Synthesis and Biological Activity of Pyrazines and Pyrazine Ribonucleosides as Pyrimidine Analogs

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Because of the marked antitumor activity which derives from structural modification of the heterocycle of the natural pyrimidine nucleosides (*e.g.*, 3-deazauridine, 5-azacytidine, 6-azauridine), various pyrazine analogs of pyrimidines were prepared. Among these, the uracil analog 1,2-dihydro-2-oxopyrazine 4-oxide, a compound which has been isolated as the antibiotic emimycin, was synthesized by an improved method, involving the oxidation of benzoyloxypyrazine. The ribonucleosides of this analog and of 1,2-dihydro-2-oxopyrazine were prepared by ribosidation of the corresponding trimethylsilyl derivatives in the presence of $TiCl_4$. Also synthesized were a number of derivatives of emimycin, among them the 6-carboxy-, 2-thio-, and the 1-, 5-, and 6-Me analogs. Emimycin and its ribonucleoside were equally active, each inhibiting 50% of the growth of *Streptococcus faecium* and *Escherichia coli* at 8×10^{-6} *M* and 1×10^{-5} *M*, respectively. The orotate analog, 1,2-dihydro-2-oxo-6-carboxypyrazine 4-oxide, interfered with the growth of these organisms at 6×10^{-7} *M* and 4×10^{-4} *M*, respectively. The inhibitory activity of the remaining analogs ranged from 10^{-3} *M* to 4×10^{-5} *M*. The corresponding compounds lacking the *N*-oxide showed no inhibitory activity in these systems. At 10^{-4} *M*, none of the compounds inhibited the *in vitro* growth of leukemia L1210 or Ehrlich ascites cells in excess of 10–20%. Strains of *S. faecium* resistant to emimycin or its ribonucleoside were cross-resistant to each other, but not to the orotate analog. Similarly, a strain resistant to the latter compound retained its sensitivity to the uracil and uridine analogs. The inhibitory effects of emimycin and its ribonucleoside were reversed competitively by uracil, cytosine, and their nucleosides over the concentration range of 10^{-3} to 10^{-6} *M*. Over this range, the growth inhibition exerted by the orotate analog was prevented noncompetitively by these metabolites, but competitively by orotate. Because of the selectivity of the pyrazine derivatives for bacterial cells, their possible use in antibacterial chemotherapy appears worthy of exploration.

Modifications in the heterocycle of the natural nucleosides have resulted in a number of analogs, which have demonstrated marked activity against a variety of experimental tumors. Included among these are compounds such as 3-deazauridine and 3-deazacytidine,[1] 5-azacytidine,[2,3] 6-azathymidine,[4,5] and 6-azauridine.[6-8] An examination of possible further alterations within the pyrimidine ring led us to consider the pyrazine ring, substituted in such a way as to provide analogs of the natural pyrimidines. Investigation revealed the existence of an antibiotic, emimycin,[9,10] which is the 1,2-dihydro-2-oxopyrazine 4-oxide, and which structurally resembles uracil. Indeed, reversal of the emimycin inhibition of *Escherichia coli* by pyrimidines confirmed the structural analogy.[11]

Since no data have been published on the effect of emimycin on mammalian, in particular, tumor cells, we undertook the synthesis of this compound by a new and facile route, and examined its effect upon various cell systems. We also prepared other substituted pyrazines, among which the orotic acid analog showed particularly marked biological activity.

