exists predominantly in its protonated form, a reaction path given by eq 12e and 12f which is parallel to 12c

$$N_2O_4 + HOC_6H_4N^+OH \longrightarrow HOC_6H_4N\overset{+}{O}_2H + N_2O_3 \quad (12e)$$

$$HOC_6H_4N\overset{+}{O}_2H + H_2O \longrightarrow HOC_6H_4NO_2 + H_3O^+ \quad (12f)$$

has to be assumed. The reactions which cause the autocatalysis are 12c and 12e at low and high acidities, respectively. Reaction 12d may be considered to be fast except at very low values of $a_w$.[15]

The true rate law, eq 8, was derived with the assumption that the preceding steps are fast. This is obviously true for the protolytic equilibrium, 12a. The rate constant for the reverse reaction of eq 12b, determined by pulse radiolytic measurements, is $1 \times 10^3$ sec$^{-1}$ $\times a_w$.[13] With this value and the previously mentioned value of the equilibrium constant, the forward rate constant is calculated to be $1.9 \times 10^3$ $M^{-1}$ sec$^{-1}$.

Since the initial concentration of *p*-nitrosophenol is of the order of $10^{-3}$ $M$, the equilibrium approximation appears to be applicable. Consequently, it is justified to assume that eq 12c is the rate-limiting step as long as the relative concentration of protonated *p*-nitrosophenol is not significant. The positive slope of the acidity profile of the rate constant is due to the formation of undissociated nitric acid which is known to increase according to the $H_0$ acidity function.

(15) M. Graetzel, S. Taniguchi, and A. Henglein, *Ber. Bunsenges. Physik. Chem.*, **74**, 488 (1970).

Since it is reported[16] that the nitrate ion is half-protonated at $H_0 = -2.8$, the rate constant should become acidity independent at $H_0 \simeq -4$. The experimental acidity profile, however, exhibits a maximum at $H_0 = -4.5$ and a decrease of the rate constant at higher acidities. This effect may be connected with a significant heterolytic dissociation of $N_2O_4$ at high acidity, which would reduce its equilibrium concention.[17,18] Alternatively, a protonated $N_2O_4$ species might be formed which could also account for the observed behavior, since its reaction with the equally charged protonated nitrosophenol is expected to be slow.

Most likely, however, a change in the rate-determining step from eq 12c to eq 12f is responsible for the rate decrease. This is supported by the slope of the Bunnett plot, Figure 3A, where the high acidity part with $w = 5$ is typical for a reaction whose rate is limited by a transfer of a proton to the solvent.[19]

**Registry No.**—*p*-Nitrosophenol, 104-91-6; nitric acid, 7697-37-2; nitrous acid, 7782-77-6.

**Acknowledgment.**—We wish to thank Professor N. C. Deno for helpful discussions and for written comments to improve the manuscript.

(16) N. C. Deno, H. J. Peterson, and E. Sacher, *J. Phys. Chem.*, **65**, 199 (1961).
(17) T. A. Turney and G. A. Wright, *J. Chem. Soc.*, 2415 (1958).
(18) F. Seel and R. Winkler, *Z. Phys. Chem.*, **25**, 217 (1960).
(19) C. H. Rochester, "Acidity Functions," Academic Press, London, 1970, p 117.

