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Synthesis and Biological Activity of Some Imidazo[4,5-*b*]pyrazines and Their Ribonucleosides as Purine Analogs

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The 1-β-D-ribofuranosyl derivative **9** of 1*H*-imidazo[4,5-*b*]pyrazine (**5**), a structural analog of the antibiotic nebularine (9-β-D-ribofuranosylpurine), has been prepared by acid-catalyzed fusion of **5** with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose and subsequent removal of the acetyl groups. Oxidation of **5** with peracetic acid furnished 1*H*-imidazo[4,5-*b*]pyrazine 4(7)-oxide (**6**). Oxidation of the triacetyl derivative of **9** with *m*-chloroperoxybenzoic acid, followed by the removal of the protecting groups, provided the inosine analog 1-(β-D-ribofuranosyl)imidazo[4,5-*b*]pyrazine 4-oxide (**11**). The site of attachment of the ribosyl moiety in **9** and **11** was substantiated by comparison of their uv spectra with those of the model 1-methylimidazo[4,5-*b*]pyrazine (**13**) and its 4-oxide **15**, synthesized by unequivocal routes. The structure of **11** was also confirmed by X-ray crystallography. In the crystal, the nucleoside exists in the anti conformation, the oxygen of the *N*-oxide function participating in hydrogen bonding with the 2′-hydroxyl group of a neighboring molecule. Compounds **6** and **11** inhibited the *in vitro* growth of *Escherichia coli* K_{12} by 50% at 1×10^{-5} and 6×10^{-6} *M*, respectively. The nebularine analog **9** was inactive. The inhibition of growth was reversed competitively by the natural purines. None of the analogs interfered with the *in vitro* growth of leukemia L-1210, mammary carcinoma TA-3, and Burkitt's lymphoma cells. In a cell free extract from *E. coli*, the ribofuranosyl derivative **11** underwent cleavage of its glycosidic bond to furnish the base **6**. Imidazopyrazine 4(7)-oxide (**6**) interfered with the activity of xanthine oxidase from milk, whereas the nucleoside **11** was ineffective.

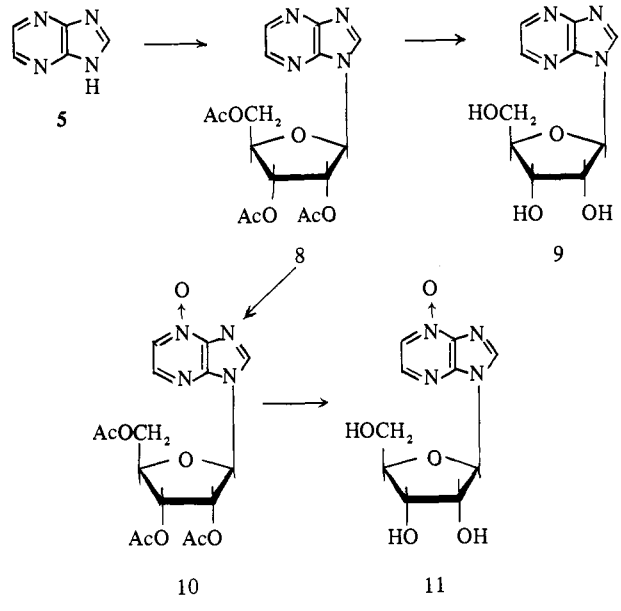
The antibiotic 1,2-dihydro-2-oxopyrazine 4-oxide (emimycin)[1] and its ribofuranosyl[2] and deoxyribofuranosyl[3] derivatives, have recently been synthesized. These compounds, which are structural analogs of uracil and its nucleosides, showed marked inhibitory activity in various microbial systems. Because of this activity, structural analogs of various naturally occurring purines have been prepared, wherein a pyrazine ring replaces the pyrimidine moiety of the purine heterocycle. These compounds, too, have shown biological activity, and this communication reports their synthesis and some of their biological effects. Part of this study was presented in a preliminary report.[4]

