A Study on the Relative Stability and a Quantitative Approach to the Reaction Mechanism of the Acid-Catalyzed Hydrolysis of Certain 7- and 9-β-D-Ribofuranosylpurines[1a,b]

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Abstract: The rates of acid-catalyzed hydrolyses of a number of 7-β-D-ribofuranosylpurines and 9-β-D-ribofuranosylpurines have been measured polarimetrically and the kinetic parameters calculated. The relative stability of the glycosidic bond for the 7- *vs.* the 9-β-D-ribofuranosylpurines has been determined and with one exception, that of guanosine and 7-(β-D-ribofuranosyl)guanine, the 7 isomer was found to hydrolyze at a faster rate. The kinetic data, as well as possible steric and electronic factors, have been considered in view of previously proposed mechanisms for the hydrolysis of purine nucleosides and strongly suggest an A-1 mechanism, *i.e.*, a preequilibrium protonation of the purine followed by rate-limiting cleavage of the glycosyl–purine bond.

The recent synthesis of a number of 7-glycosylpurines[3–6] in our laboratory made possible the study of the relative stability of the glycosidic bond of these compounds as compared to their 9-glycosylpurine counterparts. A limited amount of work on the acid-catalyzed hydrolysis of purine nucleosides has appeared in the literature.[7–11] These studies have involved only a few ribosylpurines[12] in a qualitative[7–10] or semiquantitative manner.[11] Furthermore, there are conflicting reports in the literature[13,14] on the relative stability of certain glycosylpurines (7 *vs.* 9) toward acid-catalyzed hydrolysis. The 7- and the 9-glucosides of xanthine[13] were hydrolyzed with 1 *N* hydrochloric acid for 1 hr at 100° and paper chromatography showed that the 9-glucoside was completely hydrolyzed whereas the hydrolysis of the 7 isomer was incomplete. A similar hydrolysis study[14] involving adenosine and 7-(β-D-ribofuranosyl)adenine indicated the reverse pattern. In this investigation, 7-(β-D-ribofuranosyl) adenine was completely hydrolyzed after 4 hr in 0.1 *N* hydrochloric acid at 56°, while it took more than 25 hr for the hydrolysis of adenosine. In view of these findings, the kinetic investigation of the acid-catalyzed hydrolysis of 7- and 9-β-D-ribosylpurines was begun in order to accomplish two objectives: (1) determine the relative stability of the glycosyl linkage of the 7 isomers as compared to their 9-counterparts; and (2) propose a plausible reaction mechanism for the acid-catalyzed hydrolysis of ribofuranosylpurines.

Experimental Section

Materials. (a) Reactants. The physical properties of the 7- and 9-β-D-purine nucleosides used in this kinetic investigation are given in Table I. The purity of these nucleosides was ascertained by descending paper chromatography on Whatman No. 1 paper. The solvent systems used were: A, 1-propanol–ammonium hydroxide–water (6:3:1, v/v); B, 5% ammonium bicarbonate; C, ethanol–water (7:3, v/v); D, ethyl acetate–1-propanol–water (4:1:2, v/v) upper phase; E, *n*-butyl alcohol saturated with water.

(b) Products. The purine bases of the nucleosides used were subjected to stringent reaction conditions[15] to preclude the conversion of any functional group on the purine ring and to ensure that hydrolysis of the glycosidic bond was the only parameter being measured. That cleavage of the glycosidic bond was the only reaction occurring during hydrolysis was further substantiated by chromatographic and ultraviolet analyses of the final solutions which showed only the expected products, D-ribose and the heterocyclic moiety. The likelihood of ribose decomposition[16a,b] was also considered. This possibility was eliminated when D-ribose was found to be stable at the temperatures (34.85 and 44.90°) and reaction conditions employed in this study.

Kinetic Measurements. (a) Mechanics of Measurements. A calibrated Perkin-Elmer Model 141 automatic digital readout polarimeter (sodium source, 589 nm) was used.[17] The hydrolyses were carried out in a jacketed microcell which was supplied with this polarimeter. The cell was thermostated by circulating water from

(1) (a) This investigation was supported by Research Contract No. PH-43-65-1041 with Drug Research and Development, National Cancer Institute, National Institutes of Health, Public Health Service. (b) R. J. Rousseau, R. P. Panzica, R. K. Robins, and L. B. Townsend, presented in part before the Carbohydrate Chemistry Division, 155th National Meeting of the American Chemical Society, San Francisco, Calif., April 1968, No. 53.

(2) The recipient of a University of Utah Research Committee Fellowship, 1968–1970.

(3) R. J. Rousseau, L. B. Townsend, and R. K. Robins, *Chem. Commun.*, 265 (1966).

(4) R. J. Rousseau, R. K. Robins, and L. B. Townsend, *J. Amer. Chem. Soc.*, **90**, 2661 (1968).

(5) R. J. Rousseau and L. B. Townsend, *J. Org. Chem.*, **33**, 2828 (1968).

(6) R. J. Rousseau, R. P. Panzica, R. K. Robins, S. M. Reddick, and L. B. Townsend, *ibid.*, **35**, 631 (1970).

(7) H. Venner, *Hoppe-Seyler's Z. Physiol. Chem.*, **339**, 14 (1964).

(8) B. I. Sukhorukov, V. I. Poltev, and L. A. Blyumenfel'd, *Biofizika*, **9**, 266 (1964); *Chem. Abstr.*, **61**, 7263 (1964).

(9) A. Wacker and L. Träger, *Z. Naturforsch. B*, **18**, 13 (1963); *Chem. Abstr.*, **59**, 4022 (1963).

(10) H. Venner, *Abh. Deut. Akad. Wiss. Berlin, Kl. Med.*, 45 (1964); *Chem. Abstr.*, **62**, 14971 (1965).