# Kinetics and Mechanism of the Hydrolysis of Guanosine and 7-Methylguanosine Nucleosides in Perchloric Acid

JOHN A. ZOLTEWICZ* AND D. FRED CLARK

*Department of Chemistry, University of Florida, Gainesville, Florida 32601*

*Received September 7, 1971*

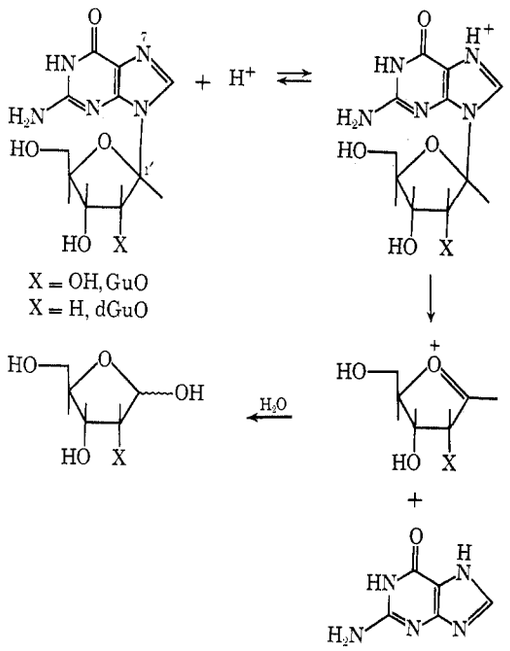
Rates of hydrolysis of guanosine (Guo) and 7-methylguanosine (7-MeGuo) to ribose and guanine or 7-methylguanine were obtained spectrophotometrically and polarimetrically in 1–9 $M$ $HClO_4$ at 30.0°. In 1–7 $M$ $HClO_4$ the substrates are converted to their diprotonated forms to the extent of about 1–90%. Rate changes in this region are approximated by the expression $k_\psi = kh_0/(h_0 + K_a)$, where $h_0$ is the Hammett acidity function and $k$ is the first-order rate constant for fragmentation of the nucleoside. For Guo $k$ is $8.8 \pm 0.6 \times 10^{-4}$ sec$^{-1}$ and for 7-MeGuo $k$ is $1.2 \pm 0.1 \times 10^{-3}$ sec$^{-1}$. "Kinetic" (equilibrium) dissociation constants or p$K_a$ values for Guo and 7-MeGuo are −2.42 (−2.42) and −2.48 (−2.61), respectively. In 7–9 $M$ $HClO_4$ rates continue to increase slightly for Guo but decrease slightly for 7-MeGuo. A mechanism involving cleavage of a protonated ribose ring to give a Schiff-base intermediate is ruled out. Instead, a mechanism (A-1) involving reversible protonation of the purine ring to give a dication followed by rate-limiting cleavage of the heterocycle–sugar bond is advanced. This is similar to the mechanism of hydrolysis of the monocationic forms of these substrates in dilute acid.

Nucleosides, fragments of nucleic acids containing a heterocyclic base bonded to a sugar, are model compounds for the study of nucleic acid hydrolysis.[1] We have shown that the acid-catalyzed hydrolysis of some purine nucleosides takes place by the reaction of mono- as well as diprotonated forms.[2] Guanosine (Guo) and deoxyguanosine (dGuo) hydrolyze first by undergoing reversible protonation of the purine ring to give a monocation and then by rate-determining fragmentation to give a purine and a cyclic carboxonium ion form of the sugar. This cation on reaction with water gives the sugar, ribose or deoxyribose (Scheme I). This mechanism of hydrolysis of a monoprotonated species is supported, for example, by the fact that 1,7-dimethylguanosinium ion (1,7-diMeGuO$^+$) reacts at very nearly the same rate as monoprotonated Guo. The dimethyl cation, since it already bears a positive charge, need not protonate in order to react and hence it undergoes ready fragmentation.

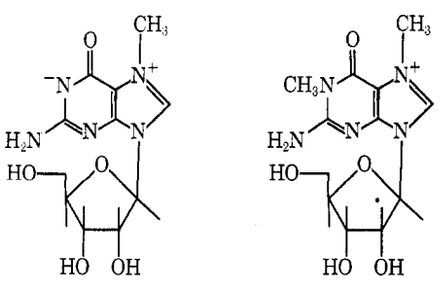
In this paper we consider and eliminate the possibility that mono- and diprotonated purine nucleosides react by different mechanisms. We report the results of the hydrolysis of Guo and 7-methylguanosine (7-MeGuo) in perchloric acid. Our results allow a distinction to be made between two mechanistic possibil-

(1) H. S. Loring in "The Nucleic Acids," E. Chargaff and J. N. Davidson, Ed., Academic Press, New York, N. Y., 1955, Chapter 5.
(2) J. A. Zoltewicz, D. F. Clark, T. W. Sharpless, and G. Grahe, *J. Amer. Chem. Soc.*, **92**, 1741 (1970). Additional references are included in this paper.

