[Chem. Pharm. Bull. 29(1) 88—97 (1981)]

Anticoccidials. VI.[1] An Improved Synthesis of 1,6-Dihydro-6-oxo-2-pyrazinecarboxylic Acid 4-Oxide and Some Related Derivatives and Determination of Anticoccidial Activity

Kin-ichi Imai, Mitsuhiko Mano,* Takuji Seo, and Toshimi Matsuno

Animal Health Products Division, Takeda Chemical Industries, Ltd., Jusohonmachi, Yodogawa-ku, Osaka 532, Japan

(Received July 25, 1980)

1,6-Dihydro-6-oxo-2-pyrazinecarboxylic acid 4-oxide (**1**) has been synthesized by two different methods. The first is a hydrolysis of methyl 6-chloro-2-pyrazinecarboxylate 4-oxide, which was in turn obtained from methyl 6-chloro-2-pyrazinecarboxylate by reaction with *m*-chloroperbenzoic acid. The second is an oxidation of 6-hydroxymethyl-2(1*H*)-pyrazinone 4-oxide with nickel peroxide. Compound **1** was converted to amine salts, esters and amides. 6-Methoxy, 6-mercapto and 1-alkyl derivatives were also prepared. The compounds prepared were tested for anticoccidial activity in chickens against *Eimeria tenella* and marked activity was seen with compound **1** and amine salts of **1**. The activity of **1** was counteracted by the simultaneous administration of an equal weight of orotic acid or adenine.

Keywords——pyrazine; 1,6-dihydro-6-oxo-2-pyrazinecarboxylic acid 4-oxide; anticoccidial activity; orotic acid; adenine; reversal

Some anticoccidial activity has been reported for 6-azauracil.[2] This drug is converted *in vivo* into 6-azauridylic acid, which is an inhibitor of the enzyme orotidylate decarboxylase.[3] This knowledge led to the screening of orotic acid antagonists for anticoccidial activity, and we now report the discovery that 1,6-dihydro-6-oxo-2-pyrazinecarboxylic acid 4-oxide (**1**) has potent activity. We also describe an improved synthetic method for **1**, the preparation of related derivatives, and the biological effects of various nucleic acid-related compounds on the anticoccidial activity of **1**.

Synthesis

Compound **1** was first prepared as an orotic acid analog by Bobek and Bloch[4] starting from methyl 1,6-dihydro-6-oxo-2-pyrazinecarboxylate. The low overall yield of **1** led us to investigate alternative routes to **1**. Oxidation of methyl 6-chloro-2-pyrazinecarboxylate (**2**)[5] with *m*-chloroperbenzoic acid provided, after removal of the unchanged starting material by column chromatography on silica gel, methyl 6-chloro-2-pyrazinecarboxylate 4-oxide (**3**) in 35% yield. Hydrolysis of **3** with aqueous sodium hydroxide afforded **1** in 55% yield, together

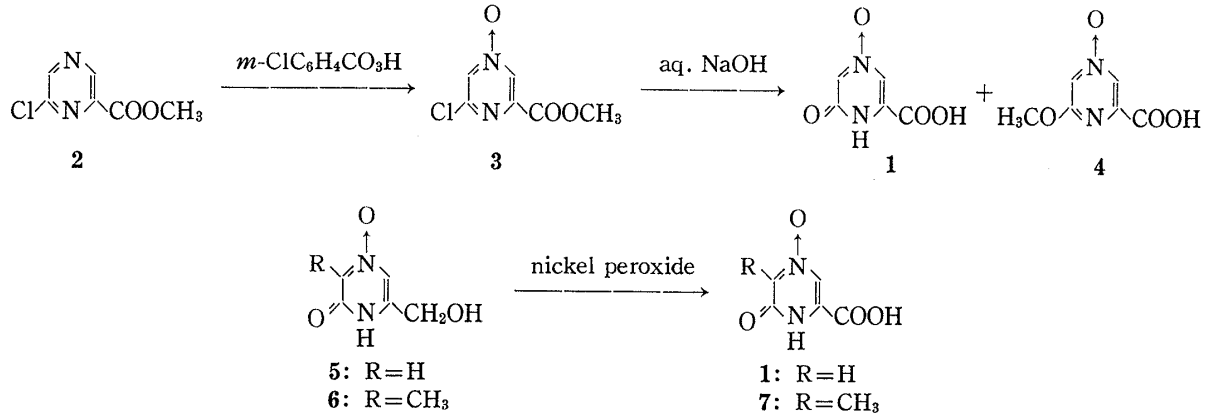
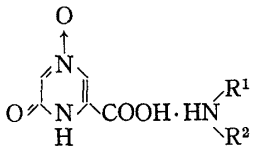


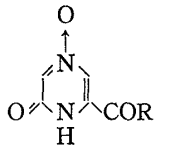
Chart 1

with a small amount of 6-methoxy-2-pyrazinecarboxylic acid 4-oxide (**4**), which was detected by paper electrophoresis (PE). Alternatively, oxidation of the readily accessible 6-hydroxymethyl-2(1*H*)-pyrazinone 4-oxide (**5**)[6] with nickel peroxide[7] gave **1** in 40% yield. This procedure provides the most convenient route to **1**. In addition, this method was extended to the preparation of the 5-methyl derivative **7**. The structure of **7** was confirmed by comparison with 4,5-dihydro-6-methyl-5-oxo-2-pyrazinecarboxylic acid 1-oxide[8] (Chart 1).

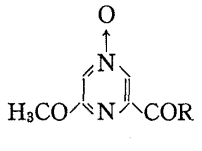
Amine salts (**8** and **9**) of **1** were obtained (Table I). The ester (**10** and **11**) and amide (**12**—**14**) derivatives of **1** were prepared by the standard procedure (Table II). 6-Methoxy derivatives (**15**, **4** and **16**) and 6-mercapto derivatives (**17** and **18**) were synthesized from **3**.