Results and Discussion

Chemistry. The synthesis of the target compound 1*H*-imidazo[4,5-*b*]pyrazine 4- (or 7-) oxide (**6**) was envisioned to proceed from the known[5] precursor 2-amino-3-chloropyrazine (**1**) *via* its oxidation to 2-amino-3-chloropyrazine 1-oxide (**2**), amination to 2,3-diaminopyrazine 1-oxide (**3**), and ring closure with ethyl orthoformate. Although these reactions proceeded smoothly, **3** was obtained only in very low yield. Therefore, an alternate synthetic route was chosen utilizing 1*H*-imidazo[4,5-*b*]pyrazine (**5**), prepared from 2,4-diaminopyrazine (**4**) according to the procedure of Day, *et al.*[5] Oxidation of **5** with H_2O_2 (30%) in AcOH provided a mixture consisting of **6** and 1*H*-imidazo[4,5-*b*]-pyrazine 4,7-dioxide (**7**), the former being the major product. The 4- (7-) oxide derivative **6** was separated from the mixture by fractional crystallization in 50% yield. Paper chromatography of the remaining mixture in *n*-BuOH–AcOH–H_2O (65:15:20) afforded the 4,7-di-*N*-oxide derivative **7** in about 8% yield. Although the site of attachment of the oxygen in **6** was not determined directly, the similarity of the uv spectra of **6** in MeOH and in 0.1 *N* HCl with those of **11** and **15** favors N-4 as the likely site of attachment.

Acid-catalyzed fusion (Scheme I) of **5** with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose gave a crude product which was purified by silica gel column chromatography to afford the blocked nucleoside **8** in 55% yield. Subsequent removal of the blocking groups with methanolic ammonia furnished

Scheme I

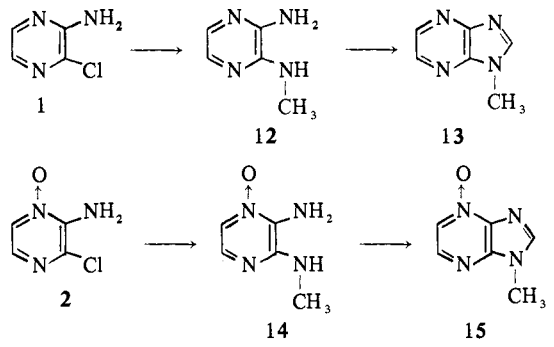


1-(β-D-ribofuranosyl)imidazo[4,5-*b*]pyrazine (**9**), an analog of nebularine,[6,7] in 75% yield.

Treatment of **8** with *m*-chloroperoxybenzoic acid in 1,2-dichloroethane afforded the 4-*N*-oxide derivative **10**. Purification of **10** by column chromatography, followed by deblocking with methanolic ammonia, gave 1-(β-D-ribofuranosyl)imidazo[4,5-*b*]pyrazine 4-oxide (**11**) in 68% yield. That N-1 is the site at which the ribosyl moiety is attached in the compounds **9** and **11** was substantiated by comparison of the uv spectra of these nucleosides with the unambiguously synthesized model compounds **13** and **15** (Scheme II).

1-Methylimidazo[4,5-*b*]pyrazine (**13**) was prepared in 75% yield by reacting **1** with 40% aqueous $MeNH_2$ in the presence of a catalytic amount of Cu powder under pressure at 140–150°, followed by ring closure with ethyl orthoformate (Scheme II). An analogous route was utilized for the preparation of 1-methylimidazo[4,5-*b*]pyrazine 4-oxide

Scheme II



(**15**) starting with the intermediate **2**. The uv spectral data pertaining to the newly synthesized compounds are summarized in Table I.

Crystallography. The crystal structure of 1-(β-D-ribofuranosyl)imidazo[4,5-*b*]pyrazine 4-oxide was solved by direct methods[8] and was refined to an *R* index of 0.045, all of the hydrogens being identified. The three-dimensional structure of the molecule is shown in Figure 1, identifying N-1 as the site of attachment of the ribosyl moiety and N-4 as the site of the *N*-oxide function. In the crystal, the molecule exists in the anti conformation, with a torsion angle of 7.96° defined by the ring oxygen, by C-1 of the carbohydrate, and by N-1 and C-2 of the heterocycle. The N → O bond distance is 1.302 Å and, in the crystal, the *N*-oxide forms a hydrogen bond with the proton of the 2′-hydroxyl group of a neighboring molecule with a bond distance of 2.85 Å. A more detailed analysis of the structure will be published elsewhere.