SCHEME I




ities. First, the purine ring becomes diprotonated and then leaves in the slow step. This is an extension of the reaction pathway in dilute acid.[2] Second, the ribose ring opens following protonation of the annular oxygen atom. The resultant Schiff base then undergoes hydrolysis. This type of ring-opening pathway has long been considered to be a distinct possibility for the hydrolysis of nucleosides.[2-4]



7-MeGuo 1,7-diMeGuo+

## Experimental Section

**Materials.**—Guanosine (Guo), guanine, and ribose were obtained from CalBiochem. 7-Methylguanosine (7-MeGuo) was prepared by methylation of Guo.[2,5] Perchloric acid was 70% Baker analyzed or 60 and 70% Mallinckrodt analytical reagent grade. Acid solutions were standarized using Fisher primary "THAM."

**Equipment.**- Absorbance changes were followed with either a Zeiss PMQ II or a Beckman DU spectrophotometer. The wavelengths employed in kinetic runs were 265 nm for Guo and 285 or 295 nm for 7-MeGuo.

Rotation of polarized light was monitored with a Perkin-Elmer 141 polarimeter. Solutions in a jacketed polarimeter cell were thermostated at 30.0 ± 0.1° by a Haake EDe constant temperature circulator. Changes generally were followed at 546 nm. All temperatures were measured with a National Bureau of Standards certified thermometer.

**Stability Studies.**—Some kinetic runs employed a sodium hydroxide quench technique. We provided evidence earlier that guanosine, guanine, and 7-methylguanine solutions in ~1 *M* NaOH at room temperature are stable.[2] 7-Methylguanosine under these conditions undergoes a rapid imidazole ring-opening reaction but the resultant hydrolysis product is stable.[2,5]

Guo was exposed to 9.75 *M* $HClO_4$ at room temperature for 345 min and the liberated guanine was recovered following neutralization with sodium hydroxide. The collected solid was reprecipitated from alkaline solution using acetic acid; a 97% yield of guanine, mp >360°, was obtained. Authentic guanine has mp >360°. Recovered material was chromatographically homogeneous.

Ribose degraded during some kinetic runs. Mixtures at the highest acidities employed in the kinetic studies acquired a yellow coloration; acidic samples which stood beyond the period of the run eventually turned black. For example, a 0.1 *M* solution of ribose or guanosine in 9.75 *M* $HClO_4$ acquired a yellow color within 0.5 hr and after about 3 hr at 30° solutions were too black for polarimetric measurements.

Large rotational changes may accompany nucleoside hydrolysis; larger changes result with increasing acid concentration. For example, about a 2.8° change was observed during the hydrolysis of a 0.14 *M* solution of guanosine in 9.75 *M* $HClO_4$. However, small rotational changes are associated with the degradation of ribose. Hence, measured rotational changes are essentially associated with the hydrolysis of the nucleoside. In practice first-order kinetic plots using polarimetry to follow the reaction were linear over at least 3 half-lives. There was no indication of ribose degradation from the kinetic plots themselves. There was no evidence of rotational changes such as those which might occur if anomerization of a sugar preceded hydrolysis.

The presence of guanine seems to have no influence on ribose degradation. Solutions of ribose in 9.75 *M* $HClO_4$ and of ribose and an equivalent of guanine in 9.75 *M* $HClO_4$ show essentially identical rotations.

Ribose degradation was unimportant for hydrolyses carried out at lower acidities. For example, in the hydrolysis of guanosine in 4.68 *M* $HClO_4$ the "infinity" optical rotation (20 hr) was the same as that for ribose in 4.68 *M* $HClO_4$ taken within 10 min after mixing and no color was visible. No attempt was made to establish the acidity and time at which significant ribose degradation occurs.

**Kinetics. A. Alkaline Quench Method.**—Reactions followed spectrophotometrically employed a sodium hydroxide quenching solution in order to increase the difference in absorbance between reactant and products. Approximately 30–40 mg of guanosine or 7-methylguanosine was weighed into a 100-ml volumetric flask and diluted to volume. Solutions were maintained at 30.0 ± 0.1°; at various intervals 6-ml aliquots were withdrawn and diluted to 50 ml with 2.5 *M* NaOH quenching solution. Pseudo-first-order rate plots using the infinity method were employed.[2] Plots generally were linear over 2–3 half-lives. Results are given in Table I.