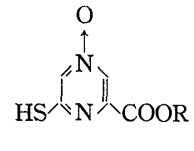
8: $R^1=R^2=CH(CH_3)_2$
9: $R^1=C_6H_{11}$, $R^2=H$



10: $R=OCH_3$
11: $R=O(CH_2)_3CH_3$
12: $R=NH_2$
13: $R=NHCH_3$
14: $R=N(CH_3)_2$



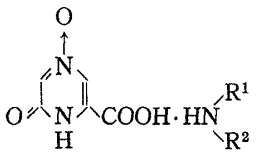
15: $R=OCH_3$
16: $R=NH_2$



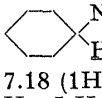
17: $R=CH_3$
18: $R=H$

An attempted synthesis of the N-methyl derivatives by alkylation of **10** with methyl iodide in the presence of sodium hydride in N,N-dimethylformamide (DMF) resulted in the formation of the O-methyl derivative **15** as a major product along with minor amounts of the N-methyl derivative **19**. However, when **10** was treated with methyl iodide in the presence

Table I. Amine Salts of 1,6-Dihydro-6-oxo-2-pyrazinecarboxylic Acid 4-Oxide

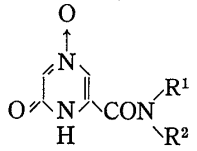


Compound No.	R^1	R^2	Recrystn solvent	Yield (%)	mp (°C)	Formula
8	$CH(CH_3)_2$	$CH(CH_3)_2$	Acetone	59	170—175 (dec.)	$C_5H_4N_2O_4\cdot C_6H_{15}N$
9	C_6H_{11}	H	EtOH–ether	45	223—225 (dec.)	$C_5H_4N_2O_4\cdot C_6H_{13}N$

Compound No.	Analysis (%) Calcd (Found) C	H	N	IR ν_{max}^{KBr} cm^{-1}	PMR[a]
8	51.35 (51.31	7.44 7.45	16.33 16.20)	1665 1640	2.22 (12H, d, $J=6$ Hz, $4\times CH_3$), 3.12—3.55 (2H, m, $2\times CH$), 7.18 (1H, d, $J=2$ Hz, 3-H or 5-H), 7.46 (1H, d, $J=2$ Hz, 5-H or 3-H), 7.7—9.7 (2H, br, COOH and NH)
9	51.76 (52.04	6.71 6.96	16.46 16.05)	1655 1635 1615	0.67—2.13 (10H, m, $5\times CH_2$), 2.60—3.20 (1H, br, ), 5.6—7.6 (3H, br, COOH and NH_2), 7.18 (1H, d, $J=2$ Hz, 3-H or 5-H), 7.45 (1H, d, $J=2$ Hz, 5-H or 3-H)

a) Measured in dimethyl sulfoxide (DMSO)-d_6 with a Varian EM-390 spectrometer.

TABLE II. 1,6-Dihydro-6-oxo-2-pyrazinecarboxamide 4-Oxides



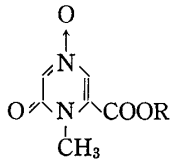
Compound No.	R^1	R^2	Recrystn solvent	Yield (%)	mp (°C)	Formula	Analysis (%) Calcd (Found) C	H	N
12	H	H	H_2O	97	272—275 (dec.)	$C_5H_5N_3O_3$	38.72 (38.39	3.25 3.43	27.09 26.71)
13	CH_3	H	EtOH	75	220—222 (dec.)	$C_6H_7N_3O_3$	42.61 (42.38	4.17 4.19	24.84 24.54)
14	CH_3	CH_3	EtOH	21	192—193	$C_7H_9N_3O_3$	45.90 (45.86	4.95 4.94	22.94 22.67)

Compound No.	IR ν_{max}^{KBr} cm^{-1}	PMR[a]
12	1690	7.76 (1H, d, J=1.5 Hz, 3-H or 5-H), 7.90 (1H, d, J=1.5 Hz, 5-H or 3-H), 7.7—8.1 (2H, br, NH_2)
13	1650 1595	[b] 2.90 (3H, d, J=5 Hz, CH_3), 7.79 (1H, d, J=1.5 Hz, 3-H or 5-H), 7.92 (1H, d, J=1.5 Hz, 5-H or 3-H), 8.4—8.7 (1H, br, NH)
14	1630	2.98 (6H, s, 2×CH_3), 7.55 (1H, d, J=1.5 Hz, 3-H or 5-H), 7.63 (1H, d, J=1.5 Hz, 5-H or 3-H)

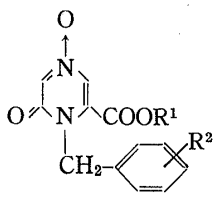
a) Measured in DMSO-d_6 with a Varian A-60A spectrometer.
b) Taken with a Varian XL-100-12 spectrometer.

of lithium carbonate in N,N-dimethylacetamide (DMA), 1,6-dihydro-1-methyl-6-oxo-2-pyrazinecarboxylic acid 4-oxide (**20**) was obtained as a major product together with **4**, **15** and **19**.

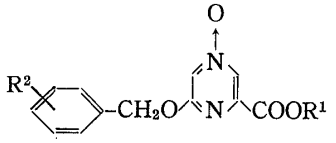
Alkylation of **10** with benzyl, 4-methylbenzyl and 3,4-dichlorobenzyl bromides in the presence of lithium carbonate in DMA gave both the N-alkyl (**21**—**23**) and O-alkyl (**27**—**29**) derivatives, which were separated by column chromatography on silica gel. In the case of 2-methylbenzyl and 2-chlorobenzyl bromides, only the O-alkyl derivatives (**30** and **31**) were obtained. Hydrolysis of the resulting products (**21**—**23** and **27**—**31**) with aqueous potassium carbonate gave the corresponding carboxylic acids (**24**—**26** and **32**—**36**) (Tables III—VI).



