Na_2SO_4 at 45 °C. One hour later, samples were collected on 0.45-μm HA filters (Millipore Corp.), washed with 15 mL of 1% CPC and 40 mM Na_2SO_4, and dried, and radioactivity was measured in Biofluor (New England Nuclear Corp.) with a Beckman 7500 scintillation spectrometer.

Acknowledgment. This research was supported in part by U. S. Public Health Service Grants CA-02817, CA-28852, and CA-16359 from the National Cancer Institute.

Registry No. 1, 86195-92-8; 2, 86195-93-9; 3, 4103-32-6; 4, 78990-31-5; 5, 86195-94-0; 6, 86195-95-1; 7, 72880-47-8; 8, 86163-12-4; 9, 86163-13-5; 10, 86195-96-2; 11, 86195-97-3; 12, 86163-14-6; 13, 86163-15-7; D-arabinopyranose, 28697-53-2; D-ribopyranose, 10257-32-6; D-glucopyranose, 2280-44-6; D-galactopyranose, 10257-28-0; D-mannopyranose, 530-26-7; L-rhamnopyranose, 73-34-7; 4-aminobenzoic acid, 150-13-0.

Studies of *N*-Hydroxy-*N'*-aminoguanidine Derivatives by Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy and as Ribonucleotide Reductase Inhibitors[1]

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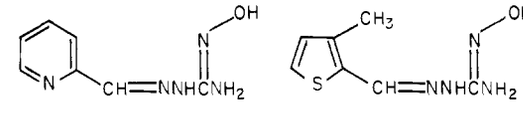
Hydroxyguanidine, with the imino group of guanidine and the hydroxyamino group of hydroxyurea, has functional groups believed to be important for both anticancer and antiviral activities (Adamson, R. H. *Nature (London)* **1972**, *236*, 400–401). Three new *N*-hydroxy-*N'*-aminoguanidine derivatives have been synthesized and found to be 20–30 times more active than the hydroxyguanidine itself as inhibitors of ribonucleotide reductase from rat Novikoff tumors (Tai, W. A.; Lai, M. M.; Lien, E. J. "Novel *N*-Hydroxyguanidine Derivatives as Antiviral Agents", North American Medicinal Chemistry Symposium, University of Toronto, Toronto, Canada, June 20–24, 1982; Abstr, p 144). The character of the tautomeric equilibria, the pK_a values, and the protonation sites of these hydroxyguanidine derivatives have been determined by ^{15}N NMR spectroscopy.

Hydroxyguanidine, a compound with the combined functional groups of anticancer hydroxyurea and antiviral guanidine, has been reported to have anticancer activity, especially against solid tumors like Walker 256 carcinoma in rats.[3] In vitro, hydroxyguanidine has an ID_{50} of 2 μg/mL against Moloney sarcoma virus.[4] Young has reported that *N*-hydroxyguanidine is equal to hydroxyurea in its ability to inhibit DNA synthesis in HeLa cells.[5] The biochemical target is generally believed to be ribonucleotide reductase, an enzyme needed for the reduction of ribonucleotides required for de novo DNA synthesis.[5] Brockman et al. have reported that 2-formylpyridine thiosemicarbazone increased the life span of mice bearing L1210 leukemia.[6] Later, French and Blanz synthesized 1-formylisoquinoline thiosemicarbazone and other α-N-heterocyclic-substituted carboxaldehyde thiosemicarbazones.[7] These compounds have shown anticancer activity against a wide spectrum of transplanted rodent neoplasms, including sarcoma 180, Ehrlich carcinoma, leukemia L1210, Lewis lung carcinoma, hepatoma 129, hepatoma 134, adenocarcinoma 755, and B16 melanoma.[7,8] The α-N-heterocyclic-substituted carboxaldehyde thiosemicarbazones have demonstrated potential as both antineoplastic and antiviral agents. these agents have been shown to inhibit DNA synthesis as a consequence of inhibiting ribonucleoside diphosphate reductase.[9] However, low water solubilities and high toxicities have limited practical therapeutic applications of these compounds.

We report here three new *N*-hydroxy-*N'*-aminoguanidines (**1**–**3**; shown here as particular tautomers in accordance with the NMR data to be discussed later), which have been designed to combine structural elements



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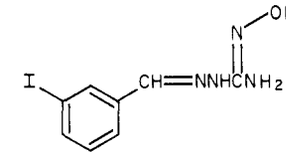

3

of hydroxyguanidine with carboxaldehyde thiosemicarbazones. The *N*-hydroxy group was expected to enhance water solubilities, and the ring substituent groups were chosen to provide a range of lipophilic/hydrophilic balance.