**B. Polarimetric Method.**—Approximately 0.1 g of guanosine or 7-methylguanosine was dissolved in 2 ml of $HClO_4$ and immediately placed in a jacketed polarimeter cell maintained at 30.0 ± 0.1°. Pseudo-first-order rate plots (20–50 points) were constructed in a manner similar to that used for spectrophotometric data. Infinity rotations had finite values, as required for the formation of optically active product. Plots were linear over 3–5 half-lives.

$H_0$ values for perchloric acid were converted from the original weight per cent scale[6] to molarity using known densities.[7] $H_0$ values were then obtained from a molarity–$h_0$ plot.

## Results

When Guo and 7-MeGuo are hydrolyzed to guanine and 7-methylguanine in moderately concentrated perchloric acid, their ultraviolet absorption spectra undergo only small changes. This prohibits direct monitoring of the acidic reaction mixture to obtain kinetic data. However, larger absorbance changes result when the acidic mixtures are quenched with concentrated sodium hydroxide and the alkaline reaction

(3) B. Capon, *Chem. Rev.*, **69**, 407 (1969).
(4) A. M. Michelson, "The Chemistry of Nucleosides and Nucleotides," Academic Press, New York, N. Y., 1963.
(5) J. W. Jones and R. K. Robins, *J. Amer. Chem. Soc.*, **85**, 193 (1963).
(6) K. Yates and H. Wai, *ibid.*, **86**, 5408 (1964).
(7) A. E. Markham, *ibid.*, **63**, 874 (1941); G. F. Smith and O. E. Goehler, *Ind. Eng. Chem., Anal. Ed.*, **3**, 61 (1931).

solutions are examined as a function of reaction time. This procedure results in imidazole ring opening of 7-MeGuo in alkali but the ring-opened product is stable.[2,5] Simple ionization occurs in the case of Guo, guanine, and 7-methylguanine. Although the liberated ribose may degrade in acid, this side reaction does not interfere with the spectrophotometric study. Good kinetic data were obtained by this spectrophotometric method for Guo and 7-MeGuo for the region 1–9 *M* $HClO_4$ at 30.0°. Results are summarized in Table I.

A few hydrolysis reactions were followed polarimetrically. Kinetic runs were carried out using a jacketed polarimeter tube and rotations of the acid solutions were measured directly. Ribose degraded in the more acidic solutions[8] but was stable over a period of a kinetic run at acidities less than 5 *M* $HClO_4$. Even when ribose degraded, good pseudo-first-order plots were obtained over as much as 5 half-lives. This obtains because rotational changes associated with the hydrolysis reaction are considerably greater than those resulting from ribose degradation. It was possible to obtain essentially constant rotational values after 10 half-lives (infinity) even when ribose degraded. The appearance of a yellow coloration provided visual evidence of ribose degradation; with time these yellow solutions turned black. Studies using polarimetry were carried out at the higher acid concentrations where reactions are fast (Table I).

First-order rate constants obtained spectrophotometrically and polarimetrically agree to within 4% on the average (Table I). This agreement between results obtained by two different physical methods clearly establishes the validity of the rate constants. The spectrophotometric method measures the concentration of the purines while the polarimetric method essentially measures the concentrations of the sugars. Since the concentration of nucleoside is about 150 times larger in the polarimetric than in the spectrophotometric studies and since results from the two approaches agree so well, then it must be concluded that there is no evidence in our results for any kind of molecular aggregation which perturbs chemical reactivity. This is consistent with the knowledge that interactions among purine bases are greatly diminished when the bases are protonated.[9] Moreover, our data show that, in spite of ribose degradation, polarimetry is a valuable method for obtaining solvolysis rates. The method is particularly attractive because of its experimental simplicity.