19: R=CH_3
20: R=H

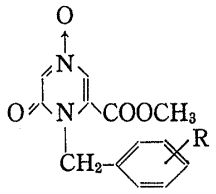


21: R^1=CH_3, R^2=H
22: R^1=CH_3, R^2=4-CH_3
23: R^1=CH_3, R^2=3,4-Cl_2
24: R^1=H, R_2=H
25: R^1=H, R_2=4-CH_3
26: R^1=H, R_2=3,4-Cl_2



27: R^1=CH_3, R^2=H
28: R^1=CH_3, R^2=4-CH_3
29: R^1=CH_3, R^2=3,4-Cl_2
30: R^1=CH_3, R^2=2-CH_3
31: R^1=CH_3, R^2=2-Cl
32: R^1=H, R^2=H
33: R^1=H, R^2=4-CH_3
34: R^1=H, R^2=3,4-Cl_2
35: R^1=H, R^2=2-CH_3
36: R^1=H, R^2=2-Cl

TABLE III. Methyl 1-Benzyl-1,6-dihydro-6-oxo-2-pyrazinecarboxylate 4-Oxides

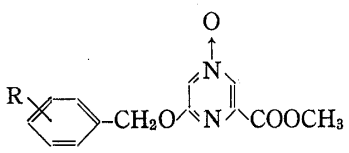


Compound No.	R	Recrystn solvent	Yield (%)	mp (°C)	Formula	Analysis (%) Calcd (Found) C	H	N
21	H	AcOEt–petroleum ether	14	120—122	$C_{13}H_{12}N_2O_4$	60.00 (59.96	4.65 4.58	10.76 10.69)
22	4-CH_3	—	9	Oil	—	—	—	—
23	3,4-Cl_2	—	16	Oil	—	—	—	—

Compound No.	IR ν_{max}^{KBr} cm^{-1}	PMR[a]
21	1735 1655 1610	[b] 3.76 (3H, s, OCH_3), 5.51 (2H, s, CH_2), 7.07—7.37 (5H, m, Ar-H), 7.40 (1H, d, J=2 Hz, 3-H or 5-H), 7.68 (1H, d, J=2 Hz, 5-H or 3-H)
22	1735 1650 1605	2.28 (3H, s, CH_3), 3.43 (3H, s, OCH_3), 5.45 (2H, s, CH_2), 7.02 (4H, s, Ar-H), 7.39 (1H, d, J=2 Hz, 3-H or 5-H), 7.85 (1H, d, J=2 Hz, 5-H or 3-H)
23	1740 1665 1615	3.80 (3H, s, OCH_3), 5.36 (2H, s, CH_2), 6.90—7.47 (4H, m, 3-H or 5-H and Ar-H), 7.52 (1H, d, J=2 Hz, 5-H or 3-H)

a) Measured in $CDCl_3$ with a Varian T-60 spectrometer.
b) Taken with a Varian EM-390 spectrometer.

TABLE IV. Methyl 6-Benzyloxy-2-pyrazinecarboxylate 4-Oxides

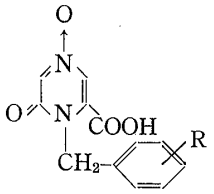


Compound No.	R	Recrystn solvent	Yield (%)	mp (°C)	Formula	Analysis (%) Calcd (Found) C	H	N
27	H	AcOEt–petroleum ether	30	64— 65	$C_{13}H_{12}N_2O_4$	60.00 (60.13	4.65 4.53	10.76 10.63)
28	4-CH_3	MeOH	32	105—106	$C_{14}H_{14}N_2O_4$	61.31 (61.25	5.15 5.13	10.21 10.10)
29	3,4-Cl_2	AcOEt–petroleum ether	36	123—125	$C_{13}H_{10}Cl_2N_2O_4$	47.44 (47.49	3.06 2.96	8.51 8.59)
30	2-CH_3	MeOH	18	97— 98	$C_{14}H_{14}N_2O_4$	61.31 (61.29	5.15 5.12	10.21 10.05)
31	2-Cl	AcOEt–petroleum ether	65	87— 88	$C_{13}H_{11}ClN_2O_4$	52.98 (52.96	3.76 3.64	9.51 9.52)

Compound No.	IR ν_{max}^{KBr} cm^{-1}	PMR[a]
27	1750 1600	[b] 3.98 (3H, s, OCH_3), 5.47 (2H, s, CH_2), 7.25—7.53 (5H, m, Ar-H), 7.84 (1H, d, J=1.5 Hz, 3-H or 5-H), 8.36 (1H, d, J=1.5 Hz, 5-H or 3-H)
28	1745 1595	2.34 (3H, s, CH_3), 3.97 (3H, s, OCH_3), 5.43 (2H, s, CH_2), 7.12 (2H, d, J=9 Hz, Ar-H), 7.33 (2H, d, J=9 Hz, Ar-H), 7.80 (1H, d, J=1.5 Hz, 3-H or 5-H), 8.32 (1H, d, J=1.5 Hz, 5-H or 3-H)
29	1745 1595	3.99 (3H, s, OCH_3), 5.42 (2H, s, CH_2), 7.17—7.67 (3H, m, Ar-H), 7.83 (1H, d, J=1 Hz, 3-H or 5-H), 8.35 (1H, d, J=1 Hz, 5-H or 3-H)
30	1740 1720 1600	2.43 (3H, s, CH_3), 4.02 (3H, s, OCH_3), 5.53 (2H, s, CH_2), 7.17—7.60 (4H, m, Ar-H), 7.88 (1H, d, J=1.5 Hz, 3-H or 5-H), 8.40 (1H, d, J=1.5 Hz, 5-H or 3-H)
31	1725 1610	4.00 (3H, s, OCH_3), 5.59 (2H, s, CH_2), 7.10—7.73 (4H, m, Ar- H), 7.89 (1H, d, J=1 Hz, 3-H or 5-H), 8.38 (1H, d, J=1 Hz, 5-H or 3-H)

a) Measured in $CDCl_3$ with a Varian T-60 spectrometer.
b) Taken with a Varian EM-390 spectrometer.

TABLE V. 1-Benzyl-1,6-dihydro-6-oxo-2-pyrazinecarboxylic Acid 4-Oxides

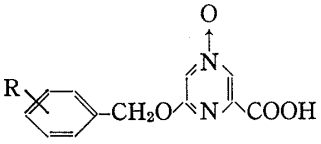


Compound No.	R	Recrystn solvent	Yield (%)	mp (°C)	Formula	Analysis (%) Calcd (Found) C	H	N
24	H	EtOH	73	180—182	$C_{12}H_{10}N_2O_4$	58.54 (58.34	4.09 3.98	11.38 11.22)
25	4-CH_3	—[b]	61	170—172	$C_{13}H_{12}N_2O_4 \cdot 1/5H_2O$	59.18 (59.27	4.74 4.54	10.62 10.65)
26	3,4-Cl_2	EtOH–ether	34	172—175	$C_{12}H_8Cl_2N_2O_4 \cdot 1/4H_2O$	45.09 (44.88	2.68 2.43	8.76 8.69)

