Thermodynamic pK, $\Delta H°$, $\Delta S°$, and $\Delta C_p°$ Values for Proton Dissociation from Several Purines and Their Nucleosides in Aqueous Solution*

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ABSTRACT: Values of pK, $\Delta H°$, and $\Delta S°$ valid at 25° and zero ionic strength are reported for proton ionization from protonated adenosine and for the consecutive ionization of two protons from protonated adenine ($\Delta H°$ and $\Delta S°$ values only) inosine, xanthine, and xanthosine, and three protons from protonated guanosine and protonated hypoxanthine. The $\Delta S°$ values are generally of the magnitudes expected for the charge types involved in the reactions. Thermodynamic quantities for ionizations with p$K \sim 12$ are also given at 10 and 40° in the cases of guanosine, hypoxanthine, inosine, xanthine, and xanthosine. Values of $\Delta C_p°$ of −6, −27, 7, −34, and −9 cal per deg mole, respectively, are reported for these ionizations.

Relationships between the $\Delta C_p°$ values and the charge types, and between the $\Delta H°$ values and the sites of proton ionization are discussed.

In previous papers of this series a calorimetric titration procedure was used to identify sites of proton ionization from adenosine (Izatt *et al.*, 1965, 1966) and pyrimidines (Christensen *et al.*, 1967b) and to determine the pK, $\Delta H°$, and $\Delta S°$ values associated with proton ionization from several components of nucleic acids, *i.e.*, purines (Christensen and Izatt, 1962; Izatt and Christensen, 1962), pyrimidines (Christensen *et al.*, 1967a,b, 1970a), monosaccharides (Izatt *et al.*, 1966; Christensen *et al.*, 1970b), and nucleosides (Christensen and Izatt, 1962; Izatt and Christensen, 1962; Izatt *et al.*, 1965, 1966; Christensen *et al.*, 1966b, 1967b, 1970a). In several cases the thermodynamic quantities have been determined as a function of temperature and $\Delta C_p°$ values calculated for the ionization reactions (Christensen *et al.*, 1970a,b).

The pK, $\Delta H°$, and $\Delta S°$ values for proton ionization from cytosine, cytidine, thymine, thymidine, uracil, and uridine at 10, 25, and 40° and zero ionic strength, μ, have been reported (Christensen *et al.*, 1967, 1970a). A similar study of the purine bases adenine, hypoxanthine, and xanthine and their respective nucleosides and guanosine at 25° is described in the present paper. In addition, the ionizations where p$K \sim$ 12 have been studied at 10 and 40° and the probable sites for the various ionization steps are presented and discussed.

The ionization steps for which thermodynamic data are given in the present study are represented by Schemes I and II with the H^+ being omitted in each step. The ionization sites are the most probable ones based on results reported in the present study and those of previous workers. As will be discussed later, there is also probably some involvement of neighboring group(s) in several of the ionization processes. The subscripts 1, 2, and 3 will be used with pK, $\Delta H°$, and $\Delta S°$ to denote first, second, and third ionization steps where necessary.

Several investigators (see references in Table I) have reported pK values and/or proposed sites for proton ionization from the purine bases and nucleosides studied here as well as from the protonated forms of adenine, adenosine, guanosine, and hypoxanthine. Previous workers have assigned the pK values for protonated adenine and protonated adenosine to both the N_1H^+ (Cochran, 1951; Zubay, 1958; Jardetsky and Jardetsky, 1960; Christensen and Izatt, 1962) and the $C_6NH_3^+$ (Levene and Simms, 1925; Taylor, 1948; Alberty *et al.*, 1951; Beers and Steiner, 1958; Cheney *et al.*, 1959; Lewin, 1964) groups while the pK_2 value for adenine has been assigned to the N_9 position (Taylor, 1948; Alberty *et al.*, 1951; Christensen and Izatt, 1962). The presence of both the 2′- and 3′-hydroxyl groups has been shown to be necessary for the ionization of a proton from the ribose group of adenosine (Izatt *et al.*, 1965, 1966). It has been demonstrated (Miles *et al.*, 1963b) using infared and nuclear magnetic resonance spectroscopy, that proton ionization from protonated guanosine occurs from the N_7H^+ group. This assignment is supported by an earlier (Jardetsky and Jardetsky, 1960) nuclear magnetic resonance study of guanosine triphosphate. Proton ionization from neutral guanosine was assigned (Miles *et al.*, 1963b) to the N_1H group with the oxygen at the 6 position assuming the negative charge. Proton dissociation steps for hypoxanthine (pK_2) and xanthosine (pK_1) have been attributed to the 6-hydroxyl group, sequences 4 and 7, enol forms (Albert, 1953). The pK_1 value of xanthine has been assigned (Cavalieri *et al.*, 1954) from spectroscopic data to the N_3H group. However, there is disagreement on the assignment of the pK_2 value. Pfleiderer and Nübel (1961) assign the pK_2 value to the N_9H group based on pH and spec-