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(1) (a) Taken in part from the Ph.D. dissertation of A.W.T., University of Southern California, 1982. Supported in part by BRSG Grant 2S07RR 05792-05 to the USC School of Pharmacy and by the Clinical Cancer Education Program of the NCI (Grant 5R 25 CA 24426) to A.W.T. (b) Supported by the American Cancer Society (Grant CH122). (c) Supported by the National Science Foundation and by the Public Health Service (Grant No. GM-11072) from the Division of General Medical Sciences.
(2) Contribution No. 6720.
(3) Adamson, R. H. *Nature (London)* **1972**, *236*, 400–401.
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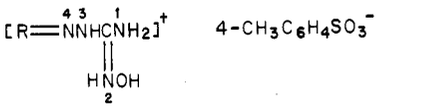
0022-2623/83/1826-1326$01.50/0

Table I. Inhibition of Ribonucleoside Diphosphate Reductase by Aminohydroxyguanidine and Related Derivatives

compd	ID_{50}, M
1	3.8×10^{-5}
2	7.0×10^{-5}
3	3.1×10^{-5}
$H_2NC(NHOH)=NH_2^+ \cdot 0.5H_2SO_4$	9.0×10^{-4}

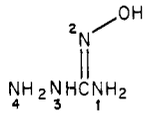
Table II. ^{15}N NMR Chemical Shifts for Salts of Aminohydroxyguanidine Derivatives in $(CH_3)_2SO$



compd	chem shift, ppm N1	N2	N3	N4
1	300.6	235.0	237.9	58.0
2	302.0	235.9	239.2	57.4
3	300.6	235.5	238.3	*a*
4	305.9	241.6	280.5	320.9

[a] Not observed.

The in vitro activities of 1–3 (as the corresponding 4-methylbenzenesulfonate salts) have been tested against ribonucleoside diphosphate reductase from rat Novikoff tumors. The structural and acid–base properties of the representative compounds have also been studied by ^{15}N nuclear magnetic resonance (NMR) spectroscopy, in hope of providing clues for further molecular modifications. To facilitate assignment of the NMR resonance positions of the 4-methylbenzenesulfonates of 1 and **3**, ^{15}N NMR spectra of *N*-hydroxy-*N'*-aminoguanidine (4) as its 4-methylbenzenesulfonate salt (2-diazanyl-*N*-hydroxymethanamidinium 4-methylbenzenesulfonate) were obtained in water and dimethyl sulfoxide.



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Results and Discussion

Table I shows the ID_{50} for inhibition of ribonucleoside diphosphate reductase by hydroxyguanidine sulfate and 1–3. The results indicate that compounds 1 and **3** are some 20–30 times more active than hydroxyguanidine. The detailed data from which ID_{50} was determined indicate that the inhibition is nonlinear with concentration for compound 1 (linear for compound **2** and uncertain for compound **3**), which may indicate either irreversible inhibition or that more than one inhibitor molecule is involved in the inhibition process.

The mechanism of inhibition by hydroxyurea and similar compounds of ribonucleoside diphosphate reductase appears to involve destruction of a radical at the active site of the enzyme.[10] The inhibition is reversed through regeneration of the radical by the combination of dithiol, iron, and oxygen under the conditions of the enzyme assay.[11] Inhibition by the iron chelates of the heterocyclic carboxaldehyde thiosemicarbazones seems to involve the same general mechanism. Hydrophobic groups on the thiosemicarbazone increase the inhibition, probably by binding to the enzyme at or near the active site.[12] Similar binding may explain the greater activity of these aromatic compounds compared to hydroxyguanidine.

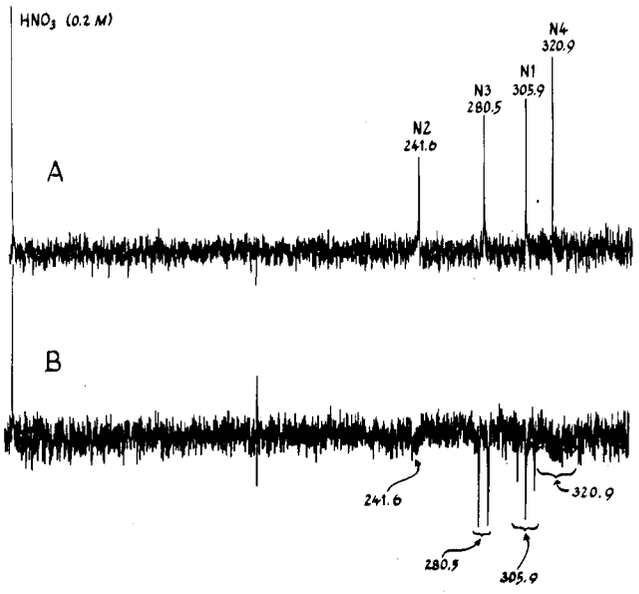
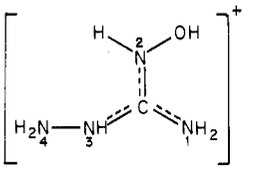