Compound No.	IR ν_{max}^{KBr} cm^{-1}	PMR[a]
24	1725 1640 1565	5.39 (2H, s, CH_2), 7.07—7.47 (5H, m, Ar-H), 7.54 (1H, d, J=2Hz, 3-H or 5-H), 7.83 (1H, d, J=2 Hz, 5-H or 3-H)
25	1720 1635 1560	2.25 (3H, s, CH_3), 5.33 (2H, s, CH_2), 7.07 (4H, s, Ar-H), 7.51 (1H, d, J=2 Hz, 3-H or 5-H), 7.80 (1H, d, J=2 Hz, 5-H or 3-H)
26	1725 1635 1570	5.30 (2H, s, CH_2), 7.19 (1H, dd, J=2 Hz, 9 Hz, Ar-H), 7.51 (1H, d, J=2 Hz, Ar-H), 7.52 (1H, d, J=9 Hz, Ar-H), 7.55 (1H, d, J=2 Hz, 3-H or 5-H), 7.82 (1H, d, J=2 Hz, 5-H or 3H)

a) Measured in DMSO-d_6 with a Varian EM-390 spectrometer.
b) Not recrystallized.

TABLE VI. 6-Benzyloxy-2-pyrazinecarboxylic Acid 4-Oxides[a)]



Compound No.	R	Yield (%)	mp (°C)	Formula	Analysis (%) Calcd (Found) C	H	N
32	H	79	190—193	$C_{12}H_{10}N_2O_4$	58.54 (58.47	4.09 4.01	11.38 11.36)
33	4-CH_3	82	181—183 (dec.)	$C_{13}H_{12}N_2O_4$	60.00 (59.73	4·65 4.48	10.76 10.67)
34	3,4-Cl_2	81	205—208	$C_{12}H_8Cl_2N_2O_4$	45.74 (45.90	2.56 2.56	8.89 8.80)
35	2-CH_3	83	204—205	$C_{13}H_{12}N_2O_4$	60.00 (60.15	4.65 4.50	10.76 10.65)
36	2-Cl	94	223—224	$C_{12}H_9ClN_2O_4$	51.35 (51.35	3.23 3.25	9.98 9.83)

Compound No.	IR ν_{max}^{KBr} cm^{-1}	PMR[b)] Solvent	Chemical shift
32	1720 1600	DMSO-d_6	5.43 (2H, s, CH_2), 7.23—7.63 (5H, m, Ar-H), 8.25 (2H, s, 3-H and 5-H)
		DMSO-d_6 +D_2O	5.50 (2H, s, CH_2), 7.23—7.63 (5H, m, Ar-H), 8.23 (1H, d, J= 1.5 Hz, 3-H or 5-H), 8.36 (1H, d, J=1.5 Hz, 5-H or 3-H)
33	1720 1600	DMSO-d_6	2.30 (3H, s, CH_3), 5.38 (2H, s, CH_2), 7.16 (2H, d, J=8 Hz, Ar-H), 7.36 (2H, d, J=8 Hz, Ar-H), 8.25 (2H, s, 3-H and 5-H)
		DMSO-d_6 +D_2O	2.38 (3H, s, CH_3), 5.47 (2H, s, CH_2), 7.25 (2H, d, J=8 Hz, Ar-H), 7.44 (2H, d, J=8 Hz, Ar-H), 8.26 (1H, d, J=1.5 Hz, 3-H or 5-H), 8.37 (1H, d, J=1.5 Hz, 5-H or 3-H)
34	1720 1595	DMSO-d_6	5.43 (2H, s, CH_2), 7.45 (1H, dd, J=2 Hz, 8 Hz, Ar-H), 7.63 (1H, d, J=8 Hz, Ar-H), 7.77 (1H, d, J=2 Hz, Ar-H), 8.20—8.37 (2H, m, 3-H and 5-H)
35	1715 1600	DMSO-d_6	2.35 (3H, s, CH_3), 5.42 (2H, s, CH_2), 7.03—7.52 (4H, m, Ar-H), 8.26 (2H, s, 3-H and 5-H)
36	1710 1595	DMSO-d_6	5.50 (2H, s, CH_2), 7.28—7.71 (4H, m, Ar-H), 8.25—8.35 (2H, m, 3-H and 5-H)

a). Recrystallized from EtOH.
b) Taken with a Varian EM-390 spectrometer.

Anticoccidial Activity

Anticoccidial screening in chickens against *Eimeria tenella* was carried out in battery experiments as described in the preceding paper.[9)] Indicators of efficacy included measurements of bloody bloody droppings, mortality, cecal lesions and relative weight gain. Bloody droppings per bird were graded as follows: −(normal), +(mild), ++(moderate), +++ (severe). The cecal lesions were scored by the procedure of Johnson and Reid.[10)]

Of the compounds tested (**1**—**4**, **7**—**18** and **20**—**36**), **1**, **8** and **9** showed potent activity; biological data for these three potent compounds are listed in Table VII as the minimum effective concentrations in feed required to control coccidiosis.

In reversal experiments, addition of orotic acid or adenine to the feed (at feed levels of 0.0125% or higher) caused a marked decrease in the activity of **1** (0.0125% in feed). These

Table VII. Anticoccidial Activity

Compound No.	Minimum effective concentration in feed (%)	Bloody droppings (day after infection) 4	5	6	7	Mortality	Cecal lesion score (number of birds) ###	##	#	+	−	Relative weight gain (%)
1	0.0125	−	−	−	−	0/3					3	110.4
8	0.025	−	−	−	−	0/3					3	93.6
9	0.025	−	−	−	−	0/3					3	96.6
Infected unmedicated control		##	##	##	##	1/3	2					50.2
Uninfected unmedicated control		−	−	−	−	0/3					3	100.0

Table VIII. Loss of the Activity against *Eimeria tenella* of 1,6-Dihydro-6-oxo-2-pyrazinecarboxylic Acid 4-Oxide upon Coadministration of Orotic Acid or Adenine