(11) J. A. Zoltewicz, D. F. Clark, T. W. Sharpless, and G. Grahe, *J. Amer. Chem. Soc.*, **92**, 1741 (1970).

(12) (a) Adenosine, deoxyadenosine, guanosine, deoxyguanosine, and the methyl analogs of the latter two nucleosides. (b) Psicofuranine, a ketonucleoside, has also been investigated by E. R. Garrett, *J. Amer. Chem. Soc.*, **82**, 827 (1960).

(13) J. Baddiley, J. G. Buchanan, and G. O. Osborne, *J. Chem. Soc.*, 3606 (1958).

(14) J. A. Montgomery and H. J. Thomas, *J. Amer. Chem. Soc.*, **87**, 5442 (1965).

(15) The purines used in this study were heated at 80° for 2 days in 1.02 *N* hydrochloric acid and checked for possible degradation by ultraviolet and chromatographic means; none was found. 6-Chloropurine and 6-methoxypurine were found to be unstable at 35°; therefore the corresponding ribosides were eliminated from this study.

(16) (a) F. A. H. Rice and L. Fishbein, *J. Amer. Chem. Soc.*, **78**, 1005 (1956). (b) We found ribose to undergo decomposition in 1 *N* HCl at 80° with the appearance of an absorption peak at 277 nm in the ultraviolet spectrum.

(17) A standard sucrose solution was used for calibration; see F. J. Bates, "Polarimetry and Saccharimetry and the Sugars," National Bureau of Standards, Washington, D. C., 1942, p 81.

Table I. Physicochemical Properties of Purine Nucleosides

Purine nucleoside	$[\alpha]^{27}D$ [lit], deg	pK^f [lit] Basic	pK^f [lit] Acidic
9-(β-D-Ribofuranosyl)adenine (adenosine)	−61.5 (*c* 1.025, H_2O) [−61.7;[11] *c* 0.706, H_2O][a]	3.62 ± 0.02 [3.55][a]	[12.5][a]
7-(β-D-Ribofuranosyl)adenine	−86.3 (*c* 1.005, H_2O) [−85.1; *c* 0.35, H_2O][b]	3.60 ± 0.10	
9-(β-D-Ribofuranosyl)hypoxanthine (inosine)	−69.2 (*c* 1.04, 0.1 *N* NaOH) [−58.8; H_2O][a]	0.96 ± 0.08 [1.2][a]	8.67 ± 0.10 [8.8, 12.3][a]
7-(β-D-Ribofuranosyl)hypoxanthine	−1.35 (*c* 1.035, 0.1 *N* NaOH) [+15.9;[26] *c* 1, 0.1 *N* NaOH][b,c]	1.25 ± 0.10	8.95 ± 0.10
9-(β-D-Ribofuranosyl)guanine (guanosine·$2H_2O$)	−67.9 (*c* 1.035, 0.1 *N* NaOH) [−72;[26] *c* 1.4, 0.1 *N* NaOH][a]	[1.6][a]	9.30 ± 0.01 [9.2, 12.4][a]
7-(β-D-Ribofuranosyl)guanine	−3.49 (*c* 0.745, 0.1 *N* NaOH) [−11.75;[26] *c* 0.6, 0.1 *N* NaOH][b,c]	2.86 ± 0.02	
9-(β-D-Ribofuranosyl)purine (nebularine)	−46.5 (*c* 0.995, H_2O) [−48.6;[25] *c* 1.0, H_2O][a]	1.89 ± 0.01 [2.1][a]	10.00
7-(β-D-Ribofuranosyl)purine	−36.7 (*c* 1.015, H_2O)[d]	1.83 ± 0.03	
9-(β-D-Ribofuranosyl)purine-6-thione	−72.7 (*c* 1.035, 0.1 *N* NaOH) [−73.0; *c* 1.0, 0.1 *N* NaOH][e]		7.47 ± 0.03 [7.56][e]
7-(β-D-Ribofuranosyl)purine-6-thione	+96.0 (*c* 1.075, 0.1 *N* NaOH)[d]	0.90 ± 0.04	7.70 ± 0.02
6-Methylthio-9-(β-D-ribofuranosyl)purine	−71.1 (*c* 1.015, pyridine)	0.78 ± 0.10	9.78 ± 0.30
6-Methylthio-7-(β-D-ribofuranosyl)purine	+30.1 (*c* 0.5, pyridine)[d]	1.4 ± 0.02	
6-Methylamino-9-(β-D-ribofuranosyl)purine	−82.5 (*c* 1.0, pyridine) [−54.0;[26] *c* 0.6, H_2O][a]	3.67 ± 0.11 [4.0][a]	
6-Methylamino-7-(β-D-ribofuranosyl)purine	−114.3 (*c* 1.015, pyridine)	3.85 ± 0.05	
6-Dimethylamino-9-(β-D-ribofuranosyl)purine	−71.8 (*c* 1.015, 60% EtOH) [−62.6;[25] *c* 2.6, H_2O][a]	3.69 ± 0.04 [4.5][a]	
6-Dimethylamino-7-(β-D-ribofuranosyl)purine	+19.6 (*c* 1.025, 60% EtOH)[d]	1.75 ± 0.05	8.40 ± 0.05

[a] D. B. Dunn and R. H. Hall, "Handbook of Biochemistry," 2nd ed, Chemical Rubber Publishing Co., Cleveland, Ohio, p 63. [b] Reference 4. [c] Rudolph polarimeter. [d] Reference 6. [e] J. J. Fox, I. Wempen, A. Hampton, and I. L. Doerr, *J. Amer. Chem. Soc.*, **80**, 1669 (1958). [f] Determined with Cary-Datex systems by Drs. P. Fromageot and W. Guschlbauer.

a controlled water bath using a Y.S.I. 403 probe.[18] The cell temperature was measured with a N.B.S. calibrated thermometer and temperature variation was observed to be less than ±0.05°. When measurements were not being made the cell was placed in an insulated container in order to minimize heat loss. A solution containing the amount of ribose that would result from complete hydrolysis was used as a blank which furnished an infinity value for the kinetic runs.[19]

In a typical experiment, 0.0203 g of adenosine was placed in a thermostated 2.0-ml volumetric flask. The standard 1.02 *N* hydrochloric acid to be used was also thermostated at the reaction temperature. At time zero, the acid was measured into the volumetric flask and the resulting solution (0.038 *M*) rapidly transferred to the polarimeter tube.