Figure 1. (A) Broad-band decoupled spectrum of 1-amino-3-hydroxyguanidine 4-methylbenzenesulfonate in $(CH_3)_2SO$. (B) ^{15}N NMR coupled spectrum of 1-amino-3-hydroxyguanidine 4-methylbenzenesulfonate in $(CH_3)_2SO$.

The Novikoff rat tumor reductase used here showed a pH optimum between 6.5 and 7.0.[13] The pK_a of the pyridyl derivative (1) is 6.7, as will be shown later from our NMR studies, while that of 1-amino-3-hydroxyguanidine, similarly determined, is 8.7, close to the 8.4 value reported for hydroxyguanidine.[14] These differences suggest that the electronic effects of the ring substituents may be comparably important to their influence on the lipophilic/hydrophilic balance.

The proton-decoupled ^{15}N NMR spectrum of a 0.3 M solution of the sulfate of 4 in $(CH_3)_2SO$ shows four ^{15}N resonance peaks (Figure 1A). In the proton-coupled ^{15}N NMR spectrum (Figure 1B), the resonance at 305.9 ppm was a triplet, and the broad resonance(s) centered on 320.9 ppm seemed to be an exchange-broadened triplet. If both of these resonances are indeed triplets, they can surely be assigned to N1 and N4, respectively. There is ample precedent for the resonance of N4 to be upfield from that of N1. The doublet 280.5-ppm resonance represents an NH nitrogen, and the one at 241.6 ppm is broad and presumably exchange broadened. Because the total number of hydrogens on these nitrogens is six, the most reasonable structure for the conjugate acid of 4 is **5**. It is not



5

certain whether the N2 resonance comes at 280.5 or at 241.6 ppm, but the latter seems more likely because this

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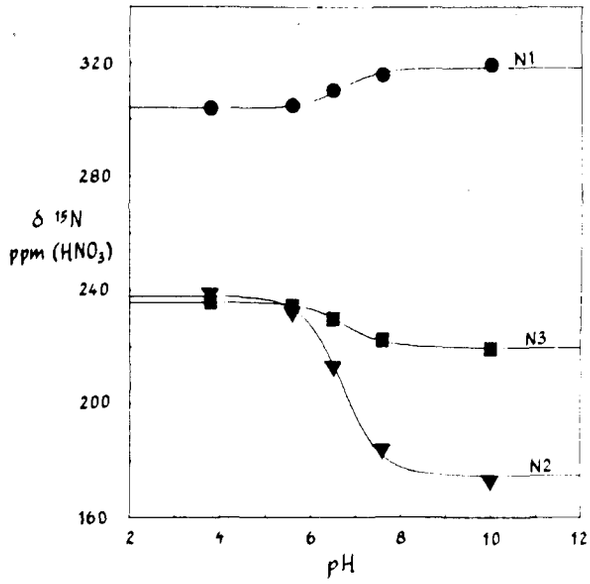
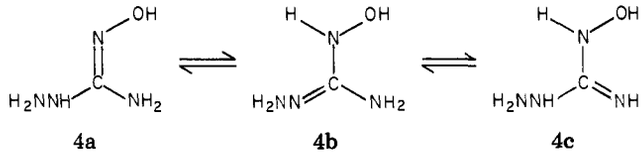


Figure 2. ^{15}N NMR chemical shift changes with pH of 2-[(2-pyridylmethylene)diazanyl]-*N*-hydroxymethanamidinium 4-methylbenzenesulfonate in water solution.

resonance is farther downfield and extensively broadened through proton exchange. The resonance of N2 is expected to experience proton-exchange broadening at a lower pH than that of N3 because N2 should be substantially less basic than N3. Essentially the same shifts are found in water solutions at pH 3.9.

In aqueous solution, structure **5** is strongly supported by the multiplicity of the ^{15}N resonances in the coupled spectrum and also by the pH dependence of the ^{15}N shifts, where **5** is converted to **4** at high pH (Figure 3). Thus, the coupled spectrum shows triplet and doublet resonances for N1 and N3, respectively. A sharp peak at 324 ppm is observed for N4, because of exchange broadening, while N2 gives a broad singlet centered on 244 ppm, also the result of exchange. On deprotonation, the resonances assigned to N1 and N3 move upfield by 12 ppm, that assigned to N4 is essentially unchanged, while that assigned to N2 moves about 30 ppm to *lower* fields, as expected for conversion of a $=NHOH^+$ to an, at least partially, =NOH nitrogen.