Level in feed[a] (%) Compound **1**	Orotic acid	Adenine	Bloody droppings (day after infection) 4	5	6	7	Mortality[b]	Cecal lesion score (number of birds) ###	##	#	+	−	Relative weight gain (%)
0.0125	—	—	−	−	−	−	0/6					6	112.9
0.0125	0.00625	—	−	−	−	−	0/6		1	1		4	98.4
0.0125	0.0125	—	##	##	##	##	0/6	3	1		1	1	78.7
0.0125	0.025	—	##	##	##	##	0/6	6					91.2
0.0125	—	0.0125	−	+	+	−	0/6	2	1		2	1	103.5
0.0125	—	0.025	+	##	##	##	0/6	4	1		1		90.0
0.0125	—	0.05	##	##	##	##	0/6	4	1		1		71.4
Infected unmedicated control			##	##	##	##	2/6	4					53.6
Uninfected unmedicated control			−	−	−	−	0/6					6	100.0

a) Commercial experimental diet for chikens (Nihon Haigoshiryo, Ltd.) was used as the basal ration.
b) 3 Birds × 2 replicates.

biological data are presented in Table VIII. On the other hand, upon simultaneous administration of uracil, cytosine, thymine, guanine, and adenosine (each at 0.025%), hypoxanthine, uridine, cytidine, guanosine, inosine or orotidine[11] (each at 0.0125%) the anticoccidial activity of **1** was not counteracted. These results support the hypothesis that the biochemical basis of anticoccidial activity of **1** is the antagonism of orotic acid metabolism in coccidia.

Experimental

Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded with a Hitachi 260-10 spectrophotometer and ultraviolet (UV) spectra were determined with a Perkin-Elmer 450 spectrophotometer. Mass spectra (MS) were obtained with a Hitachi RMS-4 mass spectrometer. Proton magnetic resonance (PMR) spectra were taken with a Varian T-60, A-60A, EM-390 or XL-100-12 spectrometer and chemical shifts are expressed in ppm (δ) from tetramethylsilane as an internal standard. When 4% NaOD in D_2O was used as a solvent, sodium 2,2-dimethyl-2-silapentane-5-sulfonate was used as an internal standard. The following abbreviations are used: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. PE was carried out in 0.1 M acetate buffer (pH 4.0) (buffer 1) or 0.05 M phosphate buffer (pH 7.5) (buffer 2) at 500 V/40 cm. Solutions were concentrated under reduced pressure with a rotary evaporator.

Methyl 6-Chloro-2-pyrazinecarboxylate 4-Oxide (3)——A solution of **2** (3.45 g, 20 mmol) and *m*-chloroperbenzoic acid (4 g, 23 mmol) in 1,2-dichloroethane (40 ml) was stirred at 65° for 7.5 hr. Further *m*-chloroperbenzoic acid (2 g, 12 mmol) was added and the mixture was stirred at 65° for 20 hr. The resulting mixture

was washed successively with saturated $NaHCO_3$, 5% $Na_2S_2O_4$ and saturated $NaHCO_3$, and dried over $MgSO_4$. The solvent was evaporated off and the residue was chromatographed on silica gel (Merck) (50 g) with $CHCl_3$ as the eluent. The eluate was concentrated and the residue was rechromatographed on silica gel (80 g). Recrystallization from AcOEt-petroleum ether gave colorless needles (1.3 g, 35%), mp 110—112°. *Anal.* Calcd for $C_6H_5ClN_2O_3$: C, 38.22; H, 2.67; N, 14.86. Found: C, 38.48; H, 2.39; N, 14.59. PMR ($CDCl_3$, T-60): 4.03 (3H, s, OCH_3), 8.22 (1H, d, J=2 Hz, 3-H or 5-H), 8.62 (1H, d, J=2 Hz, 5-H or 3-H).

1,6-Dihydro-6-oxo-2-pyrazinecarboxylic Acid 4-Oxide (1)——i) A mixture of **3** (2.92 g, 15.5 mmol) and aqueous NaOH (NaOH 1.86 g, H_2O 31 ml) was stirred at 75° for 5 hr and the ice-cooled reaction solution was adjusted with 20% HCl to pH 1. The precipitate was collected by filtration, washed with cold H_2O and suspended in MeOH (50 ml). The mixture was refluxed for 30 min, and the solution was filtered while hot. The precipitate was washed with MeOH and dissolved in hot H_2O (50 ml). After cooling, the aqueous solution was adjusted with 20% HCl to pH 0.5 and cooled. The precipitate was collected by filtration and washed with H_2O to give a crystalline powder (1.33 g, 55%). For analysis, recrystallization from H_2O gave **1** as crystals, mp>250° (lit.[4] mp>250°). *Anal.* Calcd for $C_5H_4N_2O_4 \cdot 1/4H_2O$: C, 37.39; H, 2.82; N, 17.44. Found: C, 37.58; H, 2.76; N, 17.43. PMR (DMSO-d_6, A-60A): 7.67 (1H, d, J=1.5 Hz, 3-H or 5-H), 7.76 (1H, d, J=1.5 Hz, 5-H or 3-H). IR ν_{max}^{KBr} cm^{-1}: 3450, 3110, 1730, 1650, 1630. UV $\lambda_{max}^{0.1N\ HCl}$ nm (ε): 235.5 (21600), 286 (shoulder) (4600), 330 (5600); $\lambda_{max}^{H_2O}$ nm (ε): 233 (21600), 283 (5000), 335 (5900); $\lambda_{max}^{0.1N\ NaOH}$ nm (ε): 238 (22100), 340 (6300). MS *m/e*: 156 (M^+), 140 (M^+-16), 112 (M^+-44). PE: migration distance 10.3 cm (buffer 1), 14.4 (buffer 2).

ii) Nickel peroxide (24 g) was added to a solution of **5** (5.7 g, 40 mmol) in aqueous NaOH (NaOH 8.8 g, H_2O 400 ml). The mixture was stirred at room temperature for 4 hr and then filtered through a pad of Hyflo Super-Cel, which was washed with H_2O. The filtrate and the washings were combined and concentrated to *ca.* 200 ml. The precipitate was filtered off and the filtrate was adjusted with 20% HCl to pH 3.5. The deposited crystals were dissolved by addition of H_2O, then the solution was adjusted with 20% HCl to pH 1 and cooled to give crystals (2.51 g, 40%), mp >250°. *Anal.* Calcd for $C_5H_4N_2O_4$: C, 38.47; H, 2.58; N, 17.95. Found: C, 38.00; H, 2.60; N, 17.62. PMR (DMSO-d_6, EM-390): 7.67 (1H, d, J=1.5 Hz, 3-H or 5-H), 7.77 (1H, d, J=1.5 Hz, 5-H or 3-H). pK_a': 3.35, 6.7 (determined potentiometrically).

