data for each compound can be found in Table III. Optical rotational data: (−)**XX**, $[\alpha]_{Na}$ −77.81° (*c* 0.103, C_2H_5OH); (−)**IV-14**, $[\alpha]_{Na}$ −73.44° (*c* 0.104, C_2H_5OH); (−)**V-14**, $[\alpha]_{Na}$ +36.63° (*c* 0.0982, C_2H_5OH); (−)**VI-14**, $[\alpha]_{Na}$ −47.44° (*c* 0.0978, C_2H_5OH); (−)**VII-16**, $[\alpha]_{Na}$ −62.63° (*c* 0.0942, C_2H_5OH).

Scheme IV, Step 1. *cis*-2-Propionamido-7-methoxy-1,2,3,4-tetrahydronaphthalen-1-ol (IV-17). The sodium borohydride reduction of ketone **III-11** was carried out as described in Scheme I, step 2. By TLC there was noted to be a mixture of cis and trans alcohols obtained in this reduction. These isomers were separated cleanly by medium-pressure chromatography using CH_2Cl_2 saturated with NH_3 and containing 1% CH_3OH as the eluting solvent. The cis isomer was eluted first and evaporation of the proper fractions afforded 2.3 g (16%) of **IV-17**: mp 131–134 °C; ^1H NMR ($DCCl_3$) δ 4.57 (1 H, d, J = 3 Hz). Anal. ($C_{14}H_{19}NO_3$) C, H, N. For the trans isomer, ^1H NMR ($DCCl_3$) δ 4.35 (1 H, d, J = 9 Hz).

Steps 2–4 were carried out as described in Scheme I. The phenol *cis*-**VII-18** was obtained by using the procedure of Scheme I, step 5.

Pharmacology. For the *α-receptor binding assay*, [^3H]clonidine was used as the radioligand to determine the interaction of the compounds with the α-adrenergic receptor in calf cerebral cortex in vitro. For the *dopamine receptor binding assay*, [^3H]apomorphine was used as radioligand to determine interaction with the DA receptors in rat striatal membranes in vitro. A detailed description of these test procedures is given in ref 5. A description of the assay for *contralateral turning in 6-hydroxydopamine-lesioned rats* is also provided in ref 5.

Acknowledgment. We are indebted to J. P. Moreau for chemical analysis, Dr. D. W. Cochran and J. S. Murphy for spectral data, and Mary Banker for manuscript preparation.

Registry No. **I-4**, 33892-75-0; **I-8**, 32263-70-0; **II-1**, 39262-02-7; **II-4**, 92422-31-6; **II-4·K**, 92422-37-2; **II-5**, 2471-78-5; **II-6**, 2471-80-9; **II-8**, 92422-32-7; **II-10**, 92422-33-8; (±)-**III-1**, 92421-76-6; (±)-**III-2**, 88058-53-1; (±)-**III-3**, 92421-77-7; (±)-**III-4**, 92421-78-8; (±)-**III-5**, 92421-79-9; (±)-**III-6**, 92421-80-2; (±)-**III-8**, 92421-81-3; (±)-**III-10**, 92421-82-4; (±)-**III-11**, 88058-66-6; (±)-**III-19**, 92421-83-5; (±)-**IV-1**, 92471-25-5; (±)-**IV-2**, 88058-55-3; (±)-**IV-3**, 92421-84-6; (±)-**IV-4**, 92421-85-7; (±)-**IV-5**, 92421-86-8; (±)-**IV-6**, 92421-87-9; (±)-**IV-8**, 92421-88-0; (±)-**IV-10**, 92421-89-1; (±)-**IV-11**, 88058-67-7; (+)-**IV-13**, 88058-70-2; (−)-**IV-14**, 88058-73-5; (±)-**IV-17**, 92421-90-4; (±)-**IV-19**, 92421-91-5; (±)-**V-1**, 92471-26-6; (±)-**V-2**, 92421-92-6; (±)-**V-3**, 92421-93-7; (±)-**V-4**, 92421-94-8; (±)-**V-5**, 92421-95-9; (±)-**V-6**, 92421-96-0; (±)-**V-8**, 92421-97-1; (±)-**V-10**, 92421-98-2; (±)-**V-11**, 88058-68-8; (+)-**V-13**, 88058-74-6; (−)-**V-14**, 88058-71-3; (±)-**V-17**, 92421-99-3; (±)-**V-19**, 92422-00-9; (±)-**VI-1**, 92471-28-8; (±)-**VI-1·HCl**, 92471-27-7; (±)-**VI-2**, 92422-18-9; (±)-**VI-2·HCl**, 92422-01-0; (±)-**VI-3**, 92422-19-0; (±)-**VI-3·HCl**, 92422-02-1; (±)-**VI-4**, 92422-20-3; (±)-**VI-4·HCl**, 92422-03-2; (±)-**VI-5**, 92422-21-4; (±)-**VI-5·HCl**, 92422-04-3; (±)-**VI-6**, 92422-22-5; (±)-**VI-6·HCl**, 92422-05-4; (±)-**VI-8**, 92422-35-0; (±)-**VI-10**, 92422-36-1; (±)-**VI-11**, 92422-23-6; (±)-**VI-11·HCl**, 88058-52-0; (+)-**VI-13**, 88058-98-4; (+)-**VI-13·HCl**, 88058-72-4; (−)-**VI-14**, 88059-00-1; (−)-**VI-14·HCl**, 88058-75-7; (±)-**VI-17**, 92422-24-7; (±)-**VI-17·HCl**, 92422-06-5; (±)-**VI-19**, 92422-25-8; (±)-**VI-19·HCl**, 92422-07-6; (±)-**VI-21**, 92422-26-9; (±)-**VI-21·HCl**, 92422-08-7; (±)-**VI-23**, 92422-27-0; (±)-**VI-23·HCl**, 92422-09-8; (±)-**VII-7**, 92422-28-1; (±)-**VIII-7·HCl**, 92422-10-1; (±)-**VII-8**, 92422-11-2; (±)-**VII-9**, 89292-84-2; (±)-**VII-10**, 92422-12-3; (±)-**VII-10·$C_4H_4O_4$**, 92422-13-4; (±)-**VII-12**, 89292-85-3; (+)-**VII-15**, 88058-88-2; (−)-**VII-16**, 88058-89-3; (±)-**VII-18**, 92422-29-2; (±)-**VII-18·HCl**, 92422-14-5; (±)-**VII-20**, 92422-30-5; (±)-**VII-20·HCl**, 92422-15-6; (±)-**VII-22**, 92422-16-7; (±)-**VII-24**, 92422-17-8; (+)-**XX**, 88336-54-3; (−)-**XX**, 88058-69-9; $ClCH_2COCl$, 79-04-9; (±)-*trans*-2-(ethylamino)-5-methoxy-1-tetralol, 92422-34-9; *l*-*O*-methylmandelic acid, 3966-32-3; dopamine, 51-61-6.

Supplementary Material Available: Two tables containing bond lengths and angles for structure (+)**VII-15** (4 pages). Ordering information is given on any current masthead page.

Mesoionic Pyridazine Ribonucleosides. A Novel Biologically Active Nucleoside Metabolite

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4-Cyano-3-oxido-1-β-D-ribofuranosylpyridazinium (**10a**) has been prepared from 4-cyano-3(2*H*)-pyridazinone (**4**) by using a low-temperature, kinetically controlled, silyl Hilbert–Johnson reaction followed by deblocking of the resulting triacetate derivative, **8a**, with $NaHCO_3$ in methanol. **10a** is apparently the first example of a mesoionic analogue of a pyrimidine nucleoside. It was discovered as a urine metabolite of 4-cyano-3(2*H*)-pyridazinone (**4**) in mice. **10a** possesses Gram-negative antibacterial activity in vivo against a systemic *Escherichia coli* infection in mice with an ED_{50} of 25–50 mg/kg. A series of 4-substituted 3-oxidopyridazinium ribonucleosides, **11a–h**, were synthesized as analogues of **10a**. 4-Chloro-3-oxido-1-β-D-ribofuranosylpyridazinium (**11a**) was found to be several times more active than **10a** against *E. coli* in vitro although it showed no in vivo activity.

Much attention has been given to the synthesis and biological evaluation of pyrimidine, pyridine, pyridazine, and related monoheterocyclic nucleosides.[1] There has also been extensive interest in mesoionic derivatives of these same bases.[2] The corresponding mesoionic nucleosides have been overlooked and would seem to be of interest especially as they relate to the biologically important pyrimidine nucleosides such as thymidine or cytidine. One might initially be concerned about the chemical stability of such molecules in view of the attachment of a positively charged nitrogen atom to the anomeric carbon with its high propensity to form a resonance-stabilized carbonium ion. However, the well-known chemical stability associated with related quaternary systems exemplified by nicotinamide adenine dinucleotide would suggest that the corresponding mesoionic structures may not pose a serious stability problem. Another concern in terms of biological potential is an expected increase in polar character associated with mesoionic nucleosides relative to isomeric nonmesoionic

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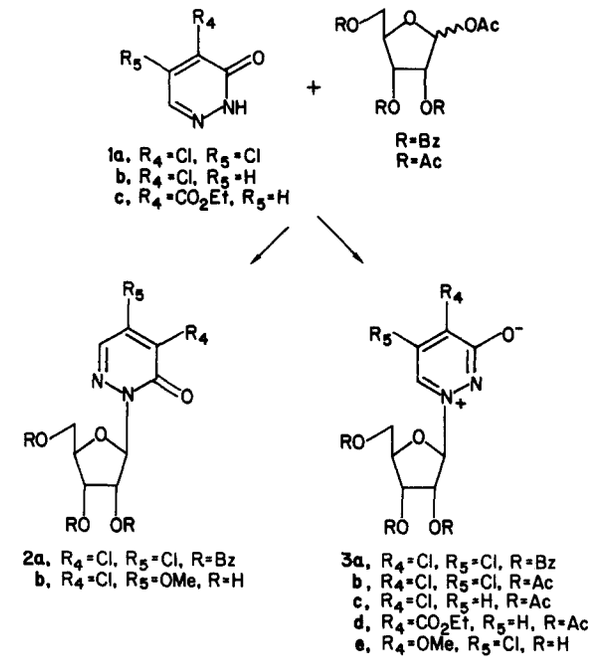
structures. This would normally reduce transport across biological membranes in the absence of some specific transport system.