The shifts of **4** in aqueous solutions (pH >10) are those anticipated for a rapidly equilibrating mixture of the tautomers **4a** ⇄ **4b** ⇄ **4c**, there being no one resonance



4a **4b** **4c**

as far downfield as expected for a double-bonded nitrogen in the structural units C=NOH (–40 ppm)[15] or $C=NNH_2$ (50 ppm)[16] and only four detectable resonances in all. It is interesting to contemplate which of the tautomers **4a–c** is the most stable. Bond energies are no help for a NHC=N ⇄ N=CNH equilibrium. The position of a tautomeric equilibrium can be correlated with the relative acidities of the separate tautomers, the most favorable isomer being the weakest NH acid. Because the electron-attracting power of an OH group is greater than that

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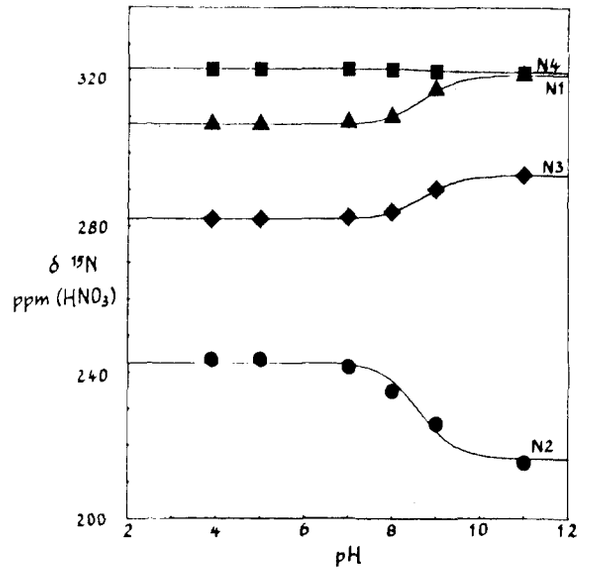
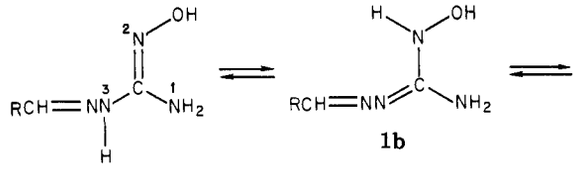


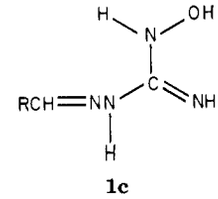
Figure 3. ^{15}N NMR chemical shift changes with pH of 1-amino-3-hydroxyguanidine 4-methylbenzenesulfonate (**4**).

of NH_2 or H groups, it seems likely that **4a** is the weakest NH acid and, hence, the most stable tautomer.

The dependence of the ^{15}N shifts of **1** and **4** is shown in Figures 2 and 3. The pK_a values derived therefrom are 6.7 ± 0.1 and 8.7 ± 0.1, respectively, which shows that **1** and **4** are much weaker bases than typical guanidines. This is surely the result of the diverse electron-attracting powers of the groups substituted on N1–N3. The general pattern of N1, N2, and N3 shifts of **1** and **4** as the 4-methylbenzenesulfonate salts is rather similar, and the assignments seem safely interpreted in the same way when account is taken of the expected downfield shift of N3 by virtue of its being bonded to a CH=N nitrogen (Figure 2). It is of special interest that on deprotonation of the conjugate acid of **1**, the resonance assigned to N3 moves *downfield*, while with the conjugate acid of **4**, the corresponding resonance moves upfield. This behavior suggests that the tautomeric equilibria **1a** ⇄ **1b** ⇄ **1c** are more on



1a **1b**



1c

R = 2-pyridyl

the side of **1b** than **4b** is with **4a** ⇄ **4b** ⇄ **4c**. This is not unexpected, because the acidity of the proton on N3 in **1a** and **1c** will be enhanced relative to **4a** and **4c** by virtue of attachment of the RCH= group.

Experimental Section

Enzyme Inhibition. The 50% inhibitory concentration was estimated from a Dixon plot of the reciprocal of the relative velocity against the inhibitor concentration.