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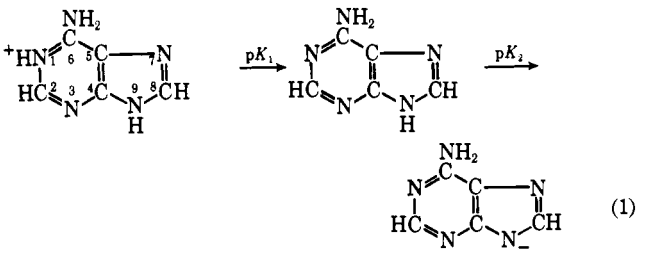
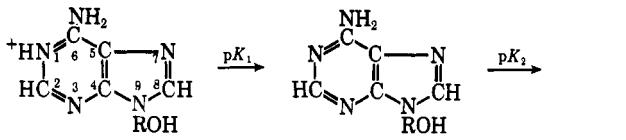
SCHEME I



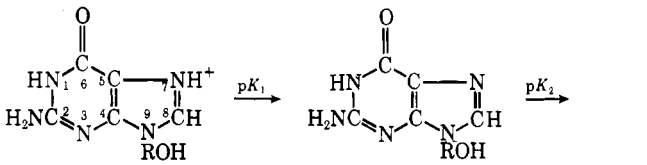
(1)

adenine



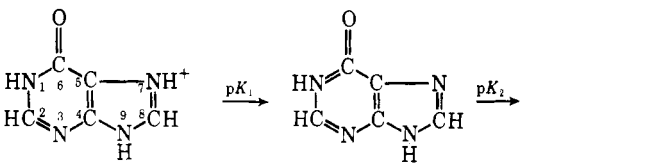
(2)

adenosine



(3)

guanosine

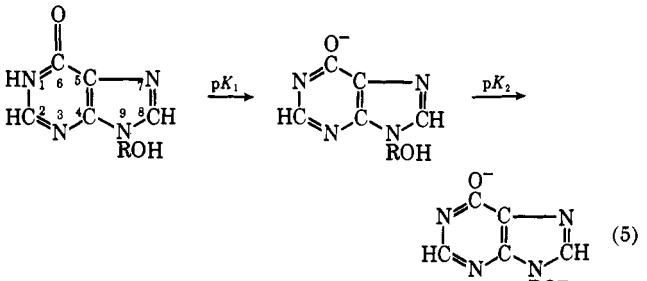


(4)

hypoxanthine

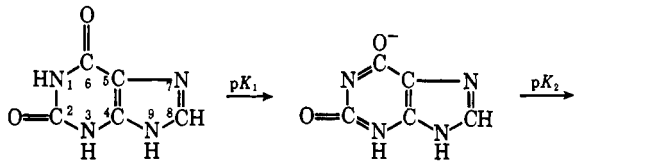
troscopic data while Cavalieri *et al.* (1954) assign this ionization to the N_7H group. Their assignment of the second dissociation to the N_7H group is a consequence of their structures of the xanthine derivatives they studied having

SCHEME II



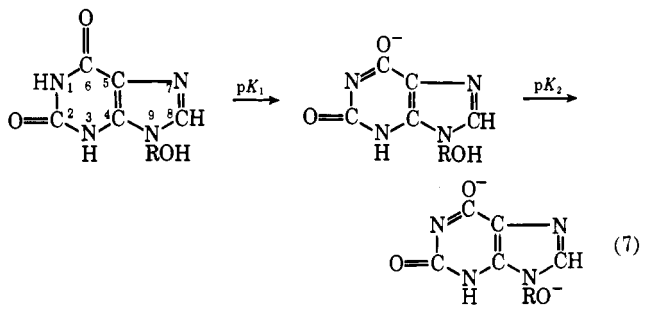
(5)

inosine



(6)

xanthine[a]



(7)

xanthosine[a]

[a] The charge distribution on the pyrimidine moiety of the ionic species is not known with certainty. Other forms are possible having the negative charge on the C_2 oxygen, partially on both the C_2 and C_6 oxygen atoms or (less likely) on the N_1 or N_3 atoms.

the R group in the N_7 rather than N_9 position (see reaction 6).