(b) Determination of Rates. The data were treated in the standard manner for first-order reactions using the graphic form of the equation

$$k = \frac{1}{t} \ln \frac{\alpha_0 - \alpha_\infty}{\alpha_t - \alpha_\infty}$$

where α_0, α_∞, and α_t are the rotations at times zero, infinity, and time "*t*," respectively, and *k* is the unimolecular rate constant in units of reciprocal time. As a check on the accuracy of the above method, the rates of some slower reactions were also determined by the Guggenheim[20] and Swinbourne[21] procedures. These methods gave results in excellent agreement with those afforded by the above procedure. In addition, the first-order rate constants as well as the energies and entropies of activation[22] were calculated with a Univac 1108 computer. The calculated standard deviation was shown to be less than 1% for all kinetic runs.[22,23]

(18) Temperature controller manufactured and distributed by Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio. Temperature can be maintained to ±0.1° in a well-stirred, well-constructed bath.

(19) In all cases in which hydrolysis reached completion the observed and calculated infinity rotation were found to be in good agreement.

(20) E. A. Guggenheim, *Phil. Mag.*, **2**, 538 (1926).

(21) E. S. Swinbourne, *J. Chem. Soc.*, 2371 (1960).

(22) L. L. Schaleger and F. A. Long, *Advan. Phys. Org. Chem.*, **1**, 1 (1963).

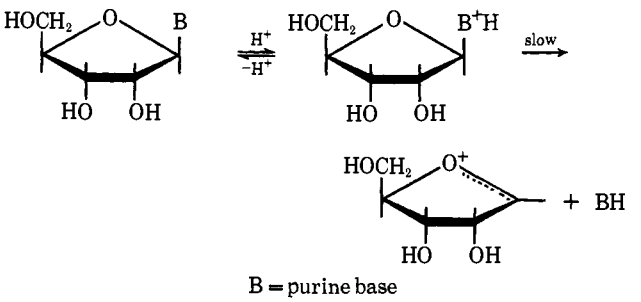
(23) S. W. Benson, "The Foundations of Chemical Kinetics," McGraw-Hill, New York, N. Y., 1960, p 86.

Results

The first-order rate coefficients were determined polarimetrically for 16 purine nucleosides using the graphic form of the equation, $k = 1/t \ln (\alpha_0 - \alpha_\infty)/(\alpha_t - \alpha_\infty)$. The results indicate (Table II, see relative rates) that the 9-ribosylpurines are more stable toward acid-catalyzed hydrolysis than the 7-ribosylpurines. 7-(β-D-Ribofuranosyl)adenine hydrolyzed 34 times faster than adenosine. In only one instance, guanosine and 7-(β-D-ribofuranosyl)guanine, did the 9 isomer hydrolyze faster.

In the process of determining the stability of the glycosidic bond in the 7- and 9-purine nucleosides, the kinetic parameters ($\Delta S^{\ddagger}$, E_a, etc.) were also calculated. The entropy of activation ($\Delta S^{\ddagger}$) values for the respective nucleosides ranged from −2.9 to +11.6 eu. Thirteen $\Delta S^{\ddagger}$ values were positive while three were negative; the mean was +4.0 eu (Table III). The mechanism which is most consistent with these data is the A-1 mechanism (Scheme I).

Scheme I



B = purine base

Discussion

This investigation was initiated with the primary objective being to determine the relative stability of the

Table II. Relative Rates and Rate Coefficients

Nucleoside	N-7/N-9 rel rates, 25°	34.85°, 10^6k, sec^{-1}	44.90°, 10^5k, sec^{-1}	25.00°, 10^5k, sec^{-1} [a]	$t^{1/2}$, hr 35°	$t^{1/2}$, hr 45°	$t^{1/2}$, hr 25°	Concn, M 35°	Concn, M 45°
Adenosine	33.8	1.03	4.10	0.235	18.7	4.7	81.8	0.0380	0.0387
7-(β-D-Ribofuranosyl)-adenine		31.00	110.56	7.945	0.6	0.2	2.4	0.0374	0.0378
Inosine	1.5	0.39	1.57	0.086	49.9	12.2	223.5	0.0386	0.0382
7-(β-D-Ribofuranosyl)-hypoxanthine		0.56	2.25	0.125	34.6	8.5	154.3	0.0382	0.0380
Guanosine·$2H_2O$	0.2	1.03	4.11	0.234	18.7	4.7	82.1	0.0384	0.0384
7-(β-D-Ribofuranosyl)-guanine		0.18	0.800	0.036	107.9	23.9	538.3	0.0383	0.0385
Nebularine	2.5	5.37	19.64	1.337	3.6	1.0	14.4	0.0388	0.0381
7-(β-D-Ribofuranosyl)-purine		12.98	46.31	3.326	1.5	0.4	5.8	0.0379	0.0381
9-(β-D-Ribofuranosyl)-purine-6-thione	3.5	0.988	4.18	0.212	19.5	4.6	90.9	0.0387	0.0382
7-(β-D-Ribofuranosyl)-purine-6-thione		3.53	15.40	0.735	5.4	1.3	26.2	0.0380	0.0380
6-Methylthio-9-(β-D-ribofuranosyl)purine	11.3	1.48	5.26	0.379	13.0	3.7	50.8	0.0377	0.0377
6-Methylthio-7-(β-D-ribofuranosyl)purine		18.02	69.01	4.284	1.1	0.3	4.5	0.0382	0.0382
6-Methylamino-9-(β-D-ribofuranosyl)purine	22.7	1.06	4.15	0.247	18.1	4.6	78.0	0.0382	0.0382
6-Methylamino-7-(β-D-ribofuranosyl)purine		20.00	65.57	5.602	1.0	0.3	3.4	0.0382	0.0382
6-Dimethylamino-9-(β-D-ribofuranosyl)purine	19.5	0.87	3.26	0.212	22.1	5.9	90.9	0.0381	0.0381
6-Dimethylamino-7-(β-D-ribofuranosyl)purine		17.00	63.64	4.138	1.1	0.3	4.7	0.0383	0.0383