Biological and Biochemical Results. The growth inhibitory activity of the newly synthesized compounds was evaluated in both microbial and mammalian cell systems. As shown in Table II, only imidazopyrazine 4(7)-oxide and, to a somewhat greater extent, its nucleoside derivative showed inhibitory activity against *E. coli* K_{12} and against a mutant strain of *E. coli* (K_{12}/pu) which, unlike K_{12}, is not sensitive to inhibition by adenine or hypoxanthine or by their nucleosides. The growth of three mammalian cell lines was not affected by any of these analogs. This finding is of interest, since nebularine is a potent inhibitor of mammalian cell growth.[9] The inactivity of these compounds in the mammalian cell systems parallels the observation made with the pyrazine 4-oxide analogs of uracil and uridine which are active against bacteria but not against mammalian cells.[2] The basis for the apparent species specificity of these *N*-oxide analogs of purines and pyrimidines has not as yet been established. The *N*-oxide function appears to be essential for biological activity, since imidazopyrazine and its nucleoside, like 2-hydroxypyrazine and its nucleoside,[2] are inactive in the microbial system.

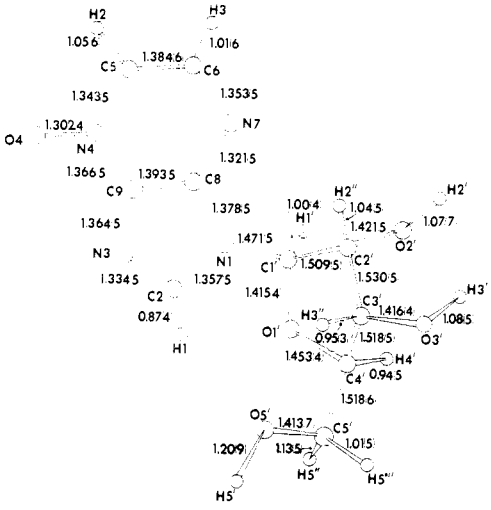


Figure 1.

That these *N*-oxide derivatives act as antimetabolites is indicated by the observation that their inhibitory effect is reversed by the corresponding metabolites. The inhibitory activity of the pyrazine analogs of uracil, uridine, or orotate is prevented competitively by the natural pyrimidines,[2] whereas the effect of imidazopyrazine 4(7)-oxide and its ribonucleoside is reversed competitively by adenine, guanine, hypoxanthine, or by their nucleosides. The inhibition indices (ratios of inhibitor concentration to substrate concentration for 50% growth) obtained with the various purines as substrates and the imidazopyrazine 4(7)-oxide and its riboside as inhibitors were quite similar, ranging from 88 to 100. Furthermore, for corresponding purines, the inhibition indices for the imidazopyrazine 4(7)-oxide coincided with those for the ribonucleoside derivative, suggesting that the two compounds act similarly. Indeed, as determined in cell free extracts of *E. coli*, the nucleoside analog is susceptible to enzymatic cleavage of its glycosidic bond. After 1 hr of incubation at 37° with 0.5 ml of the extract (6.6 mg of protein/ml), 55.4% of the nucleoside was found converted to the base. Whether the compounds undergo phosphorylation remains to be determined.

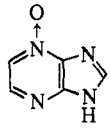
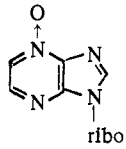
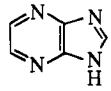
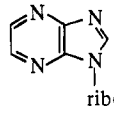
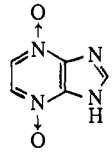
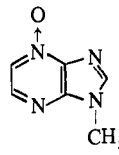
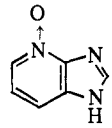
From the point of view of structure as it affects activity, it is of interest to note that the inhibitory activity depends not only on the presence of the *N*-oxide function but also on the presence of N-7 in the imidazopyrazine molecule, the position which corresponds to N-3 of the purine ring. Thus, 1*H*-imidazo[4,5-*b*]pyridine 4-oxide (**16**) was inactive in all the test systems used. Similarly, substitution of N-1, the position which corresponds to N-9 of the purine ring, with a methyl group renders the resulting imidazopyrazine 4-oxide derivative inactive. This finding may signify that ribosidation is required for activation.

Table I. Physical Characteristics of Various Pyrazines, Imidazo[4,5-*b*]pyrazines, and Imidazo[4,5-*b*]pyrazine Ribonucleosides[a]