The significance of mesoionic nucleosides was first brought to our attention by a metabolite study that we carried out on 4-cyano-3(2*H*)-pyridazinone (**4**).[3] This compound was initially found to have antibacterial activity against a systemic *Escherichia coli* infection in mice. However, it was essentially inactive in vitro, a result that suggested a mouse metabolite of **4** may be responsible for its in vivo activity. In addition to other structural types, nucleoside metabolites were considered, including the betaine **10a** and its nonmesoionic isomer **9a**. The betaine assignment was confirmed by isolation of **10a** from urine of animals dosed orally with **4** as described in detail below. Mesoionic pyrimidine, pyridine, or pyridazine nucleosides as well as mesoionic nucleoside derivatives of other monoheterocyclic bases have not been reported. Bicyclic mesoionic purine nucleosides, first characterized by Jones and Robins[4] have been found in the case of 7-methylguanosine to occur naturally in RNA isolated from several sources.[5] The lack of attention given to mesoionic nucleosides is due in part to the absence of a general synthetic approach to such compounds. Known methods for the few bicyclic examples involve methylation[4] or acylation[6] of a suitable nucleoside derivative. In this work the first mesoionic pyridazine nucleosides are described along with a convenient synthetic method for their preparation. The nucleosides have been evaluated for antibacterial activity and potent in vitro as well as in vivo activity discovered.

Results and Discussion

The silyl Hilbert–Johnson synthesis of nucleosides developed by Niedballa and Vorbrueggen[7] has proven exceedingly useful for the preparation of a variety of nonmesoionic nucleoside systems. O- and N-2-glycoside derivatives of 3(2*H*)-pyridazinones have been studied extensively[8] and the silyl Hilbert–Johnson synthesis of several normal N-2 substituted pyridazinone ribonucleosides has been reported by Townsend and co-workers.[9] Thus, for example, the *O*-trimethylsilyl derivative of 4,5-dichloro-3(2*H*)-pyridazinone (**1a**) undergoes reaction with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose and $SnCl_4$ in refluxing dichloroethane to give the N-2 substituted ribonucleoside **2a**. We have found that when this reaction is carried out at room temperature, a new nucleoside, **3a**, is formed in 55% yield as an amorphous solid following column chromatography on silica gel. This compound is



isomeric with **2a** and shows a strong IR absorption at 1605 cm^{-1} in contrast to the 1665 cm^{-1} value that we determined for **2a** prepared according to the literature method.[9] It had been reported[9] that the N-2 substituted ribonucleoside tribenzoate **2a** could be deblocked with NaOMe in MeOH to give 4-chloro-5-methoxy-2-β-D-ribofuranosylpyridazin-3-one (**2b**). A structure that is isomeric with **2b** was produced by treatment of the mesoionic nucleoside tribenzoate **3a** with NaOMe in MeOH. This compound is tentatively assigned the structure 5-chloro-4-methoxy-3-oxido-1-β-D-ribofuranosylpyridazinium (**3e**) on the basis of spectral evidence. A second low-temperature silyl Hilbert–Johnson reaction was then carried out with 4,5-dichloro-3(2*H*)-pyridazinone; this time ribose tetraacetate was used in place of the tribenzoate. This permitted comparison of UV data for the resulting mesoionic ribonucleoside triacetate (**3b**) [λ 229 nm (ε 25 800), 331 (4100)] with the known[10] 4,5-dichloro-1-methyl-3-oxidopyridazinium [λ 229 nm (ε 27 300), 328 (3600)]. The triacetate **3b** was obtained crystalline and gave a strong absorption in the IR at 1590 cm^{-1} with no significant absorption in the 1600–1700-cm^{-1} region.

A temperature-dependent process was also observed in the reaction of the *O*-trimethylsilyl derivative of 4-cyano-3(2*H*)-pyridazinone (**4**)[11] with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose. Thus at room temperature a yellow crystalline mesoionic nucleoside tribenzoate, **8b**, was isolated while at reflux in dichloroethane the normal N-2 substituted ribonucleoside **7b** was obtained as a colorless amorphous solid. Similarly two products were formed in the reaction of the *O*-trimethylsilyl derivative of 4-cyano-3(2*H*)-pyridazinone (**4**) with ribose tetraacetate. The mesoionic nucleoside triacetate **8a** was obtained in 85% yield as yellow needles at 0–5 °C. **8a** was also obtained in high yield at room temperature. However, the N-2 substituted ribonucleoside triacetate **7a** was formed in 51% yield after refluxing in dichloroethane. Apparently the mesoionic systems are the products of kinetic control while the N-2 substituted nucleosides predominate under conditions that permit thermodynamic control. A slow rearrangement of **8a** to **7a** occurs over a period of several

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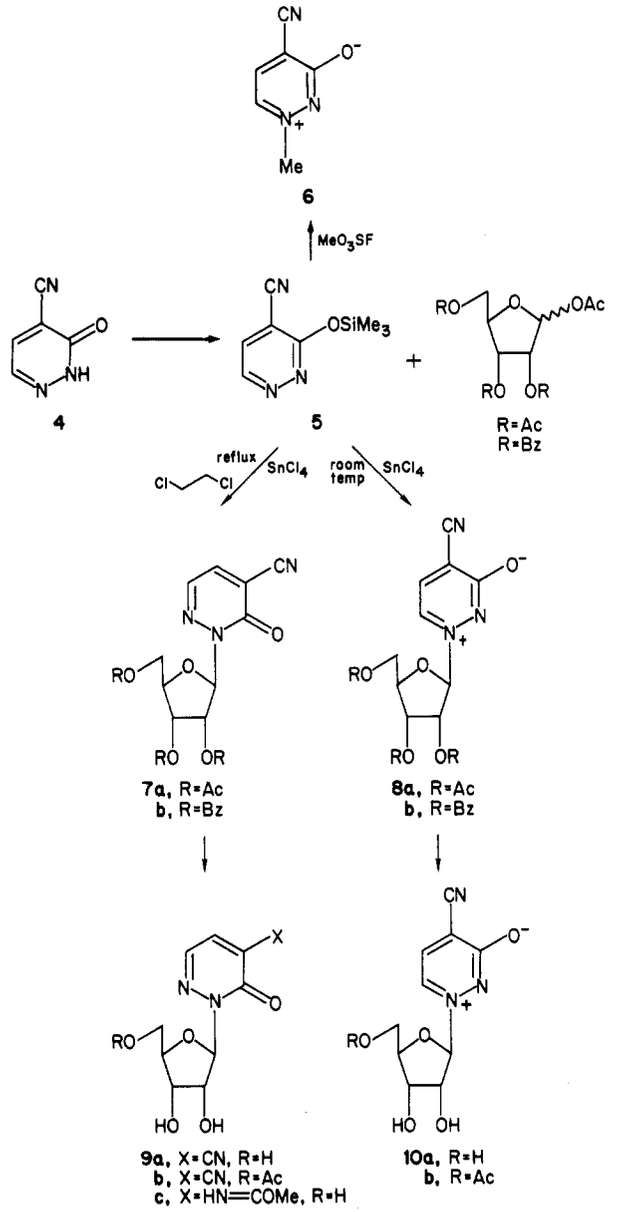
(11) Schmidt, P.; Druey, J. *Helv. Chim. Acta* **1954**, *37*, 134.

months on standing at room temperature and rapidly after refluxing in dichloroethane in the presence of $SnCl_4$.



The normal N-2 substituted cyanonucleoside derivatives **7a** and **7b** as well as the mesoionic N-1 substituted cyanonucleosides **8a** and **8b** proved to be highly sensitive to base. Our attempts to deblock these compounds using NH_3–MeOH or NaOMe–MeOH under standard reaction conditions led only to dark, intractable mixtures. However, the normal cyanoribonucleoside **9a** could be obtained in 53% yield by treating the triacetate **7a** with NaOMe in refluxing methanol for 4 min followed by quenching with Amberlite IRC-50. Some of the 5′-monoacetate **9b** and the *O*-methyl carboximidate **9c** were obtained as byproducts in this reaction. Treatment of the mesoionic ribonucleoside triacetate **8a** with NH_3 or NaOMe in methanol gave an immediate dark blue color and a complex reaction mixture. Although these methods as well as other common deblocking procedures were not effective with **8a**, we have found that the use of $NaHCO_3$ in methanol is a simple, efficient approach for deblocking **8a** as well as other base-sensitive nucleoside triacetates. We attribute the success obtained with $NaHCO_3$ to the lower pH achievable under these conditions and we recommend the use of $NaHCO_3$ with other base-sensitive nucleoside derivatives. With $NaHCO_3$–MeOH, **8a** was converted to the mesoionic ribonucleoside **10a** in 34% yield. The monoacetate **10b** was also obtained.

For comparison purposes the three methylated derivatives of 4-cyano-3(2*H*)-pyridazinone were synthesized and characterized spectrally. 4-Cyano-1-methyl-3-oxidopyridazinium (**6**) was prepared by the reaction of the *O*-trimethylsilyl derivative of **4** with methyl fluorosulfate. NMR data and TLC analysis ($CHCl_3$–MeOH, 3:1) suggested that the 1-methyl compound was the only product in this reaction. The relative TLC mobilities on silica gel of the 1-methyl, 2-methyl, and *O*-methyl derivatives of **4** were 0.01, 0.64, and 0.70, respectively, with 5% EtOH in $CHCl_3$ as eluent.

