 10, after receiving the labeled drug, and the tissues were assayed as described above.

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Studies on Anticoccidial Agents. 11. Synthesis and Anticoccidial Activity of Nitropyridinecarboxamides and Derivatives

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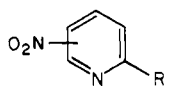
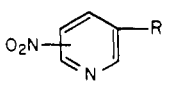
Of the nine nitropyridinecarboxamides, which are isomers of 5-nitronicotinamide, a potent anticoccidial agent, 2-nitropyridine-3-, -4-, -5-, or -6-carboxamides and 3-nitropyridine-4- or -6-carboxamides were prepared from the corresponding acids via the esters or the acid chlorides. 3-Nitropyridine-2-carboxamide was obtained from 2-methyl-3-nitropyridine by oxidation with SeO_2, oximation, dehydration with Ac_2O, and hydrolysis with H_2SO_4. 4-Nitropyridine-2-carboxamide was prepared from 2-cyano-4-nitropyridine by hydrolysis, and the 3-carboxamide analogue was obtained from 4-amino-3-cyanopyridine by oxidation with H_2O_2 and fuming H_2SO_4. Of these compounds 2-nitro- and 3-nitro- but not 4-nitropyridinecarboxamides were found to be active against *Eimeria tenella*. N-Substituted analogues of 2-nitro- and 3-nitropyridinecarboxamides were also prepared in a conventional manner and optimal anticoccidial activity was attained with 2-nitroisonicotinamide and its N-alkanoyl, N-aromatic, and N-heterocyclic acyl derivatives.

In the previous paper[1] we demonstrated that 5-nitronicotinamide and its derivatives possessed very potent anticoccidial activity. The present study was performed to determine whether a similar potency would be noted for the other nine isomeric nitropyridinecarboxamides and their derivatives. These compounds fall into three classes depending upon whether the carboxamide group is in the 2, 3, or 4 position in the pyridine ring. The first type with the carboxamide group in the 2 position consists of four isomers with the nitro group in the 3, 4, 5, and 6 positions, respectively. Schmidt-Thome et al.[2] have obtained 5-nitropyridine-2-carboxamide (**1a**) by treatment of 2-bromo-5-nitropyridine with cuprous cyanide, followed by hydrolysis. We obtained the amide **1a** by treatment of 5-nitropyridine-2-carboxylic acid (**1b**)[3] with $SOCl_2$ and then with ammonia. Similar transformations have been effected in the synthesis of the 6-nitro compound **2a** starting from 6-nitropyridine-2-carboxylic acid (**2b**).[3] 3-Nitropyridine-2-carboxamide (**3g**) had been previously prepared by Berrie et al.[4] using a somewhat tedious procedure. We have prepared this compound from 2-methyl-3-nitropyridine. Brown[3] has reported the preparation of 3-nitropyridine-2-carboxylic acid (**3c**) by the oxidation of **3a** with $KMnO_4$, but under the same oxidation conditions we isolated the acid **3c** in only 3.7% yield along with 3-nitropyridine (**3b**) and the starting material. Therefore, the synthesis of 3-nitropyridine-2-carboxamide (**3g**) was achieved in an indirect manner: oxidation of **3a** with SeO_2, oximation, dehydration with Ac_2O, and hydrolysis with H_2SO_4. 4-Nitropyridine-2-carboxamide (**4b**) was easily obtained from 2-cyano-4-nitropyridine (**4a**)[5] with H_2SO_4.



1a, R = $CONH_2$, 5-NO_2
b, R = COOH, 5-NO_2
2a, R = $CONH_2$, 6-NO_2
b, R = COOH, 6-NO_2
3a, R = Me, 3-NO_2
b, R = H, 3-NO_2
c, R = COOH, 3-NO_2
d, R = CHO, 3-NO_2
e, R = CH=NOH, 3-NO_2
f, R = CN, 3-NO_2
g, R = $CONH_2$, 3-NO_2
4a, R = CN, 4-NO_2
b, R = $CONH_2$, 4-NO_2

The second class consists of the four isomers containing the carboxamide group in the 3 position and the nitro group in either the 2, 4, 5, or 6 position; one of these has already been reported in the previous paper[1] as a potent coccidiostat. 2-Nitronicotinamide (**5c**) and 6-nitronicotinamide (**6c**) were prepared from the corresponding acids **5a** and **6c** by esterification and ammonolysis. The synthesis of 4-nitronicotinamide (**7**) was accomplished by oxidation of 4-amino-3-cyanopyridine[6] with persulfuric acid.



5a, R = COOH, 2-NO_2
b, R = COOMe, 2-NO_2
c, R = $CONH_2$, 2-NO_2
6a, R = COOH, 6-NO_2
b, R = COOMe, 6-NO_2
c, R = $CONH_2$, 6-NO_2
7, R = $CONH_2$, 4-NO_2

The compounds in the last type of nitropyridinecarboxamide are 2-nitro- and 3-nitroisonicotinamides. These two isomers were prepared by the action of am-