Compd	Mp, °C	Uv spectra, λ_{max}, nm ($\epsilon_{max} \times 10^{-3}$)		
		MeOH	0.1 *N* HCl	0.1 *N* NaOH
2	147–148	235 (20.65), 263,[b] 340 (7.17)	230 (19.46), 255,[b] 337 (6.30)	232 (19.57), 263,[b] 338 (5.87)
3	170 dec			253, 293
6	>300	221 (20.39), 305 (14.48)	215 (12.99), 303 (12.10)	220 (16.32), 246 (5.62), 317 (12.78)
7	>300	225 (4.71), 264 (2.28), 348 (4.71)	227 (7.07), 260 (1.90), 331 (6.16)	220 (6.23), 258 (2.36), 337 (2.87)
9	205–206	255,[b] 290 (11.84)	247 (2.27), 288 (8.69)	245 (6.06), 325 (5.04)
11	228–229	227 (17.55), 303 (16.35)	222 (23.05), 305 (20.77)	236 (26.8), 337 (9.92)
13	192–193	255,[b] 290 (13.94)	241 (1.61), 284 (9.90)	245,[b] 290 (9.65)
15	290–291	227 (19.79), 304 (17.54)	220 (20.91), 245,[b] 305 (17.54)	236,[b] 310 (5.85)

[a] Imidazo[4,5-*b*]pyrazine ribonucleosides are unstable in 0.1 *N* NaOH. [b] Shoulder.

Table II. Effect of Imidazopyrazines on the *in Vitro* Growth of Various Cell Systems

Compound	Structure	Molar concn for 50% inhibition of the growth of	
		E. coli K_{12}	Leukemia L-1210, mammary carcinoma TA-3, Burkitt's lymphoma
1*H*-Imidazo[4,5-*b*]pyrazine 4-oxide		1×10^{-5}	$>10^{-4}$
1-(β-D-Ribofuranosyl)imidazo[4,5-*b*]-pyrazine 4-oxide		6×10^{-6}	$>10^{-4}$
1*H*-Imidazo[4,5-*b*]pyrazine		$>10^{-3}$	$>10^{-4}$
1-(β-D-Ribofuranosyl)imidazo[4,5-*b*]-pyrazine		$>10^{-3}$	$>10^{-4}$
1*H*-Imidazo[4,5-*b*]pyrazine 4,7-dioxide		$>10^{-3}$	$>10^{-4}$
1-Methylimidazo[4,5-*b*]pyrazine 4-oxide		$>10^{-3}$	$>10^{-4}$
1*H*-Imidazo[4,5-*b*]pyridine 4-oxide		$>10^{-3}$	$>10^{-4}$

The requirement for an *N*-oxide function and the presence of N-7 is also required for imidazopyrazine 4-oxide to act as an inhibitor of xanthine oxidase. Imidazopyrazine and imidazopyridine 4-oxide were inactive, as were the nebularine analog and its 4-oxide derivative. Neither of these compounds showed any substrate activity. The inhibition exerted by the imidazopyrazine 4-oxide was competitive, with a K_i of $1 \times 10^{-5} M$.

A comparison of the crystal structure of the inosine analog (Figure 1) with that of inosine[10,11] reveals some of the differences between the two molecules which may contribute to their differential activity. Thus, the N→O bond at 1.302 Å is larger than the C=O bond distance of inosine which is 1.22 Å. Further, the *N*-oxide function binds at a distance of 2.61 Å with the hydrogen of the 2′-OH group of a neighboring molecule, whereas the oxygen at the 6 position of inosine binds at 2.85 Å with the hydrogen of the 5′-OH group of a neighboring inosine molecule. Of interest also is the fact that the proton at N-1 of inosine hydrogen bonds with N-7 of another molecule, whereas the methene hydrogen at the corresponding position in the analog cannot form such a bond.

The selectivity of the imidazopyrazine oxide analogs of hypoxanthine and inosine for bacterial systems is worthy of further evaluation, with a view toward their use as selective antimicrobial agents.

Experimental Section

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Uv spectra were recorded on a Cary Model 14 spectrophotometer. Optical rotations were determined with a Perkin-Elmer 141 polarimeter with 1-dm path length and nmr spectra on a Varian A-60 spectrometer. The mass spectra were recorded on a CEC 21-491 double focusing mass spectrometer using an ionization voltage of 70 eV. Satisfactory analyses (C, H, N, within 0.4% of the theoretical values) were obtained (Galbraith Laboratories, Inc., Knoxville, Tenn). Evaporations were carried out under reduced pressure in a rotary evaporator.

2-Amino-3-chloropyrazine 1-Oxide (2). 2-Amino-3-chloropyrazine[5] (3.30 g, 0.03 mol) was suspended in 60 ml of glacial AcOH, and 9 ml of 30% H_2O_2 in 20 ml of AcOH was added with stirring at room temperature over a period of 30 min. The reaction flask was then transferred to an oil bath preheated at 60–75°, and the progress of the reaction was monitored by tlc. After 30 hr, the reaction mixture was evaporated to dryness. The residue thus obtained was recrystallized from H_2O to yield 2.7 g (76%) of light yellow long needles: nmr (DMSO-d_6) δ 7.38 (b, 2,2-NH_2), 7.72 (d, 1, $J_{5,6}$ = 4.2 Hz, 5-H), 8.36 (d, 1, $J_{5,6}$ = 4.2 Hz, 6-H); mass spectrum *m/e* 147, 145 (M^+), 131, 129 (M − 16). *Anal.* ($C_4H_4ClN_3O$) C, H, N.