The small coupling constants (J = 1 Hz) observed for the anomeric protons in the mesoionic nucleosides prepared in this work supports the β configurational assignment,[12] which is also expected on the basis of mechanistic considerations for the silyl Hilbert–Johnson reaction.[13] In view of these same mechanistic conclusions as well as literature precedent[9] with the silyl Hilbert–Johnson ribosylation of other 3(2*H*)-pyridazinones, a β assignment is indicated for the anomeric position of the normal N-2 substituted pyridazinone nucleosides reported here. This is consistent with 1′–2′ coupling constants found for these compounds, which range from 1.0 to 4.0 Hz. The mesoionic structures **8a**, **8b**, and **6** were also characterized by ^{13}C NMR spectroscopy. The 3-position of the pyridazine base (oxido carbon) gave a chemical shift value in the range δ 165.8–166.1 while the 3-position (carbonyl carbon) of the pyridazinone base in the normal N-2 substituted compounds **7a** and **9a** and also in 2-methyl-4-cyano-3(2*H*)-pyridazinone gave values in the range δ 156.4–157.0.

On the basis of the biological activity discovered in 4-cyano-3-oxido-1-β-D-ribofuranosylpyridazinium (**10a**), it became clear that other 4-substituted 3-oxidopyridazinium ribonucleosides were important objectives for biological evaluation. We soon found that a variety of 4-substituted-3-oxidopyridazinium analogues are accessable with use of 4-chloro-3(2*H*)-pyridazinone (**1b**).[14] Thus a low-temperature silyl Hilbert–Johnson reaction employing **1b** gave a 77% yield of the mesoionic nucleoside triacetate **3c**. By treatment of **3c** with $NaHCO_3$–MeOH at room temperature, a 43% yield of 4-chloro-3-oxido-1-β-D-ribofuranosylpyridazinium (**11a**) was obtained along with 29% of the 5′-monoacetate **11b**. With more reactive deblocking conditions such as NaOMe in MeOH or NH_3 in MeOH, the corresponding 4-methoxy (**11c**) and 4-amino (**11d**) derivatives were produced. In the latter case, the nucleoside **11d** may actually exist as a proton tautomer of the structure shown. The 4-hydroxyamino derivative **11f**, or again its proton tautomer, was synthesized by using a three-step approach involving treatment of the 4-chloro triacetate **3c** with the *O*-tetrahydropyranyl derivative of hydroxylamine followed by deblocking with NH_3–MeOH to give *O*-THP derivative **11e**, which was finally hydrolyzed with HCl as catalyst to afford **11f** as a 1:1 crystalline complex with acetamide. Attempts to crystallize this compound in the absence of acetamide were unsuccessful. The 4-mercapto derivative **11g** was obtained as its crystalline ammonium salt in a two-step sequence starting with

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(14) Yanai, M.; Kinoshita, T. *Yakugaku Zasshi* **1965**, *85*, 344; *Chem. Abstr.* **1965**, *63*, 3687.

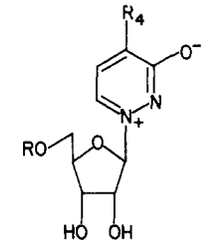
the triacetate **3c**. Thus treatment of **3c** with thioacetic acid gave the intermediate thiolacetate, which was then converted to the salt **11g** with use of NH_3 in methanol. Finally 4-carbamoyl-3-oxido-1-β-D-ribofuranosylpyridazinium (**11h**) was prepared in two steps with ethyl 2,3-dihydro-3-oxopyridazine-4-carboxylate (**1c**) as starting material.[15] The latter was converted to the mesoionic ribonucleoside triacetate **3d** by using a low-temperature silyl Hilbert–Johnson reaction. The carbethoxy group was converted to the amide and the three acetate groups removed all in one reaction employing NH_3 in methanol, thus providing **11h**.

Metabolite Study. The initial surprising result in our biological evaluation of 4-cyano-3(2*H*)-pyridazinone (**4**) was that it showed in vivo antibacterial activity against a systemic E. coli infection in mice, although it displayed no in vitro activity against E. coli as well as other Gram-negative and Gram-positive bacteria. This result suggested that the active compound was a metabolite of **4**. Frustrating our attempts to isolate and characterize this metabolite was the result that the urine of mice dosed either orally or subcutaneously with **4** showed only very weak antibacterial activity. In hindsight this result was attributed to the choice of media used in our in vitro antibacterial determinations. Initially the in vitro studies were carried out with Mueller Hinton broth. When we learned that the metabolite was a nucleoside, we reexamined our compounds in Davis minimal media.[16] The latter generally gave much lower MIC values than were observed with Mueller Hinton broth. A number of derivatives of **4** were examined in the hope of improving in vitro activity as well as activity in the urine of animals dosed either orally or subcutaneously. The most successful compound prepared in this connection was 2-(acetoxymethyl)-2,3-dihydro-3-oxopyridazine-4-carbonitrile, which had an MIC of 50 μg/mL against E. coli in Mueller Hinton broth and provided substantial antibacterial activity in urine of animals dosed orally. Thus our initial attempt to isolate the metabolite was carried out with use of this 2-acetoxymethyl derivative. This led to isolation of the mesoionic nucleoside **10a** in analytically pure form. It gave the same IR, NMR, and UV data as the synthetic mesoionic cyanonucleoside. The mixture melting point was not depressed. At a later point a similar metabolite study was carried out on 4-cyano-3(2*H*)-pyridazinone (**4**) itself and again the mesoionic ribonucleoside **10a** was isolated and found to be identical with the synthetic material.

Antibacterial Activity. The synthetic mesoionic cyanonucleoside **10a** possesses in vivo activity by subcutaneous administration against a systemic E. coli infection in mice with an ED_{50} of 25–50 mg/kg. In Davis minimal media[16] in vitro, **10a** had a minimum inhibitory concentration of 0.78 μg/mL against E. coli under aerobic conditions and 3.1 μg/mL under anaerobic conditions (Table I). It was inactive against all organisms including E. coli when tested in Mueller Hinton broth, a result that suggests interference with transport of the nucleoside across the bacterial membrane system by one or more of the Mueller Hinton nutrients. Even more active in vitro than the 4-cyano compound was the 4-chloro analogue **11a**, which gave an MIC of 0.2 μg/mL against *E. coli* in Davis media and also showed both Gram-negative and Gram-positive activity when tested in Mueller Hinton broth. However, the 4-chloro mesoionic ribonucleoside **11a** as well as its 5′-monoacetate **11b** displayed no in vivo activity. The other 4-substituted mesoionic nucleosides showed no activity or very weak in vitro activity, a result that implies a requirement for an electrophilic site at the 4-position for potent antibacterial activity. Although other mesoionic nucleosides or their nucleotide derivatives may bind to the target macromolecule, derivatives with a labile substituent at position 4 such as **10a** or **11a** may permit covalent binding either at position 4 or perhaps position 3, thus allowing for very effective inhibition of the biological target. The only other nucleoside aside from the 4-cyano mesoionic ribonucleoside **10a** to possess significant in vivo activity was its 5′-monoacetate derivative **10b**, which had an ED_{50} of the same magnitude as the parent nucleoside. The normal N-2 substituted ribonucleosides including **9a**, **9b**, and **9c** showed no in vitro or in vivo activity. They showed no overt toxicity in mice at a level of 200 mg/kg.

These nucleosides represent the first report of monocyclic mesoionic systems structurally related to the biologically important pyrimidine and pyridine nucleosides and further the first mesoionic nucleosides of any type that possess potent biological activity. Of considerable surprise is the result that certain mesoionic nucleosides may be generated in vivo in mice as metabolites by ribosylation of the parent base. An enzyme system that results in a similar transformation was discovered in erythrocytes and blood platelets by Kolis and co-workers. This work reports the transformation of 1,2-dihydro-2-oxopyridine-3-carboxylic acid to the corresponding ribonucleoside.[17] A final point of interest is the discovery of potent biological activity in a nucleoside system in which a cyano substituent is introduced at position 4 of a six-membered ring base. The cyano group is considered a bioisostere of the carbonyl

Table I. In Vitro Antibacterial Activity of 4-Substituted-3-oxido-1-β-D-ribofuranosylpyridazinium Ribonucleosides



no.	R_4	R	in vitro MIC,[a,b] μg/mL EC (anaerobic)	EC (aerobic)
10a	CN	H	3.1	0.78
10b	CN	Ac		100
11a[c]	Cl	H	0.2	0.2
11b	Cl	Ac	25	25
11c	OMe	H	100	
11d[d]	NH_2	H	100	
11e[d]	NHOTHP	H		
11f[d]	NHOH	H		
11g	$S^-NH_4^+$	H	100	
11h	$CONH_2$	H		

[a] MIC (μg/mL) is the minimum inhibitory concentration evaluated against *Escherichia coli* (EC) in Davis minimal media.[1b] Measurements were made up to a concentration of 100 μg/mL. For further details, see Experimental Section. [b] Compounds **10a**, **10b**, **11a**, **11b**, **11d**, **11g**, and **11h** gave no indication of toxicity in mice at a level of 200 mg/kg. **11c**, **11e**, and **11f** were not evaluated for toxicity. [c] The 4-chloro mesoionic ribonucleoside **11a** alone displayed broad-spectrum activity in Mueller Hinton broth, affording the following MIC values: 3.1 (EC), 50 (SC), 12.5 (CP), 6.3 (SA), 3.1 (PM). [d] These compounds may actually exist as nonmesoionic proton tautomers of the structures shown.

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oxygen substituent.[18] Although 5-cyanouridine derivatives are well known,[19] pyrimidine nucleoside analogues in which the 2- or 4-carbonyl oxygen atom is replaced by cyano are apparently new although some efforts have been directed toward their preparation.[20] Such compounds are of interest whether or not they are mesoionic in character. In this connection it should be noted that two groups have recently reported the synthesis of 9-β-D-ribofuranosylpurine-6-carbonitrile,[21] the cyanonucleoside analogue of adenine or hypoxanthine.