1,6-Dihydro-5-methyl-6-oxo-2-pyrazinecarboxylic Acid 4-Oxide (7)——A solution of 6-hydroxymethyl-3-methyl-2(1*H*)-pyrazinone 4-oxide (**6**)[6] (3.123 g, 20 mmol) in aqueous NaOH (NaOH 800 mg, H_2O 200 ml) was treated with nickel peroxide (12 g) in the manner described for **1**. The ice-cooled solution was adjusted with 20% HCl to pH 1.5 and cooled to give **7** (455 mg, 13%) as colorless crystals, mp >230° (dec.). *Anal.* Calcd for $C_6H_6N_2O_4 \cdot 1/5H_2O$: C, 41.48; H, 3.71; N, 16.12. Found: C, 41.70; H, 3.51; N, 16.15. PMR (DMSO-d_6, EM-390): 2.20 (3H, s, CH_3), 7.60 (1H, s, 3-H). IR ν_{max}^{KBr} cm^{-1}: 1720, 1650. UV $\lambda_{max}^{0.1N\ HCl}$ nm (ε): 238 (21700), 317.5 (6500); $\lambda_{max}^{H_2O}$ nm (ε): 235 (20600), 322.5 (6900); $\lambda_{max}^{0.1N\ NaOH}$ nm (ε): 237 (22400), 332.5 (7400). MS *m/e* (relative intensity): 170 (M^+) (100), 154 (M^+-16) (55), 153 (M^+-17) (60), 126 (M^+-44) (20), 125 (15), 110 (35), 108 (48), 107 (88). PE: M_1[12] 0.94 (buffer 2). The mother liquor was concentrated to *ca.* 5 ml and adjusted with 20% HCl to pH 1 to give additional **7** (345 mg, 10%) as light brown crystals.

1,6-Dihydro-6-oxo-2-pyrazinecarboxylic Acid 4-Oxide Diisopropylamine Salt (8)——Diisopropylamine (931 mg, 9.2 mmol) was added to a suspension of **1** (720 mg, 4.6 mmol) in H_2O (20 ml), and the mixture was stirred for 30 min. After removal of the solvent, the residue was dried by azeotropic distillation with EtOH and recrystallized to give crystals (700 mg).

Methyl 1,6-Dihydro-6-oxo-2-pyrazinecarboxylate 4-Oxide (10)——Dry HCl gas was passed into an ice-cooled suspension of **1** (781 mg, 5 mmol) in MeOH (100 ml) for 3 hr and then at room temperature for 2 hr. The suspension was allowed to stand at room temperature overnight, then the solvent was removed. The residue was recrystallized from MeOH to give colorless leaflets (480 mg, 56%), mp 208—212° (dec.) [lit.[4] mp 206—212° (dec.)]. *Anal.* Calcd for $C_6H_6N_2O_4$: C, 42.36; H, 3.56; N, 16.47. Found: C, 42.45; H, 3.49; N, 16.31. PMR (DMSO-d_6, A-60A): 3.90 (3H, s, OCH_3), 7.87 (1H, d, J=1.5 Hz, 3-H or 5-H), 7.93 (1H, d, J=1.5 Hz, 5-H or 3-H). IR ν_{max}^{KBr} cm^{-1}: 1745, 1640. UV $\lambda_{max}^{0.1N\ HCl}$ nm (ε): 238.5 (21500), 320 (5500); $\lambda_{max}^{H_2O}$ nm (ε): 242 (21600), 282 (shoulder) (2900), 340 (5100); $\lambda_{max}^{0.1N\ NaOH}$ nm (ε): 237 (21300), 337.5 (6400). MS *m/e*: 170 (M^+), 154 (M^+-16), 140 (M^+-30), 126 (M^+-44), 112 (M^+-58). PE: M_1 0.39 (buffer 1), M_1 0.65 (buffer 2).

Butyl 1,6-Dihydro-6-oxo-2-pyrazinecarboxylate 4-Oxide (11)——Compound **10** (700 mg, 4.1 mmol) was added to a solution of sodium (99 mg, 4.1 mg-atom) in *n*-BuOH (80 ml), and the mixture was refluxed for 3 hr with stirring. After removal of *n*-BuOH, the residue was dissolved in a small volume of H_2O. The aqueous solution was adjusted with 20% HCl to pH 1 and cooled. The precipitate was collected by filtration and recrystallized from *n*-BuOH to give colorless needles (602 mg, 69%), mp 149—150°. *Anal.* Calcd for $C_9H_{12}N_2O_4$: C, 50.94; H, 5.70; N, 13.20. Found: C, 50.80; H, 5.60; N, 13.22. PMR (DMSO-d_6, EM-390): 0.93 (3H, t, J=6 Hz, CH_3), 1.20—1.88 (4H, m, $-CH_2CH_2-$), 4.29 (2H, t, J=6 Hz, OCH_2-), 7.80 (1H, d, J=1.5 Hz, 3-H or 5-H), 7.85 (1H, d, J=1.5 Hz, 5-H or 3-H). IR ν_{max}^{KBr} cm^{-1}: 1735, 1650.

1,6-Dihydro-6-oxo-2-pyrazinecarboxamide 4-Oxide (12)——Methanolic NH_3 (30 ml) was added to a suspension of **10** (325 mg, 1.9 mmol) in MeOH (30 ml) with stirring. The mixture was stirred at room temperature for 5 hr, then concentrated, and the residue was dissolved in H_2O. The aqueous solution was adjusted with 20% HCl to pH 1 and cooled. The precipitate was collected by filtration and recrystallized to give colorless needles (286 mg). PE: M_1 0.57 (buffer 1), M_1 0.64 (buffer 2).