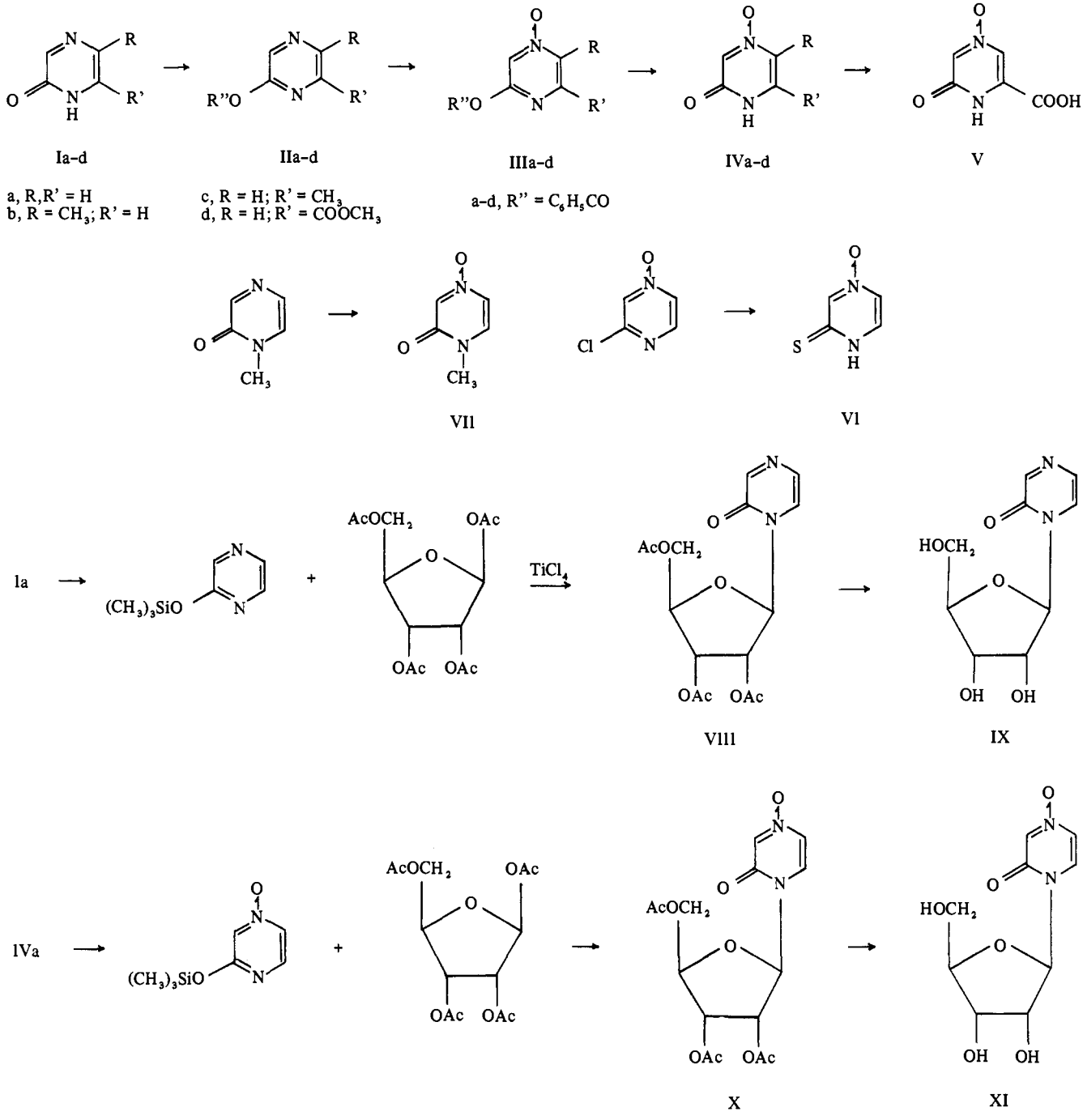
Finally, since nucleoside derivatives frequently offer metabolic advantages over the corresponding bases, particularly with respect to drug resistance, we have prepared the nucleoside derivative of emimycin, and have compared its biological activity with that of the antibiotic. The results of these studies are provided in this paper. A preliminary account of these data has been presented.[12]

Results and Discussion

(a) Chemical Results. The synthesis of 1,2-dihydro-2-oxopyrazine 4-oxide (emimycin) starting from pyrazine-2-carboxamide *via* 2-aminopyrazine 4-oxide has been reported by Terao[10] and independently by Palamidessi and Bernardi.[13] Because of the low yield of the reported procedure (overall yield based on pyrazine-2-carboxamide was 4%) and our need for the compound as a precursor of the nucleoside, an alternative route for the preparation of 1,2-dihydro-2-oxopyrazine 4-oxide was considered, starting from the readily available 1,2-dihydro-2-oxopyrazine (Scheme I). Treatment of Ia–d with PhCOCl in pyridine gave cryst IIa and syrupy IIb–d. The 2-benzoyloxy derivs (IIa–d) were oxidized with *m*-chloroperbenzoic acid, to furnish the corresponding cryst 2-benzoyloxy 4-oxides (IIIa–d). Removal of the protecting Bz group from IIIa–d with MeONa in MeOH gave 1,2-dihydro-2-oxopyrazine 4-oxides (IVa–d). Hydrolysis of the Me ester function of IVd was accomplished by treatment with KOH soln at room temp, to give 1,2-dihydro-2-oxo-6-carboxypyrazine 4-oxide (V). The position of the oxide function of IVa–c and V was established by comparison of the uv absorption spectra of IVa–c and V with those of 1,2-dihydro-2-oxopyrazine 4-oxide,[10] 1,2-dihydro-2-oxo-3,6-dimethylpyrazine 4-oxide,[14] and 1,2-dihydro-2-oxo-3-isobutyl-6-*sec*-butylpyrazine 1-oxide.[15] 1,2-Dihydro-2-thiopyrazine 4-oxide (VI) was prep from 2-chloropyrazine 4-oxide by treatment with NaHS at room temp.