Experimental Section

Melting points were obtained on a Thomas-Hoover melting point apparatus and are uncorrected. NMR (90 MHz) spectra were obtained on a Varian EM-390 spectrometer or a Bruker WH90 spectrometer. NMR spectra were recorded with tetramethylsilane as an internal standard unless otherwise noted. Infrared spectra were obtained on a Perkin-Elmer 297 spectrophotometer. UV spectra were produced on a Cary 118 UV–vis spectrophotometer. Preparative TLC was carried out with EM silica gel 60 F-254 plates of 2.0-mm thickness. Brinkmann silica gel of 70–230 mesh was used for column chromatography. Reagent grade solvents were obtained from commercial sources and used without further purification. When necessary solvents or reagents were dried by appropriate methods. Methanol was dried by distillation from magnesium. DMF was dried by distillation from calcium hydride. Pyridine was dried by storage over potassium hydroxide and dichloroethane was dried by distillation from phosphorus pentoxide.

2,3-Dihydro-3-oxo-2-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyridazine-4-carbonitrile (7a). 2,3-Dihydro-3-oxopyridazine-4-carbonitrile[11] (**4**; 4.8 g, 40 mmol) was silylated in 40 mL of hexamethyldisilazane by heating at reflux for 2 h in the presence of a catalytic amount of ammonium sulfate. The excess hexamethyldisilazane was removed under reduced pressure and the resulting oil used without further purification. The oil was dissolved in dry dichloroethane (125 mL) and ribose tetraacetate (12.4 g, 39 mmol) was added. The solution was warmed in an oil bath. $SnCl_4$ (5.2 mL, 40 mmol) was added all at once and the solution brought to a reflux, where it was maintained for 15 min. The reaction mixture was poured into 300 mL of saturated aqueous $NaHCO_3$ and methylene chloride (200 mL) was added. The layers were separated, and the aqueous layer was extracted with methylene chloride (3 × 400 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated to an oil, which was placed on a silica gel column (2.5 in. diameter × 15 in. long) eluting with chloroform–acetonitrile–hexanes (5:2:2). The fractions containing product were combined and concentrated to a gum, which was dried overnight under vacuum at 70 °C to give **7a** (8.0 g, 51%) as an amorphous solid: ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 1.99, 2.05, 2.10 (3 s, 9 H), 3.95–4.5 (m, 3 H), 5.48 (t, 1 H, J = 6 Hz), 5.63 (d, d, 1 H, J = 6 Hz, 3 Hz), 6.42 (d, 1 H, J = 3 Hz), 8.22 and 8.32 (AB q, 2 H, J = 4 Hz); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ 170.2 (s), 169.6 (s), 156.4 (s), 140.0 (d), 137.3 (d), 114.9 (s), 113.7 (s), 88.8 (d), 79.4 (d), 73.1 (d), 70.3 (d), 62.8 (t), 20.2 (q); IR (KBr) 2240 (w), 1750 (s), 1680 (s) cm^{-1}; UV (MeOH) λ_{max} 324 nm (ε 4500). Anal. ($C_{16}H_{17}N_3O_8$) C, H, N.

2,3-Dihydro-3-oxo-2-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyridazine-4-carbonitrile (7b) was prepared from 1-acetyl-2,3,5-tribenzoyl-D-ribofuranose and **4** by using the same method involving a 30-min reflux period in dichloroethane. The crude product was purified by column chromatography on silica gel with 1:1 EtOAc–hexanes as eluent to give **7b** as an amorphous solid (76%): NMR (Me_2SO-d_6, Me_4Si) δ 8.22 (s, 2 H), 8.1–7.3 (m, 15 H), 6.76 (d, 1 H, J = 1 Hz), 6.2–5.95 (m, 2 H), 5.0–4.5 (m, 3 H); IR (KBr) 2240 (w), 1730 (s), 1680 (m) cm^{-1}. Anal. ($C_{31}H_{23}N_3O_8$) C, H, N.

4,5-Dichloro-2-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-3-(2*H*)-pyridazinone (2a) was prepared according to the procedure of Katz et al.:[9] mp 164–166 °C; ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 8.14 (s, 1 H), 8.05–7.3 (m, 15 H), 6.63 (d, 1 H, J = 2 Hz), 6.2–5.9 (m, 2 H), 5.1–4.4 (m, 3 H); IR (KBr) 1735 (s), 1720 (s), 1665 (s) cm^{-1}. Anal. ($C_{30}H_{22}N_2Cl_2O_8$) C, H, N.

4-Cyano-3-oxido-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyridazinium (8a). 2,3-Dihydro-3-oxopyridazine-4-carbonitrile (**4**; 1.2 g, 10 mmol) was silylated in the usual way and the oil dissolved in dichloroethane (20 mL) along with ribose tetraacetate (3.18 g, 10 mmol). The solution was cooled in an ice bath, treated with $SnCl_4$ (1.3 mL, 10 mmol), and stirred 30 min with ice-bath cooling. The solution was poured into saturated aqueous $NaHCO_3$ (300 mL) and methylene chloride (300 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 150 mL). The combined organic layer was dried (Na_2SO_4) and concentrated to an oil, which was triturated with EtOAc (10 mL). Standing gave crystals which were washed with EtOAc–hexanes and dried under vacuum to give yellow needles (mp 165–168 °C, 3.2 g, 85%). Recrystallization from methanol gave an analytical sample: mp 168–170 °C; ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 2.01 (s, 3 H), 2.04 (s, 3 H), 2.12 (s, 3 H), 4.1–4.6 (m, 3 H), 5.43 (t, 1 H, J = 6 Hz), 5.69 (dd, 1 H, J = 6, 1 Hz), 6.20 (d, 1 H, J = 1 Hz), 8.23 (d, 1 H, J = 5 Hz), 8.62 (d, 1 H, J = 5 Hz); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ 170.3 (s), 169.4 (s), 165.8 (s), 138.2 (d), 130.8 (d), 115.4 (s), 114.0 (s), 100.3 (d), 81.6 (d), 74.3 (d), 69.9 (d), 62.5 (t), 20.5 (q), 20.2 (q); IR (KBr) 2225 (w), 1750 (s), 1735, 1610 (s) cm^{-1}; UV (MeOH) λ 223 nm (ε 19 100), 363 (4400). Anal. ($C_{16}H_{17}N_3O_8$) C, H, N.

4-Cyano-3-oxido-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyridazinium (8b) was prepared in a similar way from 2,3-dihydro-3-oxopyridazine-4-carbonitrile (**4**) and 1-acetyl-2,3,5-tribenzoyl-D-ribofuranose. The product was crystallized from EtOAc–hexanes (80%): mp 165–167 °C; ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 4.8 (br s, 2 H), 5.0–5.25 (m, 1 H), 6.13 (t, 1 H, J = 6 Hz), 6.30 (d, d, 1 H, J = 6, 1 Hz), 6.70 (d, 1 H, J = 1 Hz), 7.3–8.2 (m, 15 H), 8.33 (d, 1 H, J = 5 Hz), 8.86 (d, 1 H, J = 5 Hz); IR (KBr) 2225 (w), 1745, 1730 (s), 1720, 1625 (s) cm^{-1}; Anal. ($C_{31}H_{23}N_3O_8$) C, H, N.

4,5-Dichloro-3-oxido-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyridazinium (3b) was prepared in a similar way from 4,5-dichloro-3(2*H*)-pyridazinone (**1a**) and ribose tetraacetate. The product crystallized from EtOAc to give white needles (76%, mp 129–132 °C). This was recrystallized from EtOAc–hexanes to give an analytical sample: mp 135–136 °C; ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 2.03 (s, 6 H), 2.13 (s, 3 H), 4.2–4.6 (m, 3 H), 5.48 (t, 1 H, J = 6 Hz), 5.70 (dd, 1 H, J = 6 Hz, 1 Hz), 6.08 (d, 1 H, J = 1 Hz), 8.86 (s, 1 H); IR (KBr) 1740 (s), 1595 (s) cm^{-1}; UV (MeOH) λ 223 nm (ε 25 800), 331 (4100). Anal. ($C_{15}H_{16}N_2Cl_2O_8$) C, H, N.

4-Chloro-3-oxido-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyridazinium (3c) was prepared in a similar way from 4-chloro-3(2*H*)-pyridazinone[14] (**1b**) and ribose tetraacetate. The crude product was purified by column chromatography on silica gel eluting with ethyl acetate–hexanes–ethanol initially in a ratio of 6:3:1 with a gradual increase in polarity to 6:2:2. Fractions containing product were combined. Each fraction showed one spot on TLC. The total yield of product was 77%. A portion was dried under vacuum overnight at 50 °C to give **3c** as an amorphous solid: ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 8.58 (d, 1 H, J = 6 Hz), 8.01 (d, 1 H, J = 6 Hz), 6.23 (d, 1 H, J = 2 Hz), 5.84 (d, d, 1 H, J = 6, 2 Hz), 5.55 (t, 1 H, J = 6 Hz), 4.6–4.1 (m, 3 H), 2.12 (s), 2.05 (s), and 2.01 (s) (9 H); IR (KBr) 1755 (s), 1750 (sh), 1615 (s), 1570 (s) cm^{-1}; UV (MeOH) λ 221 nm (ε 22 400), 249 (sh), 328 (4000). Anal. ($C_{15}H_{17}N_2O_8Cl$) C, H, N.