The data in Table I show that the rates of hydrolysis of Guo and 7-MeGuo increase with increasing acid concentration. Both substrates show very similar rates of hydrolysis in the region 1–7 *M* $HClO_4$. Rates for 7-MeGuo reach a maximum at about 7.2 *M* $HClO_4$ and then decrease slightly as the acidity increases. No rate maximum is observed for the hydrolysis of Guo over the same acidity region but there is a change to a less rapid rate increase at about 7 *M* $HClO_4$. At about 7.5 *M* $HClO_4$ both substrates have identical hydrolysis rates with a half-life of 12 min. Below this acidity Guo is about 10% less reactive than 7-MeGuo but above this acidity the first real differences in reactivity between Guo and MeGuo become apparent. For example, at 9.4 *M* $HClO_4$ Guo is about 70% more reactive than 7-MeGuo.

In the region 1–7 *M* $HClO_4$ it is possible to approximate the rates of hydrolysis of Guo and 7-MeGuo by eq 1 and the observed pseudo-first-order rate constant, $k_\psi$, by eq 2. Symbols have the following meaning: $[SH]_t$ = concentration of nucleoside in all forms; [SH]

TABLE I

RATE CONSTANTS FOR THE HYDROLYSIS OF GUANOSINE AND 7-METHYLGUANOSINE AT 30.0° IN PERCHLORIC ACID[a]

Guanosine[b]

| $[HClO_4]$, *M* | $-H_0$ | $10^4k_\psi$, $sec^{-1}$ | $10^4k$, $sec^{-1}$ |
|---|---|---|---|
| 1.20 | 0.44 | 0.0848 | 8.20 |
| 1.40 | 0.58 | 0.130 | 9.15 |
| 1.76 | 0.78 | 0.196 | 8.73 |
| 2.34 | 1.06 | 0.398 | 9.54 |
| 2.40 | 1.08 | 0.392 | 8.98 |
| 3.29 | 1.47 | 0.869 | 8.69 |
| 3.61 | 1.61 | 1.25 | 9.35 |
| 3.64 | 1.63 | 1.12 | 7.18 |
| 4.68 | 2.12 | 3.61 | 10.8 |
| 4.81 | 2.21 | 3.67 | 9.60 |
| 4.86 | 2.24 | 2.90 | 7.45 |
| 5.18 | 2.41 | 4.13 | 8.35 |
| 5.26 | 2.46 | 4.99 | 9.52 |
| 5.65 | 2.67 | 5.37 | 8.40 |
| 6.01 | 2.89 | 6.17 | 8.33 |
| 6.12 | 2.96 | 6.47 | 8.26 |
| 6.59 | 3.27 | 7.65 | 8.72 |
| 7.06 | 3.57 | 8.31 | 8.90 |
| 7.25 | 3.69 | 9.17* | |
| 7.25 | 3.69 | 9.00 | |
| 7.53 | 3.83 | 9.40 | |
| 8.00 | 4.27 | 10.5 | |
| 8.25 | 4.49 | 10.9* | |
| 8.47 | 4.69 | 11.4 | |
| 9.41 | 5.54 | 12.9 | |

7-Methylguanosine[c]

| $[HClO_4]$, *M* | $-H_0$ | $10^4k_\psi$, $sec^{-1}$ | $10^3k$, $sec^{-1}$ |
|---|---|---|---|
| 1.00 | 0.30 | 0.0847 | 1.35 |
| 2.00 | 0.89 | 0.296 | 1.23 |
| 2.50 | 1.12 | 0.535 | 1.33 |
| 3.00 | 1.34 | 0.929 | 1.42 |
| 4.00 | 1.79 | 1.98 | 1.21 |
| 4.00 | 1.79 | 1.80* | 1.09 |
| 4.25 | 1.90 | 2.65 | 1.31 |
| 4.75 | 2.17 | 4.03 | 1.29 |
| 5.00 | 2.31 | 4.66 | 1.18 |
| 5.25 | 2.45 | 4.28 | 0.92 |
| 5.68 | 2.78 | 6.83 | 1.13 |
| 6.50 | 3.21 | 8.90 | 1.06 |
| 6.75 | 3.36 | 9.25 | 1.06 |
| 7.00 | 3.52 | 9.59 | 1.05 |
| 7.00 | 3.52 | 9.59* | 1.05 |
| 7.25 | 3.69 | 9.48 | |
| 7.25 | 3.69 | 10.8* | |
| 7.50 | 3.81 | 9.25 | |
| 7.50 | 3.81 | 9.51* | |
| 8.00 | 4.27 | 8.95 | |
| 8.25 | 4.49 | 9.00* | |
| 8.50 | 4.70 | 8.29 | |
| 9.37 | 5.49 | 7.95* | |
| 9.41 | 5.54 | 7.60 | |