[a] Calculated using the equation $k = (eKT/h)\exp(\Delta S^{\pm}/R)\exp(-E_a/RT)$.

Table III. Kinetic Parameters for the Hydrolysis of Purine Nucleosides in 1.020 *N* Hydrochloric Acid

Purine nucleoside	E_a, kcal mol^{-1}	$\Delta S^{\pm}$, cal deg^{-1} mol^{-1} [a]
Adenosine	26.8	3.5
7-(β-D-Ribofuranosyl)adenine	24.6	3.3
Inosine	27.2	3.0
7-(β-D-Ribofuranosyl)hypoxanthine	27.1	3.3
Guanosine·$2H_2O$	26.8	3.6
7-(β-D-Ribofuranosyl)guanine	29.2	7.8
Nebularine	25.1	1.5
7-(β-D-Ribofuranosyl)purine	24.6	1.6
9-(β-D-Ribofuranosyl)purine-6-thione	27.9	7.2
7-(β-D-Ribofuranosyl)purine-6-thione	28.5	11.6
6-Methylthio-9-(β-D-ribofuranosyl)purine	24.6	−2.8
6-Methylthio-7-(β-D-ribofuranosyl)purine	26.0	6.7
6-Methylamino-9-(β-D-ribofuranosyl)purine	26.4	2.4
6-Methylamino-7-(β-D-ribofuranosyl)purine	23.0	−2.9
6-Dimethylamino-9-(β-D-ribofuranosyl)purine	25.6	−0.7
6-Dimethylamino-7-(β-D-ribofuranosyl)purine	25.6	5.2

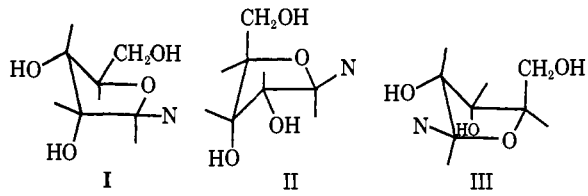
[a] Mean value +4.0 eu.

glycosidic bond for the isomeric nucleoside pairs listed in Table I. Previous investigations in this area used spectrophotometric[11] and chromatographic[7] techniques to establish the rate coefficients. The spectrophotometric method depends on the principle that there be a suitable region of the spectrum in which the ribofuranosylpurine and the heterocyclic moiety have markedly different absorptivities which, with many of the ribofuranosylpurines, is not the case. The low pH ultraviolet spectra are often very similar to those of the heterocyclic bases, *e.g.*, adenosine and adenine. Consequently, alkaline quench methods must be employed to obtain measurable spectra. Even at higher pH, the differences in the various spectra are subtle. Chromatographic techniques provide only a qualitative approach and lack the accuracy needed for calculation of the necessary kinetic parameters. Therefore, we elected to use the polarimetric method[24] which eliminates the problems associated with the above techniques. This is the first application of this technique to the hydrolysis of ribofuranosylpurines and has furnished the individual rate coefficients for the various nucleosides (Table II). The relative stability of the glycosyl linkages for the nucleoside pairs was established and furnished data which prompted us to extend the original area of investigation to include a study of the reaction mechanism.

Mechanistic Criteria. We have considered several factors, *e.g.*, structural effects (electronic and steric) and the molecularity of the rate-determining step, before suggesting a plausible reaction mechanism for the acid-catalyzed hydrolysis of ribofuranosylpurines.

Structural Effects. (a) Conformation of the Sugar Moiety. X-Ray crystallographic studies on nucleosides and nucleotides[25,26] have shown the ribo- and deoxyribofuranose ring to exist (crystalline state) in three possible conformations, *i.e.*, C-3′-endo (I), C-3′-exo (II), and C-2′-endo (III) conformations. However, in solution, it is possible that more than one conformation could be present due to interconversion. The bulky purine aglycone would be expected to reside in the more stable equatorial position regardless of the predominant form. Since D-ribose was used throughout our investigation with the configuration (β-D) about

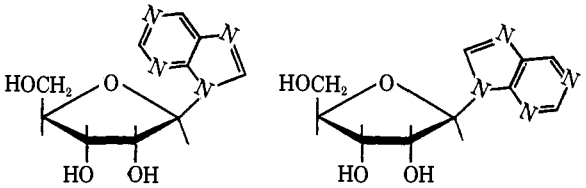
(24) J. N. BeMiller, *Advan. Carbohyd. Chem.*, **22**, 25 (1967).
(25) B. Capon, *Chem. Rev.*, **69**, 407 (1969), and references cited therein.
(26) S. T. Rao and M. Sundaralingam, *J. Amer. Chem. Soc.*, **92**, 4963 (1970).



I II III

C-1[27,28] being constant, the structural effects exerted by the sugar moiety about this center during hydrolysis, whether steric or electronic, would probably be small and equal for the nucleosides investigated and can be disregarded in determining the rate of hydrolysis.