Methyl 6-Methoxy-2-pyrazinecarboxylate 4-Oxide (15)——10% Methanolic CH_3ONa (7.5 ml) was added dropwise to an ice-cooled suspension of **3** (1.89 g, 10 mmol) in MeOH (10 ml) with stirring. The mixture was stirred at room temperature for 5 hr and allowed to stand overnight. The resulting solution was adjusted with concentrated HCl to pH 7—8 and evaporated to dryness. The residue was extracted with hot $CHCl_3$ and the extract was concentrated. Next, the residue was chromatographed on silica gel (50 g) with $CHCl_3$ as the eluent. Recrystallization of the product from AcOEt-petroleum ether gave **15** (950 mg, 52%) as colorless needles, mp 132—133°. *Anal.* Calcd for $C_7H_8N_2O_4$: C, 45.66; H, 4.38; N, 15.21. Found: C, 45.60; H, 4.32; N, 15.13. PMR ($CDCl_3$, A-60A); 3.99 (3H, s, OCH_3), 4.08 (3H, s, OCH_3), 7.86 (1H, d, $J=1.5$ Hz, 3-H or 5-H), 8.37 (1H, d, $J=1.5$ Hz, 5-H or 3-H). IR ν_{max}^{KBr} cm^{-1}: 1720, 1595. Undissolved materials after extraction with hot $CHCl_3$ were dissolved in H_2O. The aqueous solution was adjusted with 20% HCl to pH 1, then evaporated to dryness, and the residue was extracted with hot AcOEt. The extract was concentrated and diluted with petroleum ether. The precipitate was collected by filtration and recrystallized from EtOH to give **4** (105 mg, 6%) as needles, mp 249—251° (dec.).

6-Methoxy-2-pyrazinecarboxylic Acid 4-Oxide (4)——2 N NaOH (1 ml) was added to a solution of **15** (368 mg, 2 mmol) in MeOH (30 ml), and the mixture was stirred for 1 hr. The resulting solution was neutralized with 20% HCl, then concentrated, and the residue was dissolved in hot H_2O. The aqueous solution was adjusted with 20% HCl to pH 1 and cooled. The precipitate was collected by filtration and recrystallized from MeOH to give colorless needles (290 mg, 85%), mp 254—255° (dec.). *Anal.* Calcd for $C_6H_6N_2O_4$: C, 42.36; H, 3.56; N, 16.47. Found: C, 42.40; H, 3.38; N, 16.36. PMR (DMSO-d_6, A-60A): 3.99 (3H, s, OCH_3), 8.21 (1H, d, $J=1.5$ Hz, 3-H or 5-H), 8.27 (1H, d, $J=1.5$ Hz, 5-H or 3-H). IR ν_{max}^{KBr} cm^{-1}: 3410, 3100, 3070, 1730, 1600. PE: M_1 0.91 (buffer 1), M_1 0.69 (buffer 2).

6-Methoxy-2-pyrazinecarboxamide 4-Oxide (16)——Methanolic NH_3 (15 ml) was added to a solution of **15** (184 mg, 1 mmol) in MeOH (15 ml). The mixture was stirred for 1 hr and allowed to stand at room temperature for 3 days. The solvent was removed and the residue was recrystallized from H_2O to give colorless needles (105 mg, 60%), mp 269—272° (dec.). PMR (DMSO-d_6, A-60A): 4.03 (3H, s, OCH_3), 7.7—8.1 (2H, br, $CONH_2$), 8.11 (1H, d, $J=1.5$ Hz, 3-H or 5-H), 8.26 (1H, d, $J=1.5$ Hz, 5-H or 3-H). IR ν_{max}^{KBr} cm^{-1}: 1680, 1590.

Methyl 6-Mercapto-2-pyrazinecarboxylate 4-Oxide (17)——H_2S gas was passed into an ice-cooled solution of sodium (322 mg, 14 mg-atom) in MeOH (70 ml) for 30 min, then **3** (1.32 g, 7 mmol) was added. The mixture was stirred at room temperature for 3 hr and allowed to stand overnight. The reaction mixture was concentrated and the residue was dissolved in H_2O (50 ml). Undissolved materials were filtered off, then the filtrate was adjusted with 20% HCl to pH 1 and cooled. The precipitate was collected by filtration and recrystallized from MeOH to give reddish-brown needles (605 mg, 46%), mp 163—164°. *Anal.* Calcd for $C_6H_6N_2O_3S$: C, 38.71; H, 3.25; N, 15.05. Found: C, 38.97; H, 3.22; N, 15.04. PMR (DMSO-d_6, T-60): 3.92 (3H, s, OCH_3), 8.11 (1H, d, $J=1.5$ Hz, 3-H or 5-H), 8.42 (1H, d, $J=1.5$ Hz, 5-H or 3-H). IR ν_{max}^{KBr} cm^{-1}: 1715, 1620. UV $\lambda_{max}^{0.1N\ HCl}$ nm (ε): 291 (23400); $\lambda_{max}^{H_2O}$ nm (ε): 218 (16900), 284 (16000); $\lambda_{max}^{0.1N\ NaOH}$ nm (ε): 276 (14900). PE: M_1 0.80 (buffer 1), M_1 0.65 (buffer 2).

6-Mercapto-2-pyrazinecarboxylic Acid 4-Oxide (18)——2 N NaOH (2.5 ml) was added to a suspension of **17** (372 mg, 2 mmol) in MeOH (50 ml), and the mixture was stirred at room temperature for 4 hr. After removal of MeOH, the residue was dissolved in H_2O. The aqueous solution was adjusted with 20% HCl to pH 0.5 and cooled. The precipitate was collected by filtration and recrystallized from H_2O to give reddish-brown crystals (120 mg, 35%), mp 210—213°. *Anal.* Calcd for $C_5H_4N_2O_3S$: C, 34.88; H, 2.34; N, 16.27. Found: C, 34.61; H, 2.23; N, 16.24. PMR (4% NaOD in D_2O, EM-390): 8.24 (1H, d, $J=1.5$ Hz, 3-H or 5-H), 8.30 (1H, d, $J=1.5$ Hz, 5-H or 3-H). IR ν_{max}^{KBr} cm^{-1}: 1710, 1610. UV $\lambda_{max}^{0.1N\ HCl}$ nm (ε): 286 (22700); $\lambda_{max}^{H_2O}$ nm (ε): 284 (22200); $\lambda_{max}^{0.1N\ NaOH}$ nm (ε): 274 (14600). PE: M_1 0.95 (buffer 1), M_1 1.2 (buffer 2).