4-Carbethoxy-3-oxido-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyridazinium (3d) was prepared in a similar way from ethyl 2,3-dihydro-3-oxopyridazine-4-carboxylate[15] (**1c**) and ribose tetraacetate. The crude product was purified by column chromatography on silica gel eluting initially with 19:1 ethyl acetate–ethanol with increasing polarity to 7:3 EtOAc–EtOH. Finally 8:2 $CHCl_3$–MeOH was used to bring all of the betaine off

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the column. Fractions giving one spot on TLC were combined and dried under vacuum overnight at room temperature to give **3d** as an amorphous solid (45%): ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 8.61 (d, J = 5 Hz, 1 H), 7.87 (d, J = 5 Hz, 1 H), 6.23 (d, J = 2 Hz, 1 H), 5.78 (d, d, J = 2, 6 Hz, 1 H), 5.54 (t, J = 6 Hz, 1 H), 4.6–4.1 (m, 5 H), 2.14 (s), 2.08 (s), and 2.02 (s) (9 H), 1.28 (t, J = 7 Hz, 3 H); IR (KBr) 1745 (s), 1620, 1575 cm^{-1}.

4,5-Dichloro-3-oxido-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyridazinium (3a) was prepared in a similar way from 4,5-dichloro-3(2*H*)-pyridazinone and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose. In this case the silylated base and the sugar were stirred in dichloroethane in the presence of $SnCl_4$ for 2.5 h at room temperature. Workup in the usual way gave a gummy residue which was purified by chromatography on silica gel eluting with EtOAc–hexanes (3:1). **3a** was isolated as an amorphous solid (55%): ^{1}H NMR ($CDCl_3$, Me_4Si) δ 4.6–4.9 (m, 2 H), 4.9–5.25 (m, 1 H), 6.10 (t, 1 H, J = 5 Hz), 6.23 (dd, 1 H, J = 5 Hz, 1 Hz), 6.50 (d, 1 H, J = 1 Hz), 8.1–7.3 (m, 15 H), 8.93 (s, 1 H); IR (KBr) 1730 (s), 1605 (s) cm^{-1}. Anal. ($C_{30}H_{22}N_2Cl_2O_8$) C, H, N.

The 4,5-dichloro-3-oxido-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyridazinium (**3a**; 6.0 g) was added to a solution of NaOMe (0.60 g) in methanol (1.0 L). This solution was allowed to stand at room temperature for 18 h. Amberlite IRC-50 (25 g) was added and the mixture stirred for 30 min and filtered. The filtrate was concentrated to a solid. This was washed with ethyl acetate and dissolved in 100 mL of hot 95% methanol, which was then diluted with ethyl acetate (75 mL). Standing gave crystals which were collected and dried under vacuum at room temperature to give **3e** (1.45 g, 50%): mp 183–185 °C; ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 4.32 (s, 3 H) superimposed on a multiplet 3.3–4.4 (5 H), 5.05–5.4 (m, 2 H, D_2O exchangeable), 5.55 (d, 1 H, J = 1 Hz), 5.82 (d, 1 H, J = 5 Hz, D_2O exchangeable), 9.03 (s, 1 H); IR (KBr) 3240 (s), 1595 (s), 1565 (s), 1535 (s) cm^{-1}; UV (MeOH) λ_{max} 228 nm (ϵ 25 400), 320 (5200). Anal. ($C_{10}H_{13}N_2O_6Cl$) C, H, N.

2,3-Dihydro-3-oxo-2-β-D-ribofuranosylpyridazine-4-carbonitrile (9a), 2,3-Dihydro-3-oxo-2-(5-*O*-acetyl-β-D-ribofuranosyl)pyridazine-4-carbonitrile (9b), and *O*-Methyl 2,3-Dihydro-3-oxo-2-β-D-ribofuranosylpyridazine-4-carboximidate (9c). The triacetate **7a** (5.5 g) was dissolved in refluxing anhydrous methanol (150 mL) and 0.2 M NaOMe in MeOH (1.5 mL) was added. After the mixture was stirred 4 min at reflux, Amberlite IRC 50 (5.0 g) was added with stirring. The mixture was stirred several minutes and filtered and the filtrate concentrated to an oil under reduced pressure. This was dissolved in 1:1 EtOH–$CHCl_3$ and placed on a silica gel column (1 in. diameter × 30 in. long), which was then eluted with $CHCl_3$–MeOH (8:1). Fractions 14–16 were combined and concentrated to a gum, which when dried under vacuum gave **9a** as a hygroscopic amorphous solid (1.9 g, 53%): ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 3.3–3.7 (m, 2 H), 3.8–4.5 (m, 3 H), 4.68 (t, 1 H, J = 6 Hz, D_2O exchangeable), 5.13 (d, 1 H, J = 6 Hz, D_2O exchangeable), 5.41 (d, 1 H, J = 5 Hz, D_2O exchangeable), 6.29 (d, 1 H, J = 4 Hz), 8.22 (s, 2 H); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ 157.0 (s), 139.5 (d), 136.6 (d), 114.3 (s), 114.0 (s), 90.5 (d), 35.4 (d), 73.4 (d), 70.6 (d), 62.0 (t); IR (KBr) 3450 (br), 2240 (w), 1665 (s) cm^{-1}; UV (MeOH) λ 329 nm (ϵ 4700). Anal. ($C_{10}H_{11}N_3O_5$) H; C: calcd, 47.43; found: 46.8; N: calcd, 16.60; found, 16.0. Fractions 5 and 6 from the above column were combined and concentrated to a gum on the rotary evaporator. This became a hygroscopic, amorphous solid when dried under full vacuum. There was thus obtained the monoacetate **9b** (1.10 g, 26%); ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 2.00 (s, 3 H), 3.85–4.4 (m, 5 H), 5.25 (br d, 1 H, D_2O exchangeable), 5.50 (d, 1 H, J = 6 Hz, D_2O exchangeable), 6.28 (d, 1 H, J = 3 Hz), 8.22 and 8.17 (AB q, 2 H, J = 4 Hz); IR (KBr) 3450 (br), 2240 (w), 1740 (s), 1670 (s), 1600 (w) cm^{-1}. Anal. ($C_{12}H_{13}N_3O_6$) C, H, N. Fractions 8–11 from the column were combined and evaporated to a solid, which was recrystallized from methanol (30 mL). The crystals were dried overnight under vacuum at 60 °C to give the imidate **9c** (0.38 g, 9%): mp 186–188 °C dec; ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 3.3–3.6 (m, 2 H), 3.79 (s, 3 H), 3.8–4.4 (m, 3 H), 4.65 (t, 1 H, J = 6 Hz, D_2O exchangeable), 5.05 (d, 1 H, J = 6 Hz, D_2O exchangeable), 5.32 (d, 1 H, J = 6 Hz, D_2O exchangeable), 6.36 (d, 1 H, J = 3 Hz), 7.80 (d, 1 H, J = 4 Hz), 8.20 (d, 1 H, J = 4 Hz), 10.13 (s, 1 H, D_2O exchangeable); IR (KBr) 3400 (br), 3250, 1675 (s), 1590 (m) cm^{-1}. Anal. ($C_{11}H_{15}N_3O_6$) C, H, N.

4-Cyano-3-oxido-1-β-D-ribofuranosylpyridazinium (10a) and 4-Cyano-3-oxido-1-(5-*O*-acetyl-β-D-ribofuranosyl)pyridazinium (10b). 4-Cyano-3-oxido-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyridazinium (**8a**; 2.0 g) was dissolved in absolute methanol (600 mL) and solid sodium bicarbonate (4.0 g) was added. This mixture was allowed to stand at 0–5 °C for 5 days and filtered and water (6 mL) was added. The resulting solution was allowed to stand in the refrigerator overnight and then at room temperature for 3.5 h. Freshly washed Amberlite IRC-50 (50 g) was added and the mixture stirred for 10 min at room temperature. Finally Norite (10 g) was added and stirring continued for 10 min. The mixture was filtered through Celite and the residue washed with methanol (2 L). The combined filtrate was concentrated at room temperature on the rotary evaporator to 10 mL and this was placed on a silica gel column (1 in. diameter × 30 in. long) which was eluted with $CHCl_3$–MeOH (3:1). The later fractions containing product were concentrated separately to volumes of 1–2 mL, affording crystals. These were collected, combined, filtered, and washed with ethanol (10 mL) and excess ether to yield 0.53 g. This was dissolved in warm water (4 mL) and ethanol (15 mL). Ether (15 mL) was added. Cooling gave crystals which were dried under vacuum at 45 °C for 2 h to give **10a** (0.45 g, 34%): mp 168–170 °C dec; 90-MHz ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 3.5–4.0 (m, 2 H), 4.0–4.2 (m, 2 H), 4.2–4.4 (m, 1 H), 5.1–5.35 (m, 2 H, D_2O exchangeable), 5.67 (d, 1 H, J = 1 Hz), 5.94 (d, 1 H, J = 5 Hz, D_2O exchangeable), 8.31 (d, 1 H, J = 5 Hz), 8.89 (d, 1 H, J = 5 Hz); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ 165.8 (s), 137.9 (d), 127.9 (d), 115.6 (s), 112.7 (s), 102.7 (d), 86.1 (d), 76.2 (d), 68.2 (d), 59.5 (t); IR (KBr) 3390 (s), 2235 (w), 1610 (s) cm^{-1}; UV (MeOH) λ 223 nm (ϵ 19 200), 363 (4500). Anal. ($C_{10}H_{11}N_3O_5$) C, H, N. The early fractions containing product were combined with fractions from a similar column used in an identical procedure which started with 2.0 g of **8a**. The combined solution was concentrated to ~5 mL and placed on a second silica gel column (1 in. diameter × 30 in. long), which was then eluted with chloroform–methanol (6:1). From this column a solid was isolated which was recrystallized from methanol–ether. The crystals were dried under vacuum at 45 °C to give **10b** (0.45 g): mp 144–147 °C; ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 2.03 (s, 3 H), 4.0–4.5 (m, 5 H), 5.39 (d, 1 H, J = 6 Hz, D_2O exchangeable), 5.79 (d, 1 H, J = 1 Hz), 5.95 (d, 1 H, J = 5 Hz, D_2O exchangeable), 8.30 (d, 1 H, J = 5 Hz), 8.65 (d, 1 H, J = 5 Hz); IR (KBr) 3310, 2230 (w), 1750 (s), 1740 (m), 1610 (s) cm^{-1}. Anal. ($C_{12}H_{13}N_3O_6$) C, H, N.