2,3-Diaminopyrazine 1-Oxide (3). A mixture of aqueous NH_3 (28%, 15 ml) and **2** (2.90 g, 0.02 mol), along with a catalytic amount of freshly prepared Cu powder was heated at 140–150° for 18 hr in a steel bomb. Tlc of the reaction mixture showed three major spots along with some minor components. The monoxide **3** was separated from the mixture by preparative tlc using EtOAc–MeOH (70:30). The product was obtained as brown crystals: yield 0.176 g (7%); mass spectrum *m/e* 126 (M^+), 110 (M − 16). *Anal.* ($C_4H_6N_4O$)

C, H, N. The other major product was also isolated by preparative tlc and characterized as 2,3-diaminopyrazine (4) by comparison of its properties with the authentic material.

1*H*-Imidazo[4,5-*b*]pyrazine (5). This compound was synthesized by reported procedures[5] using glyoxal bisulfite and 2-aminomalonamide as intermediates. Recrystallization from H_2O afforded **5** in 70% yield: mp 258–259° (lit.[5] 257); nmr (DMSO-d_6) δ 8.65 (b s, 2, 5-H, 6-H), 9.0 (s, 1, 2-H); mass spectrum *m/e* 120 (M^+).

1*H*-Imidazo[4,5-*b*]pyrazine 4-Oxide (6). **5** (4.8 g, 0.04 mol) was suspended in glacial AcOH (20 ml), and 30% H_2O_2 (5 ml in 10 ml of glacial AcOH) was added dropwise over a period of 30 min. The reaction mixture was then transferred to a bath preheated to 70–75° and stirred at that temperature for 12 hr. An additional 5 ml of 30% H_2O_2 was added and the reaction was allowed to continue for another 24 hr. The solvent was evaporated *in vacuo* and the crude material thus obtained on fractional crystallization from H_2O gave the pure product as light yellow needles: yield 1.22 g (50%); nmr (DMSO-d_6) δ 8.47 (b s, 2, 5-H, 6-H), 8.78 (s, 1, 2-H); mass spectrum *m/e* 136 (M^+), 120 (M − 16). *Anal.* ($C_5H_4N_4O$) C, H, N.

1*H*-Imidazo[4,5-*b*]pyrazine 4,7-Dioxide (7). The mixture left after fractional crystallization of **6** was subjected to paper chromatography using *n*-BuOH–AcOH–H_2O (65:15:20) as solvent. The band corresponding to the 4,7-dioxide was eluted from the paper with H_2O. Evaporation of the solvent furnished **7**, yield 0.68 g (7%), as brownish crystals: mass spectrum *m/e* 136 (M^+ − 16), 120 (M − 32). *Anal.* ($C_5H_4N_4O_2$) C, H, N.

1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)imidazo[4,5-*b*]pyrazine (8). **5** (4.80 g, 0.04 mol) and 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (15.90 g, 0.04 mol) were pulverized and fused at 140–150° for 20–25 min. Anhydrous bis(*p*-nitrophenyl) phosphate, 400 mg, was then added. AcOH vapors which evolved almost immediately were removed by application of an H_2O aspirator vacuum. The fusion was continued at the same temperature for another 45–50 min with the flask still connected to the aspirator. The reaction flask was cooled to room temperature and the dark melt was triturated with $CHCl_3$ (500 ml). The dark brown solution was washed with saturated $NaHCO_3$ and then with H_2O until the aqueous layer became colorless. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was evaporated yielding a syrup. Tlc of this syrup showed a major product and some minor components. The major product was obtained in pure form after silica gel column chromatography using C_6H_6 with increasing proportions of EtOAc as eluent. Evaporation of the solvent gave **8** as a bright yellow syrup: yield 8.50 g (55%); nmr ($CDCl_3$) δ 8.82 (s, 1, 2-H), 8.37 (d, 1, $J_{5,6}$ = 3.5 Hz, 5-H), 8.65 (d, 1, $J_{5,6}$ = 3.5 Hz, 6-H), 6.42 (d, 1, $J_{1',2'}$ = 5.0 Hz, $H_{1'}$); mass spectrum *m/e* 378 (M^+), 320 (M − 58), 250 (M − 118).