[a] $k = k_\psi(h_0 + K_a)/h_0$. Rate constants marked with an asterisk were obtained polarimetrically. [b] $K_a = 263$; $k_{avg} = 8.8 \pm 0.6 \times 10^{-4}\ M^{-1}\ sec^{-1}$. [c] $K_a = 315$; $k_{avg} = 1.2 \pm 0.1 \times 10^{-3}\ M^{-1}\ sec^{-1}$.

(8) F. A. H. Rice and L. Fishbein, *J. Amer. Chem. Soc.*, **78**, 1005 (1956); F. A. H. Rice and A. R. Johnson, *J. Org. Chem.*, **23**, 1966 (1958).

(9) S. I. Chan and J. H. Nelson, *J. Amer. Chem. Soc.*, **91**, 168 (1969); P. O. P. Ts'O, N. S. Kondo, R. K. Robins, and A. D. Broom, *ibid.*, **91**, 5625 (1969).

$$\text{rate} = k_\psi[\text{SH}]_t = k_2h_0[\text{SH}] \quad (1)$$

$$k_\psi = \frac{kh_0}{h_0 + K_a} \quad (2)$$

= concentration of monoprotonated nucleoside; $K_a = h_0[\text{SH}]/[\text{SH}_2]$ = the ionization constant for diprotonated substrate; $h_0$ is the Hammett acidity function, and $k = k_2K_a$ where $k$ is a first-order rate constant for the hydrolysis of the diprotonated nucleoside. Significant hydrolysis of the monoprotonated species could not be detected.

In Table I are $k$ values of Guo and 7-MeGuo calculated according to eq 2. It may be seen that $k$ values are satisfactorily constant over the region 1–7 $M$ $HClO_4$; $k = 8.8 \pm 0.6 \times 10^{-4}$ sec$^{-1}$ for Guo. The kinetic p$K_a$ value employed for Guo is −2.42. The p$K_a$ value determined in a separate study where hydrolysis is unimportant is −2.42.[2]

When a kinetic p$K_a$ of −2.48 is employed for 7-MeGuo $k$ is $1.2 \pm 0.1 \times 10^{-3}$ sec$^{-1}$, but when a p$K_a$ of −2.61 is utilized $k$ is $1.4 \pm 0.3 \times 10^{-3}$ sec$^{-1}$. This latter p$K_a$ value, which is the value found under conditions where hydrolysis is unimportant,[2] gives a $k$ value with a larger (21% *vs.* 8%) uncertainty range. Nevertheless, the agreement between p$K_a$ values determined under hydrolytic and nonhydrolytic conditions is good and the range of $k$ values calculated using the two p$K_a$ values overlap. Hence, rates are adequately described by eq 2 over an acidity range which brings about the conversion of more than 90% of the monoprotonated substrate to its diprotonated form.

Inspection of the data for 7-MeGuo given in Table I indicates, with a few exceptions, that there is a small but systematic decrease in $k$ with increasing acidity. This means that the protonation of 7-MeGuo does not exactly follow the Hammett acidity function. This is not unexpected.[10-12] It is to be remembered that the indicators used in the construction of the Hammett acidity function are primary anilines which undergo monoprotonation. It is not surprising that anilines and monoprotonated purine nucleosides do not respond to changes in medium acidity in identical ways. It is remarkable how well the nucleoside hydrolysis data fit a simple Hammett acidity function.