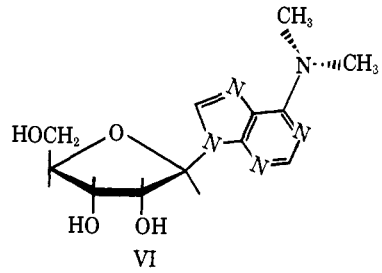
(b) Conformation of the Nucleoside (Relative Position of the Carbohydrate Moiety and the Exocyclic Groups on the Aglycone). On the premise that the atoms of the purine bases are firmly fixed and that the pentose ring can be considered a rigid unit or at least have a constant conformation (see the above discussion), then the conformation of the nucleoside can be determined by the rotation of these two essentially rigid planes about the axis of the C-1′–N-9[29] glycosidic bond. It has become the established[30] procedure to express the conformation in terms of a torsion angle, ϕ_{CN}, which specifies the relative position of the pentose residue and the purine base about the glycosidic bond. The two extreme conformations exhibited by purine ribosides are designated syn (IV) and anti (V).



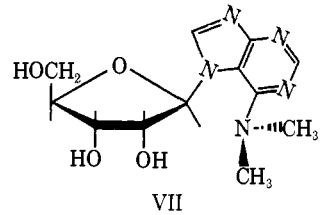
IV V

The primary physicochemical methods used in obtaining information about this torsional angle (ϕ_{CN}) are X-ray crystallography (solid),[26] ORD-CD (solution),[31] and pmr [solution, nuclear Overhauser effect].[32,33] Considerable research has been accomplished in each of these areas; however, the results for various 9-ribosylpurines are somewhat inconclusive which makes it difficult to provide an exact index for their conformation.[34]

Examination of CPK space filled models revealed that nucleosides in the 7-(β-D-ribofuranosyl)purine series with bulky substituents residing at position 6 of the purine moiety must exist in the anti conformation.[35] The steric requirement created by the different position of attachment (N-7 or N-9) of the carbohydrate moiety to the purine moiety is an important factor since this criterion can be used to differentiate between an A-1 and A-2 mechanism. If the 6-dimethylaminopurine nucleosides VI and VII are assumed to be in the anti

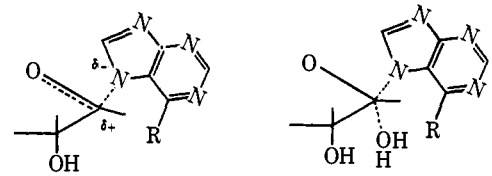


VI



VII

conformation then the 6-dimethylamino substituent of VI will not sterically interfere with the reactive center. However, attachment of the carbohydrate moiety to N-7 provides a nucleoside (VII) in which the dimethylamino group now masks the reactive center and thus should affect the rate. It is well documented[36] that atoms become more crowded in the formation of the activated complex in an A-2 (SN2) reaction (IX) but become less crowded in an A-1 (SN1) reaction (VIII).



VIII IX

Thus, in an A-2 type reaction, the presence of bulky groups near the reaction center will lead to a decrease in rate, whereas with an A-1 type reaction an increase would be expected as a result of steric acceleration. A careful examination of the data shown in Table IV illustrates that the latter type (A-1) of reaction is predominant. A comparison of the rates revealed a 20–30-fold increase for the 7 isomers with respect to their 9 counterparts. This significant increase cannot be ascribed entirely to electronic effects and most probably reflects the additional influence of steric acceleration.

Further proof of the anti conformation and concomitant steric acceleration for 6-dimethylamino-7-(β-D-ribofuranosyl)purine (VII) was obtained by an inspection of a CPK space filled model which showed the 6-dimethylamino substituent being forced out of the plane of the purine ring. This departure from planarity results in steric inhibition to resonance and is reflected

(27) In the present investigation, only those nucleosides having the β-ribo configuration were studied. The hydrolysis of nucleosides having the α- and β-arabino and the α-ribo configuration is under investigation in our laboratory.

(28) For a discussion on steric effects of the sugar moiety as well as the possible inductive effect of the 2′ position, see ref 25.

(29) For the 7-ribosylpurine series the torsional angle will be determined by the C-1′–N-7 bond.

(30) J. Donahue and K. N. Trueblood, *J. Mol. Biol.*, **2**, 363 (1960).

(31) D. W. Miles, S. J. Hahn, R. K. Robins, M. J. Robins, and H. Eyring, *J. Phys. Chem.*, **72**, 1483 (1968), and references cited therein.

(32) P. A. Hart and J. P. Davis, *J. Amer. Chem. Soc.*, **91**, 512 (1969).

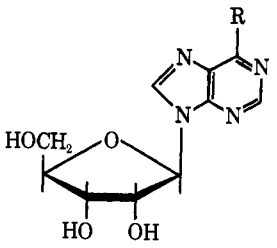
(33) The extended Hückel theory has been applied to the study of the conformation of nucleosides by F. Jordan and B. Pullman, *Theor. Chim. Acta*, **9**, 242 (1968). Their calculations predict a preferred anti conformation for adenosine and a preferred syn conformation for guanosine.

(34) Recent CD work has shown certain guanosine derivatives, *e.g.*, 7-(β-D-ribofuranosyl)guanosine, to exist in the anti conformation in alcoholic solvents and at low pH in water; see D. W. Miles, L. B. Townsend, M. J. Robins, R. K. Robins, W. H. Inskeep, and H. Eyring, *J. Amer. Chem. Soc.*, **93**, 1600 (1971).

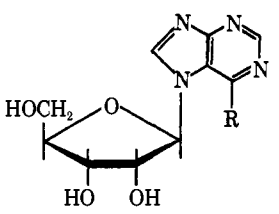
(35) A study using the magnetic anisotropy effect of the thione group led to the suggestion that 7-(β-D-ribofuranosyl)purine-6-thione probably exists predominantly in the anti conformation, R. A. Long and L. B. Townsend, *J. Chem. Soc. D*, 1087 (1970).