Wagner and Frenzel[16] and later Reisser and Pfleiderer[17] have shown that the direct glucosidation of the Ag or Hg salts of 1,2-dihydro-2-oxopyrazine furnishes the *O*-glucoside and only traces of *N*-glucoside. Their attempts to rearrange the *O*-glucoside to the *N*-glucoside were unsuccessful. As a result, a different route was chosen by us for prepn of the riboside. Ribosidation of the Me_3Si deriv of 1,2-dihydro-2-oxopyrazine in the presence of $TiCl_4$[18] in boiling 1,2-dichloroethane afforded only the *N*-glycoside. No *O*-glycoside was detected by tlc in the reaction mixt. The syrupy 1,2-dihydro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2-oxopyrazine (VIII) was deacetylated by MeONa in MeOH at 22° to give 1,2-dihydro-1-(β-D-ribofuranosyl)-2-oxopyrazine (IX), which was purified by column chromatog on silica gel. The nucleoside IX has been assigned the β configuration on the basis of the trans rule. Attempts to prep the *N*-oxide deriv of VIII or IX by oxidn with *m*-$ClC_6H_4CO_3H$ or

Scheme I



F_3CCO_3H were unsuccessful. The treatment of both VIII and IX with peracids under various conditions[19] resulted in the gradual loss of uv absorption and yielded complex mixts. Treatment of 1,2-dihydro-1-methyl-2-oxopyrazine with *m*-$ClC_6H_4CO_3H$ in boiling 1,2-dichloroethane afforded a low yield of 1,2-dihydro-1-methyl-2-oxopyrazine 4-oxide (VII), demonstrating that the oxidn of N-l-substituted 1,2-dihydro-2-oxopyrazines proceeds only with difficulty. This finding prompted us to investigate the glycosidation of the 1,2-dihydro-2-oxopyrazine 4-oxide. Reaction of the Me_3Si deriv of IVa with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose in presence of $TiCl_4$ in boiling 1,2-dichloroethane produced 1,2-dihydro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2-oxopyrazine 4-oxide (X), which was purified by column chromatog on silica gel. Deacetylation of X with MeONa in MeOH at 22° gave 1,2-dihydro-1-(β-D-ribofuranosyl)-2-oxopyrazine 4-oxide (XI). The structure of XI was established on the basis of its uv and ir absorption spectra and its elemental analysis. The spectral data pertaining to the newly prepd compds are summarized in Table I.

(b) Biological Results. The pyrazine derivs synthesized fall into 3 categories. One comprises compds with substituents at the 2 position only, which cannot strictly be considered analogs of the natural pyrimidines. The second group includes 2-substituted derivs which, in analogy with uracil, carry an O atom at the 4 position. The third class of compds is made up of nucleoside derivs of some of the analogs in the first and second categories.

The compds lacking the 4-oxide did not exert any inhibitory effects on the microbiol test systems used, whereas in the presence of the *N*-oxide, marked inhibition of cell growth resulted (Table II). The same relationship applies to

Table I. Physical Chemical Characteristics of Various Pyrazines[a]