1-(β-D-Ribofuranosyl)imidazo[4,5-*b*]pyrazine (9). **8** (3.78 g, 0.01 mol) was treated with 200 ml of MeOH saturated with NH_3 at 4° and kept at this temperature overnight. The cooled light yellow colored solution was evaporated. The amber syrup was then dissolved in H_2O and extracted several times with $CHCl_3$. The aqueous solution was evaporated to dryness, and the pale yellow crystalline solid was recrystallized from H_2O–EtOH: yield 1.89 g (75%); prismatic crystals, $\alpha^{26}D$ −49.6° (*c* 0.2, DMSO); nmr (DMSO-d_6) δ 9.18 (s, 1, 2-H), 8.62 (d, 1, $J_{5,6}$ = 3.0 Hz, 5-H), 8.75 (d, 1, $J_{5,6}$ = 3.0 Hz, 6-H), 6.2 (d, 1, $J_{1',2'}$ = 5.0, $H_{1'}$); mass spectrum *m/e* 252 (M^+), 120 (base peak). *Anal.* ($C_{10}H_{12}N_4O_4$) C, H, N.

1-(β-D-Ribofuranosyl)imidazo[4,5-*b*]pyrazine 4-Oxide (11). The blocked nucleoside **8** (3.78 g, 0.01 mol) was dissolved in dry 1,2-dichloroethane (35 ml). To this solution *m*-chloroperoxybenzoic acid (85%, 3.44 g, 0.02 mol) in 30 ml of 1,2-dichloroethane was added dropwise over a period of 30 min. The reaction mixture was heated with stirring at 70–75° in the dark. After 30 hr an additional amount (3.4 g, 0.02 mol) of the acid was added and heating was continued for 40 hr. The mixture was cooled to room temperature and was washed successively with saturated $NaHCO_3$ and H_2O until the aqueous layer was at neutral pH. The organic layer was dried over anhydrous Na_2SO_4 and then evaporated to a syrup *in vacuo*. Purification of the crude syrup by column chromatography using C_6H_6–EtOAc as the eluent gave the protected nucleoside 4-oxide (10), yield 1.80 g (45%).

To 100 ml of MeOH saturated with NH_3 at 4° was added 1.8 g of **10** and the resulting solution was stirred at 4° overnight. The solution was then evaporated to dryness and the resulting residue was triturated several times with $CHCl_3$ and recrystallized from H_2O to give 1.23 g (68%) of **11**: $\alpha^{26}D$ −56.3° (*c* 0.2, DMSO); nmr (DMSO-d_6) δ 9.02 (s, 1, 2-H), 8.5 (s, 2, 5-H, 6-H), 6.18 (d, 1, $J_{1',2'}$ = 5.0 Hz, $H_{1'}$); mass spectrum *m/e* 136 (M^+ − 132), which is the base *N*-oxide peak, 120 (M − 148) base peak. *Anal.* ($C_{10}H_{12}N_4O_5$) C, H, N.

1-Methylimidazo[4,5-*b*]pyrazine (13). A mixture of **1** (2.6 g, 0.02 mol), 10 ml of 40% aqueous $MeNH_2$, and a small amount of freshly prepared Cu powder was heated at 140–150° for 20 hr in a steel vessel. After cooling the orange solution was evaporated to dryness. The crude solid material thus obtained was dissolved in in H_2O and extracted four times with EtOAc, and the organic layer dried over anhydrous Na_2SO_4. Evaporation of the solvent gave 2-amino-3-methylaminopyrazine (**12**) as a light brown product.

12 was refluxed with ethyl orthoformate (10 ml) for 2 hr and then cooled to room temperature. The precipitate was filtered and recrystallized from MeOH as long brown needles: yield 1.85 g (70%); nmr (DMSO-d_6) δ 4.03 (s, 3, 1-CH_3), 8.60 (d, 1, $J_{5,6}$ = 2.8 Hz, 5-H), 8.72 (d, 1, $J_{5,6}$ = 2.8 Hz, 6-H), 8.95 (s, 1, 2-H); mass spectrum *m/e* 134 (M^+), 120 (M − 14), 107, 108. *Anal.* ($C_6H_6N_4$) C, H, N.