(36) E. S. Gould, "Mechanism and Structure in Organic Chemistry," Holt, Rinehart, and Winston, New York, N. Y., 1959.

Table IV



A

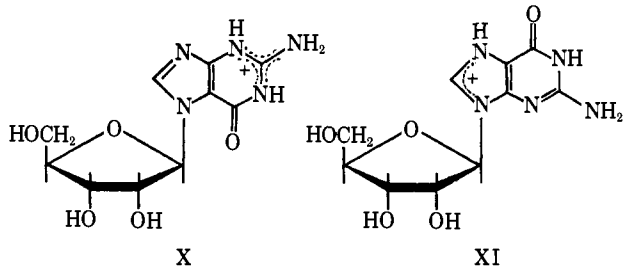


B

Uv (MeOH)	R	Rate, 35°, 10^5k sec^{-1}	$\Delta S^{\ddagger}$	E_a	pK_a
		Compound A			
	H	5.37	1.5	25.1	1.89 ± 0.01
259	NH_2	1.03	3.5	24.6	3.62 ± 0.02
265	$NHCH_3$	1.06	2.4	26.4	3.67 ± 0.11
273	$N(CH_3)_2$	0.87	−0.7	25.6	3.69 ± 0.04
		Compound B			
	H	12.98	1.6	24.6	1.83 ± 0.03
272	NH_2	31.00	3.3	24.6	3.60 ± 0.1
273	$NHCH_3$	20.00	−2.9	23.0	3.85 ± 0.05
290	$N(CH_3)_2$	17.00	5.2	25.6	1.75 ± 0.05

in the ultraviolet spectra and pK_a of the compound (Table IV).

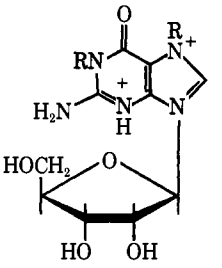
Electronic Effects. (a) Site of Protonation. Site of protonation is another factor which might be expected to influence the rate of hydrolysis of purine nucleosides. Protonation of purine nucleosides is considered to take place on the purine moiety. Evidence for initial protonation on the purine moiety is based on X-ray crystallographic studies[37] and the ultraviolet spectrum in the low pH region. The specific site of protonation on the purine moiety differs for the various nucleosides and has been based on methylation studies[38] and thermodynamic values for proton dissociation in aqueous solution.[39] For example, adenosine and guanosine have been suggested to protonate at N-1 and N-7, respectively. However, information of this nature is limited and that which is available only pertains to a few nucleosides. Additional investigations into this area, *i.e.*, site of protonation on the purine aglycone, are needed as the resultant data would indeed shed light on the nature of the charged species undergoing hydrolysis as well as how the distribution of charge density within the purine moiety affects the rate of hydrolysis. The possibility that 7-(β-D-ribofuranosyl)guanine may protonate on N-3 rather than N-9 might provide the answer for the slower rate of hydrolysis as compared to guanosine. If protonation occurred at N-3 the charge density may be localized about the guanidine portion in the pyrimidine ring (X). If this were the case then the glycosyl nitrogen would not experience the same magnitude of positive residual charge as it does for guanosine where the positive charge resides to a large extent in the imidazole moiety (XI), thus facilitating a cleavage of the glycosyl bond. This same phenomenon may be the basis for the stability of cytidine over that of isocytidine toward acid hydrolysis.[40]



X

XI

It has been suggested[11] that for certain purine nucleosides undergoing hydrolysis, *i.e.*, guanosine, 7-methylguanosine, and 1,7-dimethylguanosine iodide, the reactive species is the diprotonated purine nucleoside (XII) although unequivocal experimental evidence for its existence is lacking.[41] Purine nucleosides can be categorized as weak organic bases.[42] To measure the pK_a's of weak organic bases, acids strong enough to protonate them, *e.g.*, sulfuric acid or perchloric acid, are required. We have shown that the glycosidic bond is susceptible to cleavage under relatively mild acidic conditions, 1 *N* hydrochloric acid. Thus, at the acid strength needed to measure the pK_a at which diprotonation occurs, the nucleoside may be essentially nonexistent.[41,43] In order to provide solid evidence for the existence of the diprotonated species, the pK_a should be established. A comparative study using the various methyl analogs of the purine nucleosides would be the logical method. An investigation of this type should establish the approximate pK_a for the occurrence of diprotonation and prove or disprove the hypothesized dication.



XII

R = H or CH_3

(b) Sugar Moiety. The D-ribofuranosyl moiety has been reported[44a,b] to have an electron-withdrawing effect on the purine moiety; however, the degree of influence which this inductive effect has on the overall process of hydrolysis is probably very small. As with

(37) J. Kraut and L. H. Jensen, *Acta Crystallogr.*, **16**, 79 (1963).

(38) J. W. Jones and R. K. Robins, *J. Amer. Chem. Soc.*, **85**, 193 (1963).

(39) J. J. Christensen, J. H. Rytting, and R. M. Izatt, *Biochemistry*, **9**, 4907 (1970).

(40) (a) C. A. Dekker, *Annu. Rev. Biochem.*, **29**, 453 (1960); (b) C. A. Dekker in "The Carbohydrates, Chemistry and Biochemistry," Vol. 2A, 2nd ed, W. Pigman and D. Horton, Ed., Academic Press, New York, N. Y., 1970, pp 43–52.