Compd No.	R	R'	R''	R'''	X	Mp, °C	Yield, %	Uv spectra: $\lambda_{max}^{95\% EtOH}$, mμ	ε × 10⁻³	$\lambda_{max}^{pH 12}$, mμ	ε × 10⁻³	Formula[d]
IIIa	H	H	C_6H_5CO			106–107	56	233, 270	20.52, 15.12	262, 337	8.64, 5.24	$C_{11}H_8N_2O_3$
b	CH_3	H	C_6H_5CO			139–140	65	232, 266, 298[c]	21.99, 13.57, 3.45	263, 346	7.41, 5.37	$C_{12}H_{10}N_2O_3$
c	H	CH_3	C_6H_5CO			123–124.5	67	233, 271	21.16, 13.80	262, 336	9.20, 6.67	$C_{12}H_{10}N_2O_3$
d	H	$COOCH_3$	C_6H_5CO			144–145.5	17	238, 275, 313	26.83, 11.94, 1.37	238, 341	21.57, 6.40	$C_{13}H_{10}N_2O_5$
IVa	H	H		H	O	>250 dec	88	222, 278, 331	21.12, 7.25, 3.94	262, 337	8.52, 5.21	$C_4H_4N_2O_2$
b	CH_3	H		H	O	214–216 dec	86	223, 277, 344	23.18, 6.55, 4.53	263, 346	8.69, 5.67	$C_5H_6N_2O_2$
c	H	CH_3		H	O	>250 dec	88	224, 276, 335	16.38, 7.71, 5.19	262, 336	8.34, 6.30	$C_5H_6N_2O_2$
d	H	$COOCH_3$		H	O	206–212 dec	92	243, 285, 326	20.12, 3.54, 4.81	238, 341	21.60, 6.70	$C_6H_6N_2O_4$
V	H	COOH		H	O	>250[c]	86	233,[b] 285, 336	21.13, 5.13, 5.88	238, 341	22.03, 6.64	$C_6H_6N_2O_4 \cdot 0.7\ H_2O$
VI	H	H		H	S	204–206 dec	71	284, 215, 418	16.85, 4.69, 2.77	268, 373	13.22, 2.77	$C_4H_4N_2OS$[e]
VII	H	H		CH_3	O	190–191	7	224, 282, 336	23.46, 8.34, 4.41	281, 336	9.13, 4.72	$C_5H_6N_2O_2$
IX (*N*-oxide absent	H	H		β-D-Ribofuranosyl	O	141–142	40	222, 322	11.20, 5.08	324	5.1	$C_9H_{12}N_2O_5$
XI	H	H		β-D-Ribofuranosyl	O	196–197 dec	10	222, 283, 335	21.74, 10.50, 5.49	282, 335	10.26, 5.25	$C_9H_{12}N_2O_6$

[a] See footnote (†) to Experimental Section. [b] H_2O. [c] Shoulder. [d] All compds were analyzed for C, H, N. [e] S anal. also.

Table II. Growth Inhibitory Effect of Pyrazine Analogs of Pyrimidines[a]

Derivative of pyrazine 4-oxide	Concn for 50% growth inhibition of: *S. faecium*, *M*	*E. coli*, *M*
1,2-Dihydro-2-oxo	8×10^{-6}	1×10^{-5}
1,2-Dihydro-2-oxo-1-(β-D-ribofuranosyl)	8×10^{-6}	1×10^{-5}
1,2-Dihydro-2-oxo-6-carboxy	6×10^{-7}	4×10^{-4}
1,2-Dihydro-2-oxo-6-carbomethoxy	4×10^{-5}	$>10^{-3}$
1,2-Dihydro-2-thio	7×10^{-5}	5×10^{-5}
2-Chloro	2×10^{-5}	3×10^{-5}
2-Ethoxy	2×10^{-5}	1×10^{-5}
1,2-Dihydro-2-oxo-1-methyl	$>10^{-3}$	$>10^{-3}$
1,2-Dihydro-2-oxo-5-methyl	8×10^{-4}	$>10^{-3}$
1,2-Dihydro-2-oxo-6-methyl	9×10^{-4}	$>10^{-3}$

[a] The corresponding compds without the *N*-oxide function, including 1,2-dihydro-1-(β-D-ribofuranosyl)-2-oxopyrazine, did not produce any growth inhibition at 10^{-3} *M*.

the nucleoside derivs, the *N*-oxide being required for activity. At 10^{-4} *M*, none of the compds which showed inhibitory activity in the bacterial systems, inhibited the growth of L-1210 cells *in vitro* by more than 10–20%.

The extent of growth inhibition produced in the microbial system varied according to the nature of the substituents on the ring, and the bacterial species used for the assay. In both test systems, emimycin and its ribonucleoside deriv were equally active, suggesting that they exert their activity *via* a common metabolic intermediate. This suggestion received support from the observation that strains of *S. faecium* and *E. coli* resistant to 10^{-3} *M* emimycin are cross-resistant to the emimycin nucleoside and, similarly, that the strains resistant to 10^{-3} *M* of the nucleoside are cross-resistant to the base.