1-Methylimidazo[4,5-*b*]pyrazine 4-Oxide (15). To 2.90 g (0.02 mol) of **2** in a steel bomb were added 15 ml of 40% $MeNH_2$ and a catalytic amount of freshly prepared Cu powder. The reaction mixture was kept at room temperature for 24 hr and the orange yellow colored solution obtained was evaporated to dryness. The crude product thus obtained was recrystallized from H_2O to afford 2.22 g (80%) of 2-amino-3-methylaminopyrazine 1-oxide (**14**).

This intermediate was utilized for the synthesis of model compound **15**. To 10 ml of ethyl orthoformate was added 1.39 g (0.01 mol) of **14**, and the mixture was heated at 130–140°. A clear solution was obtained initially, but after 20 min the product started precipitating out of the solution. Heating was continued for 2 hr and the reaction mixture was then evaporated to dryness. The residue was crystallized from H_2O to give 1.05 g (70%) of **15** as analytically pure sample: nmr (DMSO-d_6) δ 3.97 (s, 3, 1-CH_3), 8.47 (s, 2, 5-H, 6-H), 8.75 (s, 1, 2-H); mass spectrum *m/e* 150 (M^+), 134 (M^+ − 16). *Anal.* ($C_6H_6N_4O$) C, H, N.

1*H*-Imidazo[4,5-*b*]pyridine 4-Oxide (16). This compound was prepared by the procedure of Ochiai[12] involving the condensation of 2,3-diaminopyridine with ethyl orthoformate and subsequent oxidation with H_2O_2 in the AcOH.

Biological and Biochemical Procedures. The techniques used for assaying the growth inhibitory activity of the analogs in the bacterial and L-1210 systems have been published previously.[13,14] *E. coli* was grown in the medium of Gray and Tatum[15] and the L-1210 cells in RPMI medium no. 1630[16,17] +10% dialyzed calf serum. The mouse mammary carcinoma cells (TA-3) were grown in no. 1640 medium[9] with 5% horse serum. The Burkitt's lymphoma cells were grown in no. 1640 medium containing 10% fetal calf serum. The Burkitt's lymphoma cells were re-fed after 60 hr of incubation, and the total assay time with this cell line was 5 days. The inhibition analyses were carried out according to published procedures.[2]

The effect of the analogs on xanthine oxidase activity was determined essentially by the method of Spector and Jones.[18] A 0.01-ml aliquot of a suspension of milk xanthine oxidase in 2.3 *M* $(NH_4)_2SO_4$ (E.C. 1.2.3.2., Sigma Chemical Co., 0.093 units/1 mg of protein/ml) was added to 2 ml of 0.05 *M* phosphate buffer, pH 7.8, and 0.1 ml of this dilution was added to the reaction mixture containing, in 3 ml of the phosphate buffer, 0.3 μmol of EDTA, 5.8 μmol of sodium dithionite, and the analog. The mixture was preincubated for 30 min at 25°, and the reaction was started by addition of hypoxanthine. The reaction, in the absence or presence of the inhibitors, proceeded linearly, whereas nonlinear kinetics were obtained in the absence of the reducing agent. As determined separately, dithionite did not interact directly with any of the pyrazine analogs.

The possible cleavage of the glycosidic linkage of 1-(β-D-ribofuranosyl)imidazo[4,5-*b*]pyrazine 4-oxide was examined with cell free extracts from *E. coli*. The reaction mixture, in a total volume of 1.0 ml, contained 0.3 ml of 0.1 *M* phosphate buffer, pH 7.5, 0.1 ml of 0.1 *M* $MgCl_2$, 0.5 ml of *E. coli* extract prepared as described previously,[19] and 0.1 ml of a 0.001 *M* nucleoside *N*-oxide solution. After incubation for 1 hr at 37°, the mixture was immersed in a boiling water bath for 2 min and the precipitated material was removed by centrifugation. The clear supernatant thus obtained was concentrated to about 0.3 ml and then subjected to paper chromatography using EtOAc-*n*-PrOH–H_2O (40:10:20, upper phase) as the solvent system. The uv-absorbing spot present on the paper, with the same R_f as imidazopyrazine *N*-oxide, was eluted with water, and its identity was confirmed by its uv absorption spectrum.