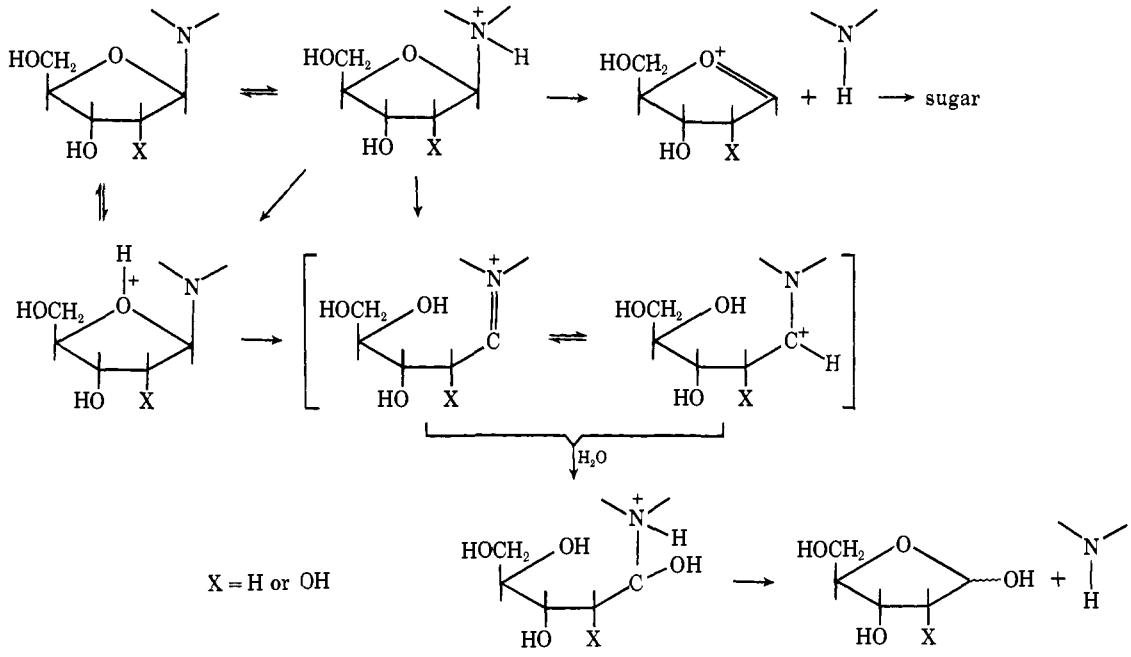
(41) It has been reported[11] "that purine nucleosides may undergo diprotonation" with the following pK_a values, −2.43 and −2.62, for the diprotonated species of guanosine and 7-methylguanosine, respectively. While determining these values, these investigators found evidence of nucleoside hydrolysis in the concentrated acid region which would suggest that their measurements may be for a mixture of components rather than for the pure diprotonated species of guanosine and 7-methylguanosine.

(42) E. M. Arnett, *Progr. Phys. Org. Chem.*, **1**, 223 (1963).

(43) The instability of the glycosidic linkage for the betaine-type nucleosides has also been demonstrated, *e.g.*, 7-methylguanosine has been shown to completely hydrolyze in methanol or water at reflux in 1 hr; see ref 38.

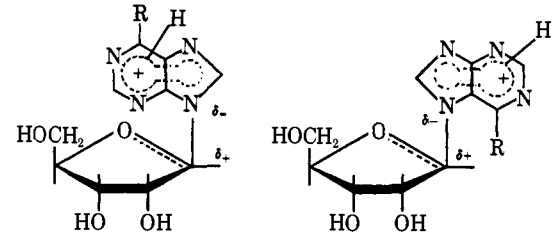
(44) (a) C. D. Jardetzky and O. Jardetzky, *J. Amer. Chem. Soc.*, **82**, 222 (1960); (b) L. B. Townsend in "Synthetic Procedures in Nucleic Acid Chemistry," Vol. 2, W. W. Zorbach and R. S. Tipson, Ed., Interscience, New York, N. Y., in press.

Scheme II



the steric effects mentioned above, the magnitude of such electronic effects exerted by the ribofuranose ring should be constant for this study.

(c) Aglycone. Substituents, such as NH_2, $NHCH_3$, and $N(CH_3)_2$, which are capable of withdrawing electron density from saturated carbon chains by induction, are also capable of supplying π-electron density to conjugated systems.[45] This phenomenon has been shown[46] to occur with the purine ring system by correlating the pmr chemical shifts of the H-8 and H-2 protons of various 6-substituted purines with the electron-donating or electron-withdrawing ability of the 6 substituent as compared to the shifts observed for the H-8 and H-2 protons on purine *per se*. This established that groups such as NH_2, $N(CH_3)_2$, and SCH_3 impart a strong resonance effect in the pyrimidine moiety while in the imidazole portion of the purine molecule they exhibit a combination of resonance and inductive effects. The electron-donating ability of substituents on the purine ring[47] (C-6) should definitely influence the rate of hydrolysis. In forming the activated complex where bond breaking is occurring, the heterolysis will be facilitated by a substituent on the purine moiety which will decrease the electron density around the *N*-glycosyl nitrogen. Substituents such as $-NH_2$, $-NHCH_3$, and $-N(CH_3)_2$ which are electron donating should increase the electron density around the *N*-glycosyl nitrogen and retard the transfer of the electron pair to the nitrogen of the purine ring during heterolysis. An examination of the rate coefficients listed in Table IV for the 9-β-D-ribofuranosylpurines shows a sixfold decrease in rate from nebularine to 6-dimethylamino-9-(β-D-ribofuranosyl)purine. Thus, the greater electron-donating capability of the dimethylamino group as compared to hydrogen in the series $N(CH_3)_2 > NHCH_3 > NHCH_3 > NH_2 > H$ was reflected by the corresponding decrease in the rate of hydrolysis. The 7-β-D-ribofuranosylpurines furnished the same correlation, *i.e.*, a decrease in the rate of hydrolysis was observed in changing the 6 substituent from NH_2 to $N(CH_3)_2$. The increase in rate for the



7-substituted series over that where the 6 substituent was H can be attributed to the added affect of steric acceleration.