Rates at >7 $M$ $HClO_4$ are not described by eq 1. It seems that medium effects specific for each substrate may be involved. This is not without ample precedent.[10-14]

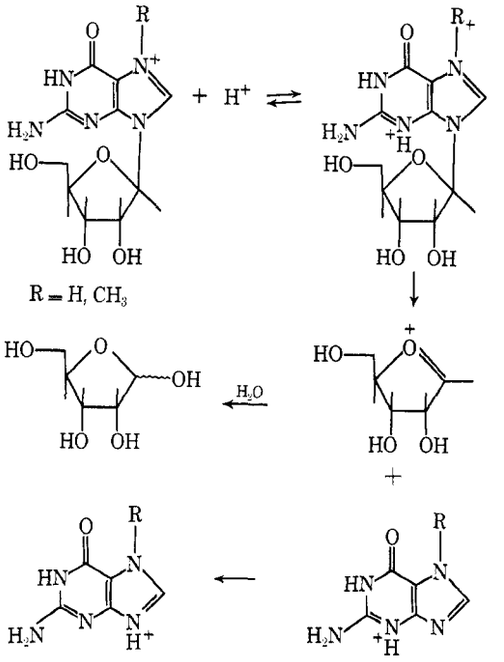
Bunnett plots[10] were constructed for the 1–7 $M$ acid region. Plots of log $k$ (Table I) *vs.* log $a_{H_2O}$ have slopes or "$w$" values of 0 and 0.2 for Guo and 7-MeGuo, respectively. According to the $w$ value criterion, water is not involved in the reaction of protonated substrate. (Our earlier study[2] of the p$K_a$ values of Guo and 7-MeGuo shows $w$ to be ~0 for equilibrium protonation.)

## Discussion

Our kinetic results dealing with the hydrolysis of Guo and 7-MeGuo suggest that these substrates react by a mechanism involving equilibrium protonation which precedes the rate-limiting step. Results supporting this conclusion are the following. (a) Rates of reaction using 1–7 $M$ $HClO_4$ at 30.0° closely follow the Hammett acidity function $H_0$. (b) Rates of hydrolysis fit eq 1 which assumes a preequilibrium protonation mechanism. The "kinetic" p$K_a$ values used in eq 2 and observed p$K_a$ values are in good agreement. They are, respectively, −2.42 and −2.42 for Guo and −2.48 and −2.61 for 7-MeGuo. (c) Bunnett $w$ values of ~0 for Guo and 7-MeGuo show that water molecules function neither as nucleophiles nor as proton transfer agents (general acids) in the rate-limiting step of the reaction.

These data suggest a mechanism involving protonation of a purine ring to give a heterocyclic dication which then fragments in the rate-determining step to give a monoprotonated guanine and a cyclic carboxonium ion. The second proton has arbitrarily been added to N-3 in Scheme II.[15] Reaction of the carboxonium

SCHEME II




ion and water generates ribose. Hence, mono- and diprotonated purine nucleosides react by the same kind of mechanism, differing in the degree of protonation. The $w$ value suggests that water does not assist the fragmentation step as in an SN2 reaction; *i.e.*, the hydrolysis mechanism is A-1 and not A-2.

The data are not consistent with a mechanism involving cleavage of a protonated ribose ring to give a Schiff base which then undergoes hydrolysis. The kinetic characteristics of such a mechanism may be discerned by a consideration of the hydrolysis of glycosylamines, compounds similar to nucleosides in that they consist of a nitrogen-containing group bonded to a sugar. Here, too, hydrolysis gives rise to CN bond cleavage products but the mechanism involves ring opening.[3] The hydrolysis of glycosylamines is specific acid–general base (general acid) catalyzed and shows rate maxima which may occur in concentrated acidic

(10) J. F. Bunnett, *J. Amer. Chem. Soc.*, **83**, 4956, 4968, 4973, 4978 (1961).
(11) C. H. Rochester, "Acidity Functions," Academic Press, New York, N. Y., 1970.
(12) K. Yates, *Accounts Chem. Res.*, **4**, 136 (1971).
(13) R. H. Boyd, *J. Amer. Chem. Soc.*, **85**, 1555 (1963).
(14) E. M. Arnett and G. W. Mach, *ibid.*, **88**, 1177 (1966).
(15) R. Wagner and W. V. Philipsborn, *Helv. Chim. Acta*, **53**, 299 (1970); J. P. Shoffner, L. Bauer, and C. T. Bell, *J. Heterocycl. Chem.*, **7**, 487 (1970).