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Preparation of 17α-Acetoxy-7-oxaprogesterone

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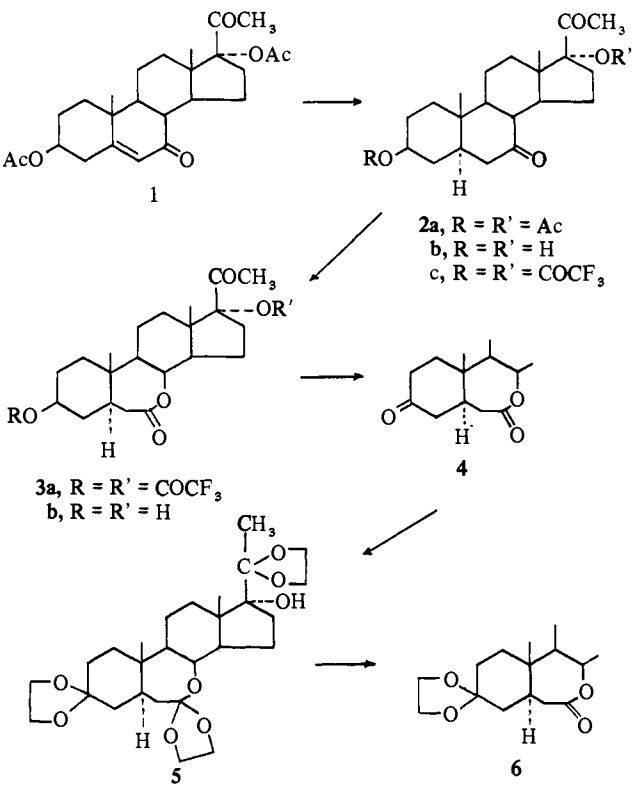
The synthesis of both 17α-acetoxy-7-oxaprogesterone and the corresponding Δ^1 derivative was accomplished. Neither compound exhibited a significant level of progestational or antiprogestational activity.

The steroid nucleus has been modified recently by the insertion of an oxygen atom at various positions. (See ref 1 for leading references.) Perhaps because of altered metabolic pathways, some of these compounds have interesting biological activity.[2-4] Although 7-oxa-5α-androstanes have been prepared,[5] the corresponding 7-oxaprogesterone analogs have not been reported so we prepared several examples of this class of compounds.

Hydrogenation of **1**[6] gave the 5α-dihydro derivative **2a** (for hydrogenation of a steroidal Δ^5-7-ketone to the 5α isomer, see ref 7) which was hydrolyzed to the 3,17-diol **2b**. The diol was then converted to the bistrifluoroacetate derivative **2c** since easily hydrolyzable protecting groups were desired. Baeyer-Villiger oxidation of **2c** with *m*-chloroperbenzoic acid yielded the *B*-homo lactone **3a**. The trifluoroacetate groups were removed very readily with potassium bicarbonate to provide **3b** which was oxidized with chromium trioxide-pyridine to give the ketone **4**. Ketalization of **4** using an extended reaction time to ensure complete reaction of the 20-ketone also caused reaction with the lactone and yielded **5**. (For other examples of this reaction, see ref 8.) The structure of **5** was established by the lack of carbonyl absorption in the infrared spectrum, the molecular ion at *m/e* 495 in the mass spectrum, and its facile hydrolysis by magnesium sulfate[9] in benzene saturated with water to the bisketal lactone **6** (Scheme I).

Scheme I



Treatment of **6** with methyllithium gave the hemiketal **7**, which was not purified but was treated directly with perphthalic acid to yield the Baeyer-Villiger product **8**. Hydrolysis of crude **8** followed by cyclization with *p*-toluenesulfonyl chloride in pyridine[10] provided the 7-oxa steroid **9**. Acid hydrolysis removed the ketal functions to yield **10a**. Acetylation with acetic anhydride-perchloric acid[11] followed by hydrolysis of the 3-enol acetate with sodium bicarbonate gave **10b**. Dehydrogenation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in refluxing dioxane yielded **11**. Hydrogenation of **11** using the soluble catalyst, tris(triphenylphosphine)chlororhodium,[12] gave the desired enone **12** along with the product of overreduction, the saturated 3-ketone **10a**. This mixture gave a single spot of tlc and could not be separated by crystallization. However, reduction of the mixture with lithium tri-*tert*-butyloxyaluminum hydride followed by oxidation with manganese dioxide gave a mixture of **12** and the saturated 3-alcohol which were readily separated by preparative tlc (Scheme II).

Biological Activity. Compounds **10b**, **11**, and **12** were tested for progestational activity in a modified Clauberg-McPhail assay.[13,14] In this procedure progesterone given subcutaneously at a dose of 40 μg/day produced significant secretory development of the uterine endometrium (+1.5 McPhail index). Compounds **10b**, **11**, and **12** were inactive by both routes of administration at a dose of 400 μg/day.

The three compounds were tested for antiprogestational