Methylation of 10——i) A solution of **10** (170 mg, 1 mmol) in DMF (5 ml) was treated with 50% NaH-mineral oil (50 mg, 1 mmol) and the mixture was stirred at 60—70° for 2 hr, then cooled to room temperature. Methyl iodide (0.07 ml, 1.1 mmol) was added and the whole was stirred at room temperature for 1 hr and at 60—70° for 5 hr. The resulting solution was concentrated, diluted with H_2O and extracted with $CHCl_3$. The extract was washed with H_2O, dried over $MgSO_4$ and concentrated. The residue was chromatographed on silica gel (6 g) with $CHCl_3$ as the eluent. Recrystallization of the products from AcOEt-petroleum ether gave **15** (23 mg, 13%) as colorless needles and **19** (5 mg, 3%) as colorless needles, mp 88—90°. PMR ($CDCl_3$, T-60): 3.67 (3H, s, NCH_3), 3.98 (3H, s, OCH_3), 7.58 (1H, d, $J=2$ Hz, 3-H or 5-H), 7.72 (1H, d, $J=2$ Hz, 5-H or 3-H). IR ν_{max}^{KBr} cm^{-1}: 1735, 1660, 1610.

ii) Methyl iodide (1.5 ml, 24 mmol) was added to a suspension of **10** (1.7 g, 10 mmol) and Li_2CO_3 (740 mg, 10 mmol) in DMA (10 ml), and the mixture was stirred at room temperature for 7 hr. After removal of DMA, the residue was extracted with hot $CHCl_3$ (150 ml). The extract was dried over $MgSO_4$ and concentrated. The residue was chromatographed on silica gel (30 g) with $CHCl_3$ as the eluent to give **15** (100 mg, 5%) and **19** (5 mg). The undissolved materials after extraction with hot $CHCl_3$ were dissolved in MeOH and the solution was filtered. The filtrate was concentrated and the residue was dissolved in H_2O (10 ml). The aqueous solution was adjusted with concentrated HCl to pH 1 and cooled. The precipitate was collected, washed with cold H_2O and recrystallized from MeOH to give **20** (514 mg, 30%) as crystals, mp 177—180° (dec.). *Anal.* Calcd for $C_6H_6N_2O_4$: C, 42.36; H, 3.56; N, 16.47. Found: C, 42.19; H, 3.59;

N, 16.35. PMR (DMSO-d_6, T-60): 3.49 (3H, s, NCH_3), 7.54 (1H, d, $J=2$ Hz, 3-H or 5-H), 7.78 (1H, d, $J=2$ Hz, 5-H or 3-H). IR ν_{max}^{KBr} cm^{-1}: 1725, 1625, 1550. The acidic mother liquor was saturated with NaCl and extracted with AcOEt. The extract was dried over Na_2SO_4 and concentrated. The residue was recrystallized from MeOH to give **4** (10 mg) as a colorless powder.

Methyl 1,6-Dihydro-1-(4-methylbenzyl)-6-oxo-2-pyrazinecarboxylate 4-Oxide (22) and Methyl 6-(4-Methylbenzyloxy)-2-pyrazinecarboxylate 4-Oxide (28)——4-Methylbenzyl bromide (2.59 g, 14 mmol) was added to a suspension of **10** (1.19 g, 7 mmol) and Li_2CO_3 (518 mg, 7 mmol) in DMA (70 ml), and the mixture was stirred at room temperature for 10 hr. After removal of DMA, the residue was diluted with H_2O and extracted with $CHCl_3$. The extract was washed with H_2O, dried over $MgSO_4$ and concentrated. The residue was chromatographed on silica gel (50 g) with toluene–AcOEt (19: 1) followed by toluene–AcOEt (9: 1) as the eluent to give **28** (613 mg) and **22** (180 mg).

1,6-Dihydro-1-(4-methylbenzyl)-6-oxo-2-pyrazinecarboxylic Acid 4-Oxide (25)——A mixture of **22** (180 mg, 0.66 mmol), 0.2 N K_2CO_3 (13.2 ml) and MeOH (6.6 ml) was stirred at 50° for 3 hr and concentrated. The concentrate was adjusted with 20% HCl to pH 1 and cooled to give a crystalline powder (104 mg).

6-Benzyloxy-2-pyrazinecarboxylic Acid 4-Oxide (32)——A mixture of **27** (390 mg, 1.5 mmol) and 0.2 N K_2CO_3 (15 ml) was stirred at 50° for 1 hr. The solution was adjusted with 20% HCl to pH 1 and cooled. The precipitate was collected by filtration, washed with cold H_2O and recrystallized to give colorless leaflets (290 mg).

Acknowledgement The authors are grateful to Dr. H. Nawa, director of this Division, for his permission to publish this paper and to Drs. S. Yamatodani and T. Kanzaki for their encouragement throughout this work. Thanks are also due to Dr. T. Yamazaki for helpful advice.

References and Notes

1) Part V: M. Mano, T. Seo, T. Hattori, T. Kaneko, and K. Imai, *Chem. Pharm. Bull.*, **28**, 2734 (1980).
2) B. Klimeš, *Berlin. München. Tierärztl. Wochenschr.*, **76**, 298 (1963).
3) R.E. Handschumacher, *J. Biol. Chem.*, **235**, 2917 (1960).
4) M. Bobek and A. Bloch, *J. Med. Chem.*, **15**, 164 (1972).
5) S. Okada, A. Kosasayama, T. Konno, and F. Uchimaru, *Chem. Pharm. Bull.*, **19**, 1344 (1971).
6) M. Mano, T. Seo, and K. Imai, *Chem. Pharm. Bull.*, **28**, 2720 (1980).
7) *a*) R.N. Warrener and E.N. Cain, *Aust. J. Chem.*, **24**, 785 (1971); *b*) *Idem*, *Tetrahedron Lett.*, **1967**, 4953.
8) M. Mano, T. Seo, and K. Imai, *Chem. Pharm. Bull.*, **28**, 3057 (1980).
9) M. Mano, T. Seo, T. Matsuno, and K. Imai, *Chem. Pharm. Bull.*, **24**, 2871 (1976).
10) J. Johnson and W.M. Reid, *Experimental Parasitol.*, **28**, 30 (1970).
11) The ammonium salt (purity 90—95%, Sigma Chemical Company) was used.
12) Relative mobility compared to **1**.