4-Cyano-1-methyl-3-oxidopyridazinium (6). 2,3-Dihydro-3-oxopyridazine-4-carbonitrile (1.2 g) was silylated in the usual way and the oil dissolved in dichloroethane (35 mL). This solution was cooled in an ice bath and methyl fluorosulfate (1.2 mL) was added (2 min). The reaction was stirred at 0–5 °C for 30 min and then brought to room temperature (20 min). Ether (175 mL) was added. Standing in the freezer gave a gum which was triturated with acetonitrile to give a tan solid (1.25 g). This was dissolved in methanol (10 mL), the solution was decolorized with charcoal, and acetonitrile (3 mL) and ether were added until cloudy. Crystals slowly formed. These were collected and recrystallized a second time from MeOH–CH_3CN–ether. The resulting crystals were dried under vacuum at room temperature for 6 h to give a salt derivative of the desired 4-cyano-1-methyl-3-oxidopyridazinium (0.43 g): mp 95–100 °C; ^{1}H NMR (Me_2SOd_6, Me_4Si) δ 4.17 (s, 3 H), 6.82 (s, D_2O exchangeable), 8.27 (d, 1 H, J = 5 Hz), 8.58 (d, 1 H, J = 5 Hz); IR (KBr) 3450 (br), 2240 (w), 1605 cm^{-1} (s). The mother liquors from the three crystallizations were combined and concentrated to dryness, and the residue was dissolved in a small amount of 1:1 $CHCl_3$–MeOH. This solution was applied to a silica gel column ($^3/_4$ in. diameter × 24 in. long) eluting with $CHCl_3$–MeOH (3:1). Fractions containing product were combined, concentrated to a solid which was triturated with ethanol, and filtered. The crystals were dried at 60 °C on the pump overnight to give **6** (0.2 g): mp 200–203 °C dec; ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 4.18 (s, 3 H), 8.22 (d, J = 5 Hz, 1 H), 8.52 (d, J = 5 Hz, 1 H); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ 166.1 (s), 138.2 (d), 131.9 (d), 115.6 (s), 111.1 (s), 52.1 (q); IR (KBr) 2235 (w), 1610 (s) cm^{-1}; UV (MeOH) λ 222 nm (ϵ 20 300), 362 (4300). Anal. ($C_6H_5N_3O$) C, H, N.

2,3-Dihydro-2-methyl-3-oxopyridazine-4-carbonitrile was prepared according to the procedure of Druey and Schmidt:[22] mp

130–131 °C; ^{1}H NMR ($CDCl_3$, Me_4Si) δ 3.88 (s, 3 H), 7.65 (d, 1 H, J = 4 Hz), 7.88 (d, 1 H, J = 4 Hz); ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ 1.56.9 (s), 140.0 (d), 139.0 (d), 114.2 (s), 113.1 (s), 40.7 (q); IR (KBr) 2240 (w), 1665 (s) cm^{-1}; UV (MeOH) λ 328 nm (ε 5100). Anal. ($C_6H_5N_3O$) C, H, N.

3-Methoxypyridazine-4-carbonitrile was obtained from 3-chloropyridazine-4-carbonitrile (1.4 g)[23] and NaOMe (0.55 g) in THF at room temperature. The crude product was purified by column chromatography on silica gel eluting with ethyl acetate–hexanes (1:1) and recrystallized from ether to give an analytical sample (0.10 g): mp 85–87 °C; ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 4.17 (s, 3 H), 8.17 (d, 1 H, J = 4 Hz), 9.13 (d, 1 H, J = 4 Hz); IR (KBr) 2240 (s), 1575 (m), 1545 (m) cm^{-1}; UV (MeOH) λ 292 nm (ε 4000). Anal. ($C_6H_5N_3O$) C, H, N.

The relative TLC mobilities of the three methylated derivatives of 4-cyano-3(2*H*)-pyridazinone using 5% EtOH in $CHCl_3$ as eluent are as follows: 0.70, 3-methoxypyridazine-4-carbonitrile; 0.64, 2,3-dihydro-2-methyl-3-oxopyridazine-4-carbonitrile; 0.01, 4-cyano-3-oxido-1-methylpyridazinium.

4,5-Dichloro-3-oxido-1-methylpyridazinium was prepared according to the procedure of Reicheneder and Kropp:[10] mp 230 °C dec; ^{1}H NMR (CF_3CO_2H, Me_4Si) δ 4.34 (s, 3 H), 9.03 (s, 1 H); IR (KBr) 1590 (s) cm^{-1}; UV (MeOH) λ 229 nm (ε 27 300), 328 (3600).

2-(Hydroxymethyl)-2,3-dihydro-3-oxopyridazine-4-carbonitrile. 4-Cyano-3(2*H*)-pyridazinone (**4**; 2.42 g) was dissolved in hot absolute ethanol (50 mL) and 37% aqueous formaldehyde (4 mL) was added. The solution was allowed to cool to room temperature. After standing 3 h it was filtered to remove a small amount of insoluble material. The solution was then allowed to stand in the freezer overnight. The next day the needles were collected, washed with ethanol and ether, and dried under vacuum at 60 °C for 1 h to give analytically pure material (1.4 g, 46%): mp 114–116 °C; ^{1}H NMR (Me_2SO-d_6, Me_2SO-d_5) δ 5.25 (s, 2 H), 8.02 (s, 2 H); IR (KBr) 3360 (br), 2235 (w), 1645 (s) cm^{-1}; Anal. ($C_6H_5N_3O_2$) C, H, N.

2-(Acetoxymethyl)-2,3-dihydro-3-oxopyridazine-4-carbonitrile. Acetyl chloride (35 mL) was added to 2-(hydroxymethyl)-4-cyano-3(2*H*)-pyridazinone (1.0 g). The mixture was stirred at room temperature for 45 min when all the solid dissolved. Stirring was continued at room temperature for another 1.75 h. The excess CH_3COCl was removed with a rotary evaporator. CCl_4 was added to the residue and evaporated (3 × 20 mL). The resulting oil was dissolved in $CHCl_3$ (5 mL) and ether added until cloudy. Upon standing in the freezer, crystals were obtained which were collected, washed with ether, and dried under vacuum overnight at room temperature to give analytically pure material (1.15 g, 90%): mp 47–49 °C; ^{1}H NMR ($CDCl_3$, Me_4Si) δ 2.15 (s, 3 H), 6.11 (s, 2 H), 7.84 (d, 1 H, J = 4 Hz), 8.02 (d, 1 H, J = 4 Hz); IR (KBr) 2235 (w), 1740 (s), 1690 (s) cm^{-1}. Anal. ($C_8H_7N_3O_3$) C, H, N.

Urine Metabolites of 2-(Acetoxymethyl)-2,3-dihydro-3-oxopyridazine-4-carbonitrile. Mice were dosed orally with an aqueous solution of 2-(acetoxymethyl)-2,3-dihydro-3-oxopyridazine-4-carbonitrile (0.60 g), and 128 mL of urine was collected and lyophilized to a gummy solid (3.5 g). The bulk of this material was treated with methanol (10 mL) and 95% ethanol (10 mL) and the mixture stirred at room temperature for 20 min. Acetonitrile (20 mL) was added and the mixture filtered. The filtrate was concentrated and the residue stirred with 95% ethanol for 15 min. Acetonitrile (10 mL) was added and the mixture centrifuged. The supernatant was placed on a silica gel column (1 in. diameter × 30 in. long) and the column eluted with CH_3CN–EtOH (8:1). Fractions (100 mL) were collected and concentrated on the rotary evaporator below 40 °C. The residue in fraction 2 was dissolved in warm ethanol (2 mL) and centrifuged to remove insoluble material. The supernatant was concentrated with an argon stream and the residue recrystallized from absolute ethanol (0.5 mL) to give tan crystals that were dried under vacuum at 60 °C for 5 h to give 4-cyano-3(2*H*)-pyridazinone: mp 184–186 °C. The mixture melting point of this material with authentic 4-cyano-3(2*H*)-pyridazinone was 184–186 °C. The IR spectrum and mass spectrum of this metabolite were identical with those obtained for authentic 4-cyano-3(2*H*)-pyridazinone.[11]

Fractions 5–9 from the above column were each dissolved in 1–2 mL of methanol. On standing crystals settled out of fractions 5–8. The mother liquors from these crystals were combined with fraction 9 from the column, and this solution was concentrated and placed in the freezer. On standing overnight more crystals were obtained. These were removed by centrifugation, and the supernatant was concentrated under an argon stream to a volume of 1 mL. A solid settled out from this solution on standing. The supernatant was spotted on a 2.0 mm thick preparative silica gel plate (8 in. × 8 in.), which was then developed with $CHCl_3$–MeOH (2:1). The band that produced a dark color under long wavelength light (365 nm) was removed from the plate and extracted with 4:1 $CHCl_3$–MeOH (3 × 20 mL). The extracts were combined and concentrated to dryness on a rotary evaporator (below 30 °C). The residue was dissolved in 2 mL of warm ethanol and centrifuged to remove insoluble material and the supernatant concentrated under an argon stream to approximately 0.75 mL. Crystals formed. These were centrifuged, separated from the supernatant, and dried under vacuum at room temperature for 30 min to give 4-cyano-3-oxido-1-β-D-ribofuranosylpyridazinium (**10a**): mp 164–168 °C dec. This material was recrystallized from 95% ethanol (0.75 mL) to give analytically pure **10a** (3.5 mg): mp 170–172 °C dec; 270-MHz ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 3.63 (m, 1 H), 3.78 (m, 1 H), 4.06 (m, 2 H), 4.26 (br s, 1 H), 5.22 (m, 2 H, D_2O exchangeable), 5.62 (d, 1 H, J = 1 Hz), 5.95 (d, 1 H, J = 5 Hz, D_2O exchangeable), 8.32 (d, 1 H, J = 5 Hz), 8.86 (d, 1 H, J = 5 Hz); IR (KBr) 3390 (br), 2235 (w), 1610 (s); UV (MeOH) λ_{max} 222 nm (ε 22 000), 363 (4600). Anal. ($C_{10}H_{11}N_3O_5$) C, H, N. The mixture melting point of this metabolite with synthetic **10a** is 168–170 °C dec.