Replacement of the 2-oxo group of emimycin by =S decreased the inhibitory activity of the antibiotic in the microbiol systems from 5- to 10-fold, whereas a 3-fold decrease in activity occurred when the hydroxyl group was replaced by Cl. The 2-ethoxy deriv was approx 3 times less effective than emimycin against *S. faecium*, but was as active as the antibiotic against *E. coli*.

The introduction of Me into the 1 position of emimycin abolished the inhibitory activity of the antibiotic, whereas Me at its 5 or 6 position allowed for some inhibitory activity in *S. faecium*, but not in *E. coli*. As detd in the *S. faecium* system, the 5-Me deriv of emimycin could not replace thymine for growth of the organism in the basic medium free of folate and contg adenine as the purine source.

Of marked interest is the finding that the pyrazine analog of orotate was more effective than emimycin against *S. faecium*, but was less active than the antibiotic against *E. coli*. This analog retained full activity in the emimycin and emimycin ribonucleoside resistant strains of *S. faecium*, suggesting a different metabolic path for its activation or a different site of action.

An inhibition analysis carried out with *S. faecium* showed (Table III) that the inhibitory effects of emimycin and its ribonucleoside were prevented competitively by uracil and cytosine and their nucleosides at concns ranging from 10^{-3} to 10^{-6} *M*. Over this concn range, the inhibition exerted by the orotate analog was reversed in a noncompetitive (product) manner, indicating that the pyrazine analog of orotate does not interfere with the conversion of exogenous uracil or uridine to UMP and to further metabolic intermediates, but likely exerts its effect along the *de novo* path leading to UMP. Further support for this deduction comes from the

Table III. Reversal, by Pyrimidines, of the Inhibition of Growth of *S. faecium* by Pyrazines

Substrate	Inhibition index[a] obtained with 1,2-Dihydro-2-oxopyrazine 4-oxide	1,2-Dihydro-1-(β-D-ribofuranosyl)-2-oxopyrazine 4-oxide	1,2-Dihydro-2-oxo-6-carboxypyrazine 4-oxide
Uridine	20	20	Noncompetitive
2'-Deoxyuridine	10	11	Noncompetitive
Cytidine	0.9	0.8	Noncompetitive
2'-Deoxycytidine	1	2	Noncompetitive
Uracil	0.7	0.9	Noncompetitive
Cytosine	0.4	0.5	Noncompetitive
Thymine	2- to 3-fold reversal at 10^{-3} *M*	2- to 3-fold reversal at 10^{-3} *M*	2- to 3-fold reversal at 10^{-3} *M*
Thymidine	10-fold reversal at 10^{-3} *M*	10-fold reversal at 10^{-3} *M*	10-fold reversal at 10^{-3} *M*
Orotic acid	2-fold reversal at 10^{-3} *M*	2-fold reversal at 10^{-3} *M*	6

[a] [I]/[S] for 50% growth inhibition, with substrate concns ranging from 10^{-3} to 10^{-6} *M*.

observation that orotate reversed the inhibitory effect of the orotate analog in a competitive manner, whereas, at the highest concn of 10^{-3} *M*, this metabolite prevented the growth inhibition exerted by emimycin and its nucleoside by only 1.5- to 2-fold.

The fact that orotate does not reverse the emimycin or emimycin riboside action extensively, might mean that the uracil analogs act at the base or ribonucleoside level *per se*, or that competition for their activation *via* a pyrophosphorylase or kinase is exerted more effectively by the exogenously supplied uracil or uridine than by UMP, obtainable in a possibly limited amount, from exogenous orotate.

At 10^{-3} *M*, the highest concn used, thymine or thymidine reversed the inhibitory effect of the analogs from 4- to 15-fold in a noncompetitive manner, the orotate analog being reversed more effectively than emimycin or its ribonucleoside. This relatively small extent of reversal suggests a sparing effect of thymine or thymidine on *de novo* pyrimidine synthesis. It might be added here, that, in analogy with our observations in *S. faecium*, the inhibition of the growth of *E. coli* by emimycin was also found to be competitively reversed by uracil.[11]

It remains to be detd why, as compared to their effect on the bacteria, the analogs are only marginally active against the mammalian cells. However, because of this pronounced selectivity, the potential use of emimycin and its derivs in antimicrobial chemotherapy appears worthy of exploration.