solutions. The rate-determining step in the acidity region where rates increase with increasing acid concentration involves addition of water to the ring-opened cation. Neither of these characteristics is found for the nucleosides considered here. Reactions in which water is acting as a nucleophile have Bunnett $w$ values in the range 1.2–3.3 and those in which water is acting as a proton transfer agent have values >3.3. Clearly these are much larger than the values of approximately 0 observed here. Our results fall into Bunnett's third group (−2.5–0), indicating the lack of the involvement of water in the rate-limiting step of the reaction.

A shallow rate maximum was observed near 7 $M$ $HClO_4$ for the hydrolysis of 7-MeGuo and this would seem to provide support for the ring-opening pathway. However, in the same acidity region where 7-MeGuo shows decreasing hydrolysis rates with increasing acidity, Guo shows just the opposite pattern. A slight increase in rate with increasing acidity results. This is in an acidity region where both substrates are largely converted to their dicationic forms and a region in which kinetic eq 2 predicts small changes in the rate constant $k_\psi$. Since it is likely that Guo and 7-MeGuo react by the same pathway in this acidity region and since the observed changes are small and in opposite directions, it seems reasonable to conclude that medium effects are influencing reactivity. It is not at all uncommon to find large and nonlinear changes in the reactivity of a substrate in concentrated electrolyte solutions.[10-14] Such changes could, for example, result from variations in solvation of the substrates.

Overall, then, there are no results in our work to support a ring-opening mechanism. Rather, the data suggest a CN bond cleavage mechanism such as that given by Scheme I. A similar conclusion is likely to extend to the hydrolysis of purine deoxyribosides in concentrated acid. Again the hydrolysis is likely to be A-1 rather than A-2 because a more stable carboxonium ion is formed in the case of the deoxyribosides.

Kinetic data now are available to describe the hydrolysis of Guo and 7-MeGuo to ribose and to guanine or 7-methylguanine over a wide acidity range. This varies from pH 3 (Guo) and pH 5 (7-MeGuo)[2] at 100° to 9 $M$ $HClO_4$ at 30°. This variation in acidity covers a factor of about $10,^{8-10}$ neglecting temperature changes. Over this entire acidity region the rate of Guo hydrolysis increases linearly with increasing acid concentration while rates for 7-MeGuo show a similar acid dependence except for pH 3–5 at 100° where rates are independent of acid concentration and except for >7 $M$ $HClO_4$ where rates decreased slightly. It is worth noting that, for example, in 1 $M$ $HClO_4$ at 30° where only about 1% of Guo and 7-MeGuo exist in their diprotonated forms, hydrolysis by way of the monocations is negligible relative to reaction by means of diprotonated structures. This clearly demonstrates that the fragmentation of the dications must be at least $10^3$ faster than that for the monocations. Diprotonated purine is a better leaving group than is monoprotonated purine.

The results of kinetic studies such as these on Guo and 7-MeGuo make it possible to select convenient conditions for the hydrolysis of these substrates with the assurance that side reactions such as deamination are unimportant. Thus, if one desires to hydrolyze Guo under conditions where the half-life is, say, 30 min, then pH 1.7 at 100° or 4.8 $M$ $HClO_4$ at 30° may be selected.

Our studies on the hydrolysis of purine nucleosides in dilute and concentrated acids provide the first truly detailed understanding of nucleoside hydrolysis. They are a first step toward an understanding of the factors which influence the rates of cleavage of sugar–heterocycle bonds in nucleic acids. Additional studies are desirable.

**Registry No.**—Guanosine, 118-00-3; 7-methylguanosine, 33686-50-9.

**Acknowledgment.**—Support of this work by a grant (11798) from the National Institutes of Health, Division of General Medical Sciences, is gratefully acknowledged. A National Institutes of Health Predoctoral Fellowship was awarded to D. F. C.