A similar procedure was employed for the isolation of **10a** in a mouse metabolite study carried out on the parent base 4-cyano-3(2*H*)-pyridazinone (**4**). The **10a** isolated from urine of mice dosed orally with **4** gave mp 167–169 °C dec. The mixture melting point with synthetic **10a** is 168–170 °C dec. The infrared spectra of this metabolite was identical with the spectra obtained for the synthetic ribonucleoside **10a**.

4-Chloro-3-oxido-1-β-D-ribofuranosylpyridazinium (11a) and 4-Chloro-3-oxido-1-(5-*O*-acetyl-β-D-ribofuranosyl)-pyridazinium (11b). 4-Chloro-3-oxido-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyridazinium (**3c**; 2.4 g) was dissolved in methanol (125 mL) and $NaHCO_3$ (1.2 g) added. This mixture was allowed to stand at room temperature for 44 h. It was then filtered and the filtrate treated with Amberlite IRC 50 (20 g). After stirring 15 min, this mixture was filtered and the Amberlite washed with methanol. The combined methanol filtrates were concentrated to ~3 mL. This solution was applied to a silica gel column ($1^1/_2$ in. diameter × 2 ft long) which was eluted with 5:1 $CHCl_3$–MeOH. The later fractions containing product were combined and concentrated to an oil that was dissolved in a small amount of methanol. Standing gave crystals. These were collected, washed with ethanol and then ether, and finally dried under vacuum at room temperature overnight to give **11a** as yellow crystals (0.70 g, 43%): mp 146–148 °C; ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 8.75 (d, 1 H, J = 6 Hz), 8.00 (d, 1 H, J = 6 Hz), 5.88 (d, 1 H, J = 5 Hz, D_2O exchangeable), 5.69 (d, 1 H, J = 2 Hz), 5.3–5.1 (m, 2 H, D_2O exchangeable), 4.5–3.5 (m, 5 H); IR (KBr) 3330 (br), 1605, 1570 (s), 1550 (s) cm^{-1}; UV (MeOH) λ_{max} 217 nm (ε 22 300), 254 (2900), 304 (5900). Anal. ($C_9H_{11}N_2O_5Cl$) C, H, N. The early fractions containing product were combined and concentrated to an oil. This was triturated with ethanol to give crystals that were collected, washed with ether, and dried under vacuum at room temperature overnight to give the monoacetate **11b** (0.55 g, 29%): mp 145–146 °C; ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 8.50 (d, J = 5 Hz, 1 H), 7.98 (d, J = 5 Hz, 1 H), 5.87 (d, J = 5 Hz, 1 H, D_2O exchangeable), 5.75 (d, 1 H, J = 1 Hz), 5.39 (d, J = 6 Hz, 1 H, D_2O exchangeable), 4.5–4.0 (m, 5 H), 2.08 (s, 3 H); IR (KBr) 3390 (br), 1750 (s), 1595 (s), 1550 (s) cm^{-1}. Anal. ($C_{11}H_{13}N_2O_6Cl$) C, H, N.

4-Methoxy-3-oxido-1-β-D-ribofuranosylpyridazinium (11c). 4-Methoxy-3-oxido-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-pyridazinium (**3c**; 2.7 g) was dissolved in anhydrous methanol (300

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mL) and sodium methoxide was added. This solution was allowed to stand at room temperature for 20 h and then treated with Amberlite IRC 50 (12 g) with stirring. The mixture was filtered and the filtrate concentrated to a gum. This was dissolved in a small amount of methanol and chromatographed on a silica gel column (1.5 in. diameter × 8 in. long) eluting with $CHCl_3$–MeOH (2:1). The fractions containing product were combined and concentrated to a gum which was dissolved in a small amount of methanol. Standing gave crystals which were collected, washed with ethanol and then ether, and finally dried under vacuum at room temperature to give pure **11c** (0.40 g, 19%): mp 179–181 °C; ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 8.60 (d, J = 6 Hz, 1 H), 6.90 (d, J = 6 Hz, 1 H), 5.67 (d, J = 5 Hz, 1 H, D_2O exchangeable), 5.45 (d, J = 1 Hz, 1 H), 5.3–4.95 (m, 2 H, D_2O exchangeable), 3.85 (s) superimposed on 4.35–3.4 (m) (8 H); IR (KBr) 3450 (br), 1600 (m), 1555 (s) cm^{-1}; UV (MeOH) λ_{max} 218 nm (ε 23 000), 325 (3800). Anal. ($C_{10}H_{14}N_2O_6 \cdot {}^1/_2H_2O$) C, H, N.

4-Amino-3-oxido-1-β-D-ribofuranosylpyridazinium (11d). 4-Chloro-3-oxido-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-pyridazinium (**3c**; 2.5 g) was dissolved in methanol (250 mL) that had been saturated with ammonia at room temperature. This solution was allowed to stand at room temperature overnight. The next day the methanol soltuion was concentrated under reduced pressure to 25 mL. The resulting crystals were collected, washed with ethanol, and dried under vacuum overnight at 90 °C to give **11d** (1.4 g, 89%): mp 185–190 °C dec; ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 8.22 (d, J = 7 Hz, 1 H), 8.0–6.7 (br D_2O exchangeable absorption, 2 H), 6.29 (d, J = 7 Hz, 1 H), 5.49 (d, J = 5 Hz, 1 H, D_2O exchangeable), 5.38 (d, J = 3 Hz, 1 H), 5.35–4.9 (m, 2 H, D_2O exchangeable), 4.5–3.3 (m, 5 H); IR (KBr) 3600–2500 (s), 1640 (s), 1580 (s) cm^{-1}; UV (MeOH) λ 228 nm (ε 13 700), 311 (13 900). Anal. ($C_9H_{13}N_3O_5$) C, H, N.

3-Oxido-1-β-D-ribofuranosyl-4-[*O*-(tetrahydropyran-2-yl)hydroxyamino]pyridazinium (11e). 4-Chloro-3-oxido-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyridazinium (**3c**; 3.0 g) was dissolved in methanol (120 mL) and *O*-(tetrahydropyran-2-yl)-hydroxylamine (1.5 g) was added. This solution was allowed to stand overnight at room temperature (18 h). More of the *O*-(tetrahydropyran-2-yl)hydroxylamine (0.5 g) was added and the solution allowed to stand another 6 h at room temperature. The reaction mixture was cooled in an ice bath and saturated with ammonia gas and the resulting solution allowed to stand overnight at room temperature. After 24 h it was concentrated under reduced pressure and the residue dissolved in methanol (5 mL) and passed through a short silica gel column ($1^3/_4$ in, diameter × 4 in. long) eluting with 8:1 chloroform–methanol. Fractions 2, 4, and 5 were combined and dried under vacuum for 2 days at 50 °C to give **11e** (2.5 g). NMR and TLC analysis of this material indicated it was contaminated with acetamide. Fraction 3 from the above column was dried under vacuum 2 days at 50 °C to give the monohydrate of **11e** as an amorphous solid (0.5 g): ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 8.11 (d, 1 H, J = 7 Hz), 6.42 (d, 1 H, J = 7 Hz), 5.37 (d, 1 H, J = 3 Hz), 5.07 (s, 1 H), 6.5–4.5 (br, D_2O exchangeable), 4.4–3.4 (m, 7 H), 2.0–1.4 (m, 6 H); IR (KBr) 3400 (br), 1600 (w), 1570, 1520 cm^{-1}; UV (MeOH) λ 232 nm (ε 9800), 316 (17 600). Anal. ($C_{14}H_{21}N_3O_7 \cdot H_2O$) C, H, N.

4-(Hydroxyamino)-3-oxido-1-β-D-ribofuranosylpyridazinium (11f). 4-[*O*-(Tetrahydropyranyl)hydroxyamino]-3-oxido-1-β-D-ribofuranosylpyridazinium (**11e**; 2.5 g) was dissolved in ethanol (150 mL) and 12 N HCl (700 μL) was added. This solution was allowed to stand at room temperature for 2 days. TLC analysis ($CHCl_3$–MeOH–HOAc, 4:1:0:5) indicated considerable starting material remained. More 12 N HCl (200 μL) was added and the solution allowed to stand another 48 h at room temperature. TLC analysis indicated the absence of starting material. The solution was concentrated to an oil. This was dissolved in ethanol and excess ether added to give a precipitate. The precipitate was redissolved in a small amount of methanol and excess ether added to give a precipitate which was taken up in methanol–water (3:1) and treated with Dowex 1X8-50 (hydroxide form, 8.5 g) to give a solution with a pH of 7. The resin was filtered off and the green filtrate concentrated to a gum. This was dissolved in water and acetamide (200 mg) was added. Methanol was then added. Standing gave crystals. This material was recrystallized again from aqueous methanol and dried at room temperature under vacuum for 5 h to give the acetamide complex of 4-(hydroxyamino)-3-oxido-1-(β-D-ribofuranosylpyridazinium (**11f**; 0.45 g, 19%): mp 151–154 °C; ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 8.10 (d, 1 H, J = 7 Hz), 6.15 (d, 1 H, J = 7 Hz) superimposed on a broad D_2O exchangeable absorption at 7.5–6.0, 5.31 (d, 1 H, J = 3 Hz), 4.4–3.75 (m, 3 H), 3.75–3.45 (m, 2 H), 1.75 (s, 3 H); IR (KBr) 3410, 3170 (s), 1650, 1615 (m), 1560 (s) cm^{-1}; UV (MeOH) λ 233 nm (ε 9600), 318 (17 800). Anal. ($C_{11}H_{18}N_4O_7$) C, H, N.