Experimental Section†

2-Benzoyloxypyrazine 4-Oxide (IIIa). 2-Benzoyloxypyrazine[20] (4.00 g, 0.02 mole) was dissolved in dry 1,2-dichloroethane ($C_2H_4Cl_2$, 50 ml). To this soln *m*-chloroperoxybenzoic acid (85% product, 4-$ClC_6H_4CO_3H$, 5.00 g) was added and the reaction mixt was heated at 55–60° for 15 hr. The soln was then cooled to 20°, washed successively with a satd soln of $NaHCO_3$ (80 ml) and H_2O (80 ml), and dried (Na_2SO_4). The solvent was removed, and the remaining syrupy product was dissolved in Et_2O (15 ml). Cryst IIIa which sepd from the soln after standing for several hours at 20° was collected by filtration and recrystd from MeOH.

2-Benzoyloxy-5-methylpyrazine 4-Oxide (IIIb). 5-Methyl-1,2-dihydro-2-oxopyrazine[21] (2.20 g, 0.02 mole) was added to dry pyridine (20 ml). To this mixt BzCl (3.1 g, 0.022 mole) was added dropwise with stirring. The reaction mixt, after standing at 20° for 15 hr, was poured into 50 ml of ice-cold H_2O. An amber oil sepd which was extd with PhMe (80 ml). This ext was washed with H_2O (4 × 100 ml) and dried (Na_2SO_4). PhMe was removed by evapn and the remaining oily residue was dissolved in xylene (50 ml). This soln was concd to a thick oil which was dissolved in $C_2H_4Cl_2$ (80 ml). 4-$ClC_6H_4CO_3H$ (5 g) was added to the soln and the work-up proceeded as described for the prepn of IIIa.

2-Benzoyloxy-6-methylpyrazine 4-oxide (IIIc) was prepd from 1,2-dihydro-2-oxo-6-methylpyrazine[21] by the procedures described under IIIb.

2-Benzoyloxy-6-carbomethoxypyrazine 4-Oxide (IIId). BzCl (3.1 g, 0.022 mole) was added dropwise with stirring to a mixt of Id[22] (3.1 g, 0.02 mole) and dry pyridine (20 ml), and the mixt was worked up as above. To this soln, 4-$ClC_6H_4CO_3H$ (4 g) was added, and the mixt was heated at reflux temp for 2 hr. The soln was cooled to 30–40° and addnl 4-$ClC_6H_4CO_3H$ (2.5 g) was added. The reaction mixt was heated at reflux temp for another 2 hr, cooled to 20°, and dild with $CHCl_3$ (80 ml). The soln was washed with a satd soln of $NaHCO_3$ (80 ml) and then with H_2O, and dried (Na_2SO_4). It was concd to an oil, which was chromatogd on a silica gel column in C_6H_6–EtOAc (6:1, v/v). Concn of the eluate containing Id (as shown by tlc) furnished cryst substance, which was recrystd from Me_2CO–Et_2O. The column was subsequently washed with Me_2CO–MeOH (6:1, v/v). The eluate was evapd to dryness and the residue was recrystd twice from EtOH furnishing 400 mg of 1,2-dihydro-2-oxo-6-carbomethoxypyrazine 4-oxide (IVd): $\bar{\nu}_{max}$ cm^{-1} 3110, 3090, 2960 (CH), 1745 (C=O), 1260 – 1250 (N→O).

1,2-Dihydro-2-oxopyrazine 4-Oxide (IVa). IIIa (2.16 g, 0.01 mole) was dissolved in MeOH (100 ml) and a 2 *N* MeONa–MeOH soln was added dropwise with stirring until pH 9–10 was achieved. MeOH-washed Dowex 50 (H^+) resin (10 ml) was added to the soln with stirring. The resin was filtered and washed with MeOH (80 ml). The MeOH soln was evapd, the residue suspended in EtOH (50 ml) and reevapd to a solid, which was recrystd from 96% EtOH.

1,2-Dihydro-2-oxo-5-methylpyrazine 4-oxide (IVb) was prepd from IIIb in the same manner as described for IVa.

1,2-Dihydro-2-oxo-6-methylpyrazine 4-oxide (IVc) was prepd from IIIc (2.30 g, 0.01 mole) following the procedure described for the prepn of IV, except that a larger vol of MeOH (200 ml) was used because of the poor solubility of both IIIc and IVc.

1,2-Dihydro-2-oxo-6-carbomethoxypyrazine 4-oxide (IVd) was prepd from IIId (2.74 g, 0.01 mole) in the same manner as described for IVc: $\bar{\nu}_{max}$ 3160, 3080, 2940, 2825 (NH, CH), 1740 (C=O carboxyl), 1660–1620 (C=O, C=C, C=N) 1295, 1260, 1230, 1025, 825 cm^{-1} (N→O, COC).