The ribonucleotide reductase was extracted from Novikoff rat tumors. The enzyme from the 100000*g* supernatant was purified

10- to 20-fold by DEAE-cellulose and ammonium sulfate precipitation. The inhibition test medium was 2.1 mM in ATP, 6 mM in magnesium acetate, 20 μM in ferrous ion, 6 mM in dithiothreitol, 0.25 μM in thioredoxin (*Escherichia coli*), 8 mM in potassium phosphate (pH 7), and either 80 μM in [^{32}P]CDP (cytidine diphosphate) or 170 μM in [^{14}C]CDP and contained sufficient enzyme to reduce the concentration of CDP by 12.5–25 μM in 30 min. The reaction product was separated as dCMP (deoxycytidine monophosphate) on Dowex 50^{10} or as dCDP and dCTP on boronate columns.[17]

The inhibitors were dissolved in water and neutralized, except for **3**, which was dissolved in dimethyl sulfoxide and diluted with water to give less than 1% dimethyl sulfoxide in the final solution. The enzyme was added to ice-cold mixtures of the substrates and inhibitors. The solutions were immediately incubated at 37 °C for 30 min. Each inhibitor was tested in four separate runs.

^{15}N NMR Studies. The *N*-hydroxyguanidine derivatives were synthesized as described elsewhere.[4] The ^{15}N NMR spectra were obtained with a Bruker WH-180 spectrometer operating at 18.25 MHz in the FT mode. The ^{15}N NMR spectra of 2-[2-pyridylmethylene)diazanyl]-*N*-hydroxymethanamidinium 4-methylbenzenesulfonate (**1**), 2-[[(3-methyl-2-thienyl)methylene]diazanyl]-*N*-hydroxymethanamidinium 4-methylbenzenesulfonate (**2**), and 2-[[(3-iodophenyl)methylene]diazanyl]-*N*-hydroxymethanamidinium 4-methylbenzenesulfonate (**3**) were taken at the natural-abundance level in 25-mm sample tubes, with broad-band decoupling, a repetition rate of 5 s, and a pulse width of 35 μs (34° flip angle). The concentrations were about 0.3 M.

The ^{15}N NMR chemical shifts are reported in parts per million upfield from an external standard made up to be 1 M $H^{15}NO_3$ in D_2O.

The pH dependence of the ^{15}N NMR chemical shifts of the salts of **1–4** were determined in water solution, with the pH values were adjusted by addition of small increments of concentrated sodium hydroxide or hydrochloric acid, with the pH being measured directly in the NMR sample tube with the aid of a Radiometer pH meter.

Registry No. 1, 85894-16-2; 2, 85894-18-4; 3, 85894-20-8; ribonucleoside diphosphate reductase, 9047-64-7.

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Aza Analogues of Lucanthone: Synthesis and Antitumor and Bactericidal Properties

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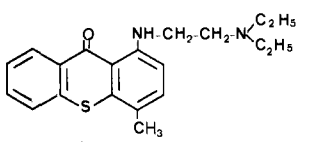
Received December 30, 1982

Three types of aza analogues of lucanthone were synthesized for evaluation as antitumor drugs. None of the compounds was found to have significant cytotoxic effects either on Friend tumor cells or on L1210 leukemia cells. However, one of the target compounds, 5,10-dihydro-10-oxo-1-[[3-(diethylamino)propyl]amino]-3-methylpyrido[4,3-*b*]quinoline, was shown to have noticeable antibiotic properties.

Lucanthone (1, Miracil D) is an important schistosomicidal agent whose antiparasitic properties have been known for a long time.[1,2] Besides this clinical property, 1 is also effective against a wide range of experimental laboratory tumors[3,4] and shows a strong affinity for nucleic acids,[5,6] including DNA, with which it interacts through intercalation.[7,8] A large variety of lucanthone analogues have been synthesized and tested for antitumor activity,[3,9,10] the most potent compound being the 7-hydroxy derivative of 1.[9]



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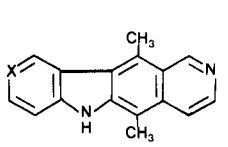
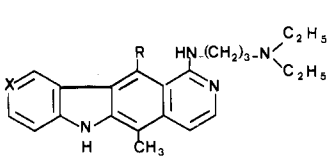
Several members of the ellipticine series, another family of intercalating drugs, are known to have antitumor properties. As observed with lucanthone derivatives, ring-hydroxylated compounds, such **2b**, as well as the methoxy analogue **2c**, are more potent than the unsubstituted ellipticine **2a**.[11,12]



2a. X = C-H
2b. X = C-OH
2c. X = C-OCH$_3$
2d. X = N



3a. X = C-OCH$_3$ R = CH$_3$
3b. X = N R = H

On the one hand, studies carried out in this laboratory on 9-azaellipticine (**2d**) have shown that replacement of

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