4-Mercapto-3-oxido-1-β-D-ribofuranosylpyridazinium Ammonium Salt (11g). 4-Chloro-3-oxido-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyridazinium (**3c**; 3.0 g) was dissolved in methanol (100 mL) and the solution cooled in an ice bath. Thioacetic acid (4.5 mL) was added all at once and the yellow solution stirred 5 min. It was then concentrated on a rotary evaporator under full vacuum. The residue was dissolved in methanol (100 mL) and this solution cooled in an ice bath and then saturated with ammonia. The resulting solution was allowed to stand 6 h at room temperature. The flask was then placed in the freezer overnight. The next day the crystals were collected, washed with alcohol, and dried under vacuum at 40 °C to give 1.3 g. The mother liquors on standing gave a second crop (0.5 g). Both crops gave acceptable elemental analyses. The total yield is 1.8 g (84%): mp 179–181 °C; ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 7.92 (d, J = 6 Hz, 1 H), 7.20 (d, J = 6 Hz, 1 H), 6.38 (br s, 7 H, D_2O exchangeable), 5.38 (d, J = 3 Hz, 1 H), 4.5–3.4 (m, 5 H); IR (KBr) 3430, 3150, 1570 (w), 1505 cm^{-1}; UV (MeOH) λ 376 nm (ε 24 000). Anal. ($C_9H_{15}N_3O_5S$) C, H, N.

4-Carbamoyl-3-oxido-1-β-D-ribofuranosylpyridazinium (11h). 4-Carbethoxy-3-oxido-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyridazinium (**3d**; 1.9 g) was dissolved in methanol (400 mL) and the solution cooled in an ice bath and then saturated with ammonia. The resulting solution was allowed to stand in a refrigerator for 16 h and then concentrated on the rotary evaporator to ~10 mL. Ethanol (10 mL) was added, causing crystal formation. These crystals were collected, washed with ether, and dried under vacuum at 70 °C for 5 h to give **11h** (1.0 g, 78%): mp 193–195 °C; ^{1}H NMR (Me_2SO-d_6, Me_4Si) δ 9.97 (br d, 1 H, D_2O exchangeable), 9.00 (d, J = 5 Hz, 1 H), 8.28 (d, J = 5 Hz, 1 H), 8.03 (br d, 1 H, D_2O exchangeable), 5.98 (d, J = 5 Hz, 1 H, D_2O exchangeable), 5.73 (d, J = 1 Hz, 1 H), 5.3–5.05 (m, 2 H, D_2O exchangeable), 4.4–3.5 (m, 5 H); IR (KBr) 3380 (s), 3210 (s), 1680 (s), 1600 (w), 1500 cm^{-1}; UV (MeOH) λ_{max} 217 nm (ε 18 800), 351 (4100). Anal. ($C_{10}H_{13}N_3O_6$) C, H, N.

Microbiological Evaluations. The mesoionic nucleosides were screened for antibacterial activity in vitro against *Escherichia coli* ATCC 23540, *Salmonella choleraesuis* ATCC 13312, *Streptococcus faecalis* AtCC 19433, *Staphylococcus aureus* (Smith) Merck 2949, *Proteus mirabilis* PR-91 and *Clostridium perfringens* ATCC 3624. The nucleoside (10 mg) was solubilized in Me_2SO (4 mL) and this solution diluted with Mueller Hinton broth or Davis minimal media[16] to provide the following concentrations (μg/mL): 62.5, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.20, and 0.10 ppm. A Dynatech MIC 2000 Dispenser-Inoculator was employed. The MIC was the lowest concentration at which growth of the inocula did not occur. Anaerobic determinations were carried out under a CO_2 atmosphere.

In vivo antibacterial evaluations in mice were carried out with use of *Escherichia coli* TR 750211 isolated from swine neonates. A virulent smooth culture (in vivo mice isolate) of the above-mentioned organisms was removed by washing from several brain–heart agar culture production plates incubated at 37 °C for 12–24 h. The culture washing solution and suspension media was sterile brain–heart broth. Organisms are evenly suspended at maximum concentration and then diluted by adding an equal volume of 5% aqueous gastric mucin. This suspension is transferred in 2-mL aliquots into heat-sealable ampules and shell frozen in a dry ice–acetone bath for storage at −20 °C. The experimental inoculum was obtained by aseptically adding the desired amount of frozen stock culture to 0.5% sterile aqueous gastric mucin. This ratio was determined by frozen pool titration for a dilution that produced an LD-90 at 120-h postchallenge in 21 ± 1 g mice. Harlan Hapi (ND/4) BR outbred Swiss white mice were used in the antibacterial evaluations in a weight range of 20–23 g infected intraperitoneally with 0.5 mL of the inoculum. A 100-mg sample of the test compound is added to 5 mL of a 2.5% suspension of (carboxymethyl)cellulose in water. The required volume of this suspension is administered subcutaneously by

injection such that a level of 200, 100, 50, 25, or 12.5 mg of test compound/kg of mouse weight is maintained. Ten mice are employed per test compound at each dose level and survivors counted at 120-h postchallenge. Streptomycin sulfate at 100 mg/kg was used as the positive control.

Registry No. **1a**, 932-22-9; **1b**, 1677-79-8; **1c**, 1445-55-2; **2a**, 56707-86-9; **3a**, 91777-77-4; **3b**, 91777-76-3; **3c**, 92187-06-9; **3d**, 92187-07-0; **3e**, 92187-08-1; **4**, 64882-65-1; **6**, 91777-79-6; **7a**, 91777-71-8; **7b**, 92187-09-2; **8a**, 91777-72-9; **8b**, 92187-10-5; **9a**, 91777-78-5; **9b**, 92187-11-6; **9c**, 92187-12-7; **10a**, 91777-70-7; 10b, 92187-13-8; **11a**, 92187-14-9; **11b**, 92187-15-0; **11c**, 92187-16-1; **11d**, 92187-17-2; **11e**, 92187-18-3; **11f**, 92187-19-4; **11g**, 92187-20-7; **11h**, 92187-21-8; 1-acetyl-2,3,5-tribenzoyl-D-ribofuranose, 14215-97-5; methyl fluorosulfate, 421-20-5; 2,3-dihydro-2-methyl-3-oxopyridazine-4-carbonitrile, 92187-25-2; 3-methoxypyridazine-4-carbonitrile, 92187-22-9; 3-chloropyridazine-4-carbonitrile, 1445-56-3; 4,5-dichloro-3-oxido-1-methylpyridazinium, 33386-96-8; 2-(hydroxymethyl)-2,3-dihydro-3-oxopyridazine-4-carbonitrile, 92187-23-0; 2-(acetoxymethyl)-2,3-dihydro-3-oxopyridazine-4-carbonitrile, 92187-24-1; *O*-(tetrahydropyran-2-yl)hydroxylamine, 6723-30-4; ribose tetraacetate, 28708-32-9.

Novel Pyrimidine and 1,3,5-Triazine Hypolipidemic Agents

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New compounds were synthesized by changing the substituents of a trisubstituted pyrimidine, i.e., [[4-chloro-6-[(2,3-dimethylphenyl)amino]-2-pyrimidinyl]thio]acetic acid, a potent hypolipidemic agent, impaired, however, by a marked hepatomegaly-inducing effect. The structural variations led to the subsidence (**14b**, i.e., 4-chloro-2-(dimethylamino)-6-[(2,3-dimethylphenyl)amino]pyrimidine) or to the reduction (**18b**, [[4-chloro-6-[(2,3-dimethylphenyl)amino]-2-pyrimidinyl]amino]acetic acid) of said untoward effect but still maintained the hypolipidemic effect that, although markedly decreased, still proves significant for serum cholesterol and triglycerides (**18b**) or for serum triglycerides only (**14b**).

Development of drugs for the treatment of hyperlipidemias has focused on (aryloxy)acetic acid derivatives (so-called "fibrates"), e.g., clofibrate, bezafibrate, tibric acid, procetofen,[1-3] nicotinic acid derivatives,[4] and agents of different chemical series, e.g., tiadenol.[5] A promising hypolipidemic activity in the pharmacological and preclinical testing was shown by [[4-chloro-6-[(2,3-dimethylphenyl)amino]-2-pyrimidinyl]thio]acetic acid (**1d**) and by its related ethanolamide **1e**.[6-11] All these new hypolipidemic agents (with the exception of nicotinic acid derivatives) produce an increase in liver weight and volume, peroxisomal proliferation, and, although not mutagenic, the onset of hepatomas in predisposed strains of rats, i.e., Fischer rats.[12-19] We therefore deemed it useful to bring some structural changes to molecules **1d** and **1e**, both in the substituents and in the pyrimidine ring, so as to maintain the hypolipidemic activity and prevent injurious effects on the liver cell.

Two types of compounds were selected, i.e., derivatives similar to **1d** and derivatives, recalling someway the metformin structure, that, according to Sirtori et al.,[6,20] counteract the onset of an experimentally induced atheroma in the rabbit aorta.

The resulting molecules were subjected to a first pharmacological investigation that checked their action on serum cholesterol, triglycerides, and lipoproteins and that assessed for the most active compounds their effect on liver weight and increases in catalase and liver enzymes related to the fatty acid β-oxidation as well as their response to peroxisomal proliferation.

Results

Chemistry. The synthesis of all 2,4,6-trisubstituted pyrimidines started from 1,2-dihydro-2-thioxo-4,6-(1*H*,5*H*)pyrimidinedione (**3**). Its sodium or tetrabutylammonium salt was alkylated with alkyl bromides or iodides, and the corresponding 2-(alkylthio)-4,6(1*H*,5*H*)-pyrimidinediones **4a–d** were obtained (Scheme I). The

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