1,2-Dihydro-2-oxo-6-carboxypyrazine 4-Oxide (V). IVd (0.340 g, 0.001 mole) was dissolved in a KOH (0.4 g) soln in H_2O (60 ml). After 2 hr at 22°, the soln was applied to a column (1 × 20 cm) of Dowex 50 (H^+) resin. The column was washed with H_2O and the acid fraction was collected and evapd. The residue was recrystd from H_2O: $\bar{\nu}_{max}$ 3500 (OH), 3080, 2820 (broad) (NH, CH), 1730 (C=O), 1630 (broad) (C=O, C=C, C=N), 1275, 1245 cm^{-1} (broad) (N→O).

1,2-Dihydro-2-thiopyrazine 4-Oxide (VI). 2-Chloropyrazine 4-oxide[23] (1.30 g, 0.01 mole) was dissolved with stirring in a soln of NaHS, prepd by satg a soln of 0.46 g Na in EtOH (50 ml) with H_2S at 5°. The reaction mixt was stirred at 22° for 15 hr and evapd, the residue was suspended in EtOH–H_2O (1:1, v/v; 40 ml), and the pH of the mixt was adjusted to 4–5 with HCl. The mixt was then evapd to dryness, the residue was extd with boiling 98% EtOH (3 × 150 ml) and filtered while hot. The filtrate was concd to 50 ml and allowed to stand at 5° for 4 hr. The product was collected by filtration and recrystd from 96% EtOH: $\bar{\nu}_{max}$ 3125, 3080, 2945, 2840 (NH, CH), 1250, 1215, 1130, 1105 cm^{-1} (C=S, N→O).

1,2-Dihydro-1-methyl-2-oxopyrazine 4-Oxide (VII). A soln of 1,2-dihydro-1-methyl-2-oxopyrazine[24] (0.550 g, 0.005 mole) and 4-$ClC_6H_4CO_3H$ (1.0 g) in $C_2H_4Cl_2$ (60 ml) was heated at reflux temp for 2 hr and was then cooled to 30–40°. Addnl 4-$ClC_6H_4CO_3H$ (1.0

†Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Optical rotation are equilibrium values and were detd on a Jasco ORD/UV Model 5 instrument at 0.2% concn in MeOH. Uv spectra were obtained on a Cary Model 14 recording spectrophotometer. The ir absorption spectra were detd in pressed KBr disks with a Perkin-Elmer Model 521 spectrophotometer. Solvent concn was conducted under reduced pressure in a rotary evaporator.

g) was added, and the reaction mixt was heated at reflux temp for another 2 hr. The soln was cooled to 22°, concd to 5-8 ml, and chromatogd on a silica gel column with PhH–Me_2CO (1:1, v/v). The fraction contg IV (as shown by tlc) was evapd, and the residue was recrystd from EtOH–Et_2O.

1,2-Dihydro-1-(β-D-ribofuranosyl)-2-oxopyrazine (IX). A mixt of 1,2-dihydro-2-oxopyrazine (4.3 g, 0.045 mole), $(Me_3Si)_2NH$ (4 ml), and Me_3SiCl (3 ml) was heated at reflux temp with stirring for 1 hr. After cooling to 22°, PhMe (80 ml) was added, and the soln was concd to an oil, which was dissolved in dry $C_2H_4Cl_2$ (250 ml). 1,2,3,5-Tetra-*O*-acetyl-β-D-ribofuranose (9.55 g, 0.03 mole) and $TiCl_4$ (3 ml) were added to this soln, and the reaction mixt was heated for 5 hr at reflux temp with stirring and exclusion of atm moisture. The mixt was cooled to 22° and poured slowly with vigorous stirring into a satd soln of $NaHCO_3$ (1500 ml). It was filtered through a Celite pad, which was then washed with $CHCl_3$. The org layer was sepd, washed with H_2O, and dried (Na_2SO_4). The solvent was evapd, and the residue was dissolved in MeOH (100 ml). A catalytic amt of MeONa was added to this soln and after 0.5 hr at 22°, the soln was neutralized with HCl and evapd. The syrupy residue was dissolved in MeOH (5 ml) and applied to a dry column (2.5 × 8 cm) of silica gel. A Me_2CO–MeOH (4:1, v/v) mixt was applied to the column and the eluate was evapd. The syrupy residue was dissolved in Me_2CO–EtOH (1:2, v/v; 30 ml); and crystn occurred after standing of the soln overnight at 22°. Recrystn from EtOH gave pure IX: $[\alpha]^{25}D$ −21.2°, $\bar{\nu}_{max}$ 3400, 3280 (OH), 2945–2920 (CH), 1645 cm^{-1} (C=O).