Enthalpy and entropy change values have been reported for proton ionization from protonated adenine (ΔH_1 and ΔS_1: Harkins and Freiser, 1958; Rawitscher and Sturtevant, 1960; Christensen and Izatt, 1962; Lewin and Tann, 1962; Suchorukow *et al.*, 1964; ΔH_2 and ΔS_2: Christensen and Izatt, 1962; Lewin and Tann, 1962; Suchorukow *et al.*, 1964; Lewin and Barnes, 1966), protonated adenosine (ΔH_1 and ΔS_1: Harkins and Freiser, 1958; Rawitscher and Sturtevant, 1960; Christensen and Izatt, 1962; Suchorukow *et al.*, 1964; ΔH_2 and ΔS_2; Izatt *et al.*, 1965, 1966), protonated guanosine (ΔH_1, ΔS_1, ΔH_2, and ΔS_2: Suchorukow *et al.*, 1964), protonated hypoxanthine (ΔH_1 and ΔS_1: Wooley *et al.*, 1970), ΔH_2 and ΔS_2: Suchorukow *et al.*, 1964; Wooley *et al.*, 1970), and inosine (ΔH_1 and ΔS_1: Suchorukow *et al.*, 1964). Of these ΔH values, those for protonated adenine (Rawitscher and Sturtevant, 1960; Christensen and Izatt, 1962), protonated

adenosine (Rawitscher and Sturtevant, 1960; Christensen and Izatt, 1962; Izatt *et al.*, 1965), protonated guanosine (Rawitscher and Sturtevant, 1960), and protonated hypoxanthine (Wooley *et al.*, 1960) were determined calorimetrically.

Experimental Section

Materials. The chemicals used were A grade adenine, adenosine, guanosine, hypoxanthine, inosine, xanthine, and xanthosine from Calbiochem and Reagent grade 50% NaOH and $HClO_4$ from Baker and Adamson. All solutions were prepared using boiled doubly distilled water and were prepared, stored, handled, and all titrations were carried out under pure nitrogen atmospheres. The NaOH solutions were standardized against potassium hydrogen phthalate (National Bureau of Standards) and the $HClO_4$ solutions against Fisher primary standard tris(hydroxymethyl)aminomethane.

Procedure and Calculations. The pK_1 values for protonated adenosine, inosine, xanthine, and xanthosine and the pK_2 values for guanosine and hypoxanthine were determined by a pH titration method similar to that used previously (Hansen *et al.*, 1966).

The pH measurements were made at appropriate intervals with a Beckman Research Model pH meter (Model 1019) using glass (Beckman E-2) and saturated calomel electrodes. The electrodes were calibrated throughout the titration range using reagents and instructions obtained from the National Bureau of Standards. The procedure used to calculate pK values from the pH titration data has been given (Hansen *et al.*, 1966). A value of 5 Å was used for a in the Debye–Hückel expression for calculation of activity coefficients.

The pK_1 values for protonated guanosine and protonated hypoxanthine, the pK_2 values for inosine, xanthine, and xanthosine, and the pK_3 values for guanosine and hypoxanthine were determined directly from the calorimetric titration data by a method described previously (Christensen *et al.*, 1967b). The operation and calibration of the titration calorimeter used to make all heat determinations have been described (Christensen *et al.*, 1965) together with the method used to calculate pK values from the calorimetric titration data (Christensen *et al.*, 1966a).

The general method used to calculate ΔH values from the calorimetric titration data is available (Christensen and Izatt, 1962; Christensen *et al.*, 1966a). Literature values were used for the heats of dilution of NaOH (Sturtevant, 1940) and $HClO_4$ (Vanderzee and Swanson, 1963) at 25°, the ion product of water (Harned and Owen, 1958) at 10, 25, and 40° and the heat of ionization of water (Hale *et al.*, 1963) at 25°. Heat of dilution data for NaOH at 10 and 40° were determined experimentally and are available.[1] Values for the heat of ionization of water at 10 and 40° and $\mu = 0$ were determined to be 14.216 and 12.61 kcal per mole, respectively (unpublished data, this laboratory). At the low ionic strength ($\mu < 0.05$) used in the present calorimetric determinations, ΔH values have been found (Christensen *et al.*, 1967a) not to vary significantly with μ; therefore, ΔH values in the present study are assumed to be equal to $\Delta H°$ values. The standard state to which $\Delta H°$ refers is defined to be an ideal 1 M solution behaving as an infinitely dilute solution (Lewis and Randall, 1961).

Calculations were aided by using an IBM 360 computer.

Results

In Table I are tabulated the calculated pK, $\Delta H°$, and $\Delta S°$ values for proton ionization from the purines and nucleosides studied, together with literature values. The probable site of proton ionization as given in Schemes I and II is also indicated together with the reaction involved in each case. The pH titration and calorimetric titration data from which the pK, $\Delta H°$, and $\Delta S°$ values are calculated are available.[1]

Discussion

The pK values reported in Table I generally agree well with available literature values considering differences in temperature and ionic strength. The $\Delta H_1°$ value (4.81) determined by us for adenine is in good agreement with our earlier calorimetric value (4.9), but not with those reported by other workers (4.2, 4.2, and 3.99). Our $\Delta H_2°$ value (9.65) for adenine agrees well with previously reported values except that of 11.0 which appears to be too high. Our $\Delta H_1°$ value (3.2) for guanosine is considered to be in fair agreement with earlier values (1.0, 0.99, and 2.22) considering the difficulties the earlier workers had in making accurate determinations of the small pK values from which their $\Delta H°$ values were calculated. The earlier ΔH_1 value for inosine and the ΔH_2 values for guanosine (see footnote *e*, Table I) and hypoxanthine (Suchorukow *et al.*, 1964) differ from our values by approximately 1 kcal/mole.