Kinetic Parameters. Entropy of Activation. The use of $\Delta S^{\ddagger}$ has been proposed[48] as a criterion for distinguishing between the A-1 and A-2 mechanisms in acid-catalyzed hydrolysis reactions. A literature survey for entropies of activation for an assortment of acid-catalyzed hydrolyses including their own findings led these investigators to postulate that the A-1 mechanism is generally associated with a much more positive $\Delta S^{\ddagger}$ than the A-2 mechanism, It was later suggested[22] that all A-1 reactions could be expected to have small entropies of either sign and all A-2 reactions large negative entropies.

This rationale has been used as a means to resolve the question of mechanism for the acid-catalyzed hydrolysis of certain pyranosides[24] and furanosides.[49] To date, this criterion has not been applied to the acid-catalyzed hydrolysis of purine ribosides since very little quantitative data have appeared in the literature pertaining to this subject. Therefore, kinetic parameters

(45) J. March, "Advanced Organic Chemistry: Reactions, Mechanisms, and Structure," McGraw-Hill, New York, N. Y., 1968, pp 382–387.

(46) W. C. Coburn, Jr., M. C. Thorpe, J. A. Montgomery, and K. Hewson, *J. Org. Chem.*, 30, 1114 (1965).

(47) Those substituents which appear on the purine ring in our study, *i.e.*, $-NH_2$, $-NHCH_3$, $-N(CH_3)_2$, =S, $-SCH_3$, =O, all fall in the +R, −I category.

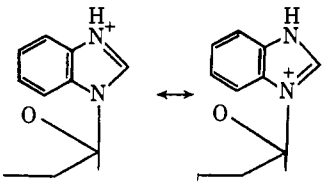
(48) F. A. Long, J. G. Pritchard, and F. E. Stafford, *J. Amer. Chem. Soc.*, 79, 2362 (1957).

(49) W. G. Overend, C. W. Rees, and J. S. Sequeira, *J. Chem. Soc.*, 3429 (1962); (b) J. W. Green, *Advan. Carbohydrate Chem.*, 21, 95 (1966); (c) B. Capon and B. Thacker, *J. Chem. Soc. B*, 185 (1967).

listed in Table III should shed considerable light on the molecularity of the rate-determining step. As was pointed out earlier, the $\Delta S^{\ddagger}$ values (Table III) for the purine ribosides in this study ranged from -2.9 to $+11.6$ eu with a mean value of $+4.0$ eu. Thus, when the entropy criterion discussed above was applied to our study it would suggest an A-1 mechanism.

Prior Mechanistic Views. There has been considerable controversy over the originally proposed reaction mechanism[40,50] for the acid-catalyzed hydrolysis of purine nucleosides (Scheme II).[51] Several investigators have criticized this mechanism,[40,50] *i.e.*, the intramolecular proton transfer requirement and the Schiff base pathway (Scheme II), based on theoretical and experimental findings.[52] We have also found fault with this mechanism, *vide infra*.

It was stated[50] that a mechanism involving protonation on nitrogen followed by heterolysis to a glycosyl cation would be unfavorable. This assumption was based on the observed stability of tri-*N*-methylammonium glucoside,[53] as well as the slow rate of hydrolysis observed for benzimidazole nucleosides in acid. It was suggested[50] that with a benzimidazole nucleoside the charge is shared equally between the two nitrogens of the imidazole moiety which inhibits the availability of the electron pair on the glycosyl nitrogen needed for ring opening. Also the proton which resides on the benzimidazole moiety is far removed from the annular ring oxygen and prevents possible proton transfer. It was thought[50] that a combination of these factors was involved in the slow hydrolysis of benzimidazole nucleosides. On the basis of the above factors, it was predicted[50] that 9-(β-D-ribofuranosyl)purine (nebularine) should hydrolyze slower than adenosine since the positive charge would be shared exclusively between the N-1 and N-9 nitrogens of the purine ring whereas with adenosine the exocyclic amino can accommodate part of the charge density. In direct contrast to this, we have reported[1b] that nebularine hydrolyzes five times faster than adenosine.



We have also obtained evidence which indicates that the occurrence of proton transfers is nonexistent. The "transfer sites" on 6-dimethylamino-7-(β-D-ribofuranosyl)purine (VII) because of its geometry (anti-conformation, *vide supra*) are not in a suitable juxtaposition to the annular oxygen of the ribose moiety; thus proton transfer should be prohibited. However, there was observed a tremendous increase in the hydrolysis rate of IX (20 times faster) as compared to 6-dimethylamino-9-(β-D-ribofuranosyl)purine (VI).

Conclusion

During the course of this study, we have confirmed an earlier conclusion[11] in regard to the mechanism of acid-catalyzed hydrolysis of ribofuranosylpurines which involves preequilibrium protonation of the purine followed by rate-limiting bond breaking of the glycosyl–nitrogen bond to give a C-1′ carbonium ion. The composite of our mechanistic considerations, *i.e.*, electronic, steric, and entropy criterion, strongly favor the A-1 rather than the A-2 pathway for the acid-catalyzed hydrolysis of ribofuranosylpurines.

Acknowledgments. The authors wish to thank Mr. C. L. Schmidt for his assistance in preparing the computer program used in this investigation, and Drs. C. D. Poulter, E. M. Eyring, and R. H. Boyd for helpful suggestions.

(50) G. W. Kenner, "Ciba Foundation Symposium on the Chemistry and Biology of Purines," Little, Brown and Co., Boston, Mass., 1957, p 312.

(51) E. R. Garrett, J. K. Seydel, and A. J. Sharpen, *J. Org. Chem.*, **31**, 2219 (1966).

(52) For a critical evaluation of the Kenner–Dekker mechanism[40,50] (Scheme II), the reader is referred to ref 11 and 25 and R. Shapiro, *Progr. Nucl. Acid Res. Mol. Biol.*, **8**, 73 (1968), and references cited therein.

(53) P. Karrer and J. Ter Kuile, *Helv. Chim. Acta*, **5**, 870 (1922).