1,2-Dihydro-1-(β-D-ribofuranosyl)-2-oxopyrazine 4-Oxide (XI). A mixt of IVa (1.12 g, 0.01 mole), $(Me_3Si)_2NH$ (3 ml), and Me_3SiCl (3 drops) was heated for 0.5 hr at 90–95° with stirring and exclusion of atm moisture. The resulting soln was cooled to 22°, dild with dry PhMe (40 ml), and evapd. The remaining solid was evapd once more from dry PhMe (40 ml), and dissolved in dry $C_2H_4Cl_2$ (100 ml). To this soln was added 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (3.20 g, 0.01 mole) and $TiCl_4$ (3 ml), and the reaction mixt was heated at reflux temp with stirring and exclusion of atm moisture for 4 hr. It was cooled to 22° and poured slowly, with vigorous stirring, into a satd soln of $NaHCO_3$ (500 ml). The mixt was filtered through a Celite pad, which was subsequently washed with $CHCl_3$. The org layer was sepd, washed with H_2O, and dried (Na_2SO_4). The syrup remaining after removal of the solvent was purified by column chromatog on silica gel with $CHCl_3$–Me_2CO (7:1, v/v) as the eluent. The syrupy 1,2-dihydro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2-oxopyrazine 4-oxide (X) (480 mg) obtd by evapn of the solvent was dissolved in MeOH (50 ml). A catalytic amt of MeONa was added to this soln and after 0.5 hr at 22° the soln was neutralized with Dowex 50 (H^+) resin. The resin was filtered and washed with MeOH (20 ml). The syrup remaining after removal of the MeOH was dissolved in 96% EtOH and purified by column chromatog on silica gel with Me_2CO as the eluent. Me_2CO was removed by evapn and the remaining solid was recrystd from 96% EtOH: $[\alpha]^{25}D$ −204.2°, $\bar{\nu}_{max}$ 3400, 3300 (OH), 2960, 2925 (CH), 1660 (C=O), 1230, 840, 830 cm^{-1} (N→O).

Biological Assays. The techniques used for these detns have been published previously.[25]

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Synthesis and Biological Activity of 4′-Thio Analogs of the Antibiotic Toyocamycin

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The 4′-thio analog of the antibiotic toyocamycin was prepared by condensation of 2,3,5-tri-*O*-acetyl-4-thio-D-ribofuranosyl chloride with the chloromercuri derivative of 4-acetamino-6-bromo-5-cyanopyrrolo-[2,3-*d*]pyrimidine, followed by removal of the protecting groups with MeOH–NH_3 and removal of Br with H_2/Pd catalyst. Condensation with the chloromercuri derivative of 4-chloro-6-bromo-5-cyanopyrrolo-[2,3-*d*]pyrimidine, followed by treatment with MeOH–NH_3 at 5°, effected removal of the protecting groups and nucleophilic substitution of the Br group to furnish 4-chloro-6-amino-5-cyano-7-(4-thio-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine. When treatment with MeOH–NH_3 was carried out at 120°, 4,6-diamino-5-cyano-7-(4-thio-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine was formed. The 4′-thio derivatives proved to be effective inhibitors of the growth of leukemia L-1210 cells *in vitro*, their concn for 50% reduction of growth ranging from 4×10^{-7} to 5×10^{-6} *M*. 4′-Thiotoyocamycin retained full inhibitory activity against a strain of *Streptococcus faecium* resistant to 10^{-3} *M* toyocamycin.

The antibiotic toyocamycin[1] is an analog of adenosine in which N-7 of the imidazole ring is replaced by C, to which is attached a CN group.[2] In exptl systems, this antibiotic showed marked antitumor activity,[3] but the severe local toxocity in man[4] which it produced limited its clinical usefulness.

In an attempt at decreasing this toxicity, two structural modifications of the toyocamycin molecule were made. In one, the ring O of the carbohydrate moiety was replaced by S; the other involved, in addition to this replacement, the substitution of the 4 and 6 position of the heterocycle with Cl and amino groups, resp. The results obtained in *in vitro*