We have observed that usually a characteristic enthalpy change accompanies proton ionization from a particular donor atom (Izatt *et al.*, 1966; Christensen *et al.*, 1967a,b). We have made use of this observation to support the contention that the N_1H^+ rather than the $C_6NH_3^+$ group is the site of proton ionization from the protonated forms of adenine and adenosine. The evidence for assignment of the proton ionization site to the N_1H^+ group is seen in the much better agreement of the $\Delta H_1°$ value reported in Table I for the first ionization from protonated adenine (4.81) with those for protonated compounds where the site of ionization is known to be a nitrogen of the type of N_1H^+, *e.g.*, the protonated forms of cytosine, 5.14 (Christensen *et al.*, 1967b), and pyridine, 4.80 (Sacconi *et al.*, 1960) than with protonated aniline, 7.28 (Levi *et al.*, 1949) where the proton must ionize from the $C_6NH_3^+$ group. It has been established (Miles *et al.*, 1963a,b) by a nuclear magnetic resonance technique that the N_3 nitrogen is the site of protonation in cytosine. Since the $\Delta H_1°$ values for ionization from protonated adenine and protonated adenosine are similar to those for the protonated forms of cytosine, cytidine, and pyridine, but about 2 kcal/mole lower than that for protonated aniline, we conclude that ionization from protonated adenine and protonated adenosine is from the N_1H^+ group.

Examination of the structures in Schemes I and II reveals that proton ionization from three sites should be accompanied by similar $\Delta H°$ values from compound to

[1] Material supplementary to this Article has been deposited as Document No. NAPS—01163 with the ASIS National Auxiliary Publication Service, c/o CCM Information Corp., 909 3rd Ave, New York, N. Y. 10022. A copy may be secured by citing the document number and by remitting $2.00 for microfiche or $5.00 for photocopies. Advance payment is required. Make checks or money orders payable to: CCMIC-NAPS.

TABLE I: Values of pK, $\Delta H°$, and $\Delta S°$ for the Indicated Reactions Together with Probable Sites of Ionization as Given in Reactions 1–7 and Previous Literature Values.[a]

Temp (°C)	pK	$\Delta H°$ (kcal/mole)	$\Delta S°$ (cal/deg mole)
	Adenine, N_1H^+, pK_1, $H_2A^+ = HA + H^+$		
20	(4.22)[b,c]		
25	(4.20)[d]	4.81 ± 0.02	−3.17 ± 0.07
	(4.12)[e,f]	(4.2)[e]	(−4.7)[e]
	(4.18)[g]	(4.2)[g]	(−5)[g]
	(4.22)[h]	(4.9)[i]	(−2.7)[i]
	(4.1)[j]	(3.99)[k]	
	(4.15)[l]		
30	(4.12)[c]		
20–30		(3.8)[c]	(−5)[c]
	Adenine, N_9H, pK_2, $HA = A^- + H^+$		
20	(9.96)[c]		
25	(9.87)[d]	9.65 ± 0.05	−12.88 ± 0.07
	(9.88)[h]		
	(9.72)[e]	(9.5)[e]	(−12.4)[e]
	(9.80)[l]	(9.1)[i]	
	(9.75)[f]		
	(9.7)[g]		
30	(9.67)[c]		
	(9.75, 9.70)[m]		
20–30		(11.0)[c]	
20–50		(9.1, 9.6)[m]	(−14.8, −12.8)[m]
	Adenosine, N_1H^+, pK_1, $H_2A^+ = HA + H^+$		
20	(3.52)[b]		
	(3.703)[n]		
25	3.50 ± 0.02	3.91 ± 0.02	−2.92 ± 0.06
	(3.51)[g]	(3.4)[g]	(−5)[g]
	(3.55)[e]	(3.8)[e]	(−3.4)[e]
	(3.52)[d]	(3.1)[i]	(−5.7)[i]
	(3.6)[j]	(3.81)[k]	
	(3.63)[f]		
	(3.45)[o]		
	(3.57)[p]		
	Adenosine, ribose OH, pK_2, $HA = A^- + H^+$		
25	(12.35)[q]	(9.7)[q]	(−24.0)[q]
	(12.5)[r]		
	Guanosine, N_7H^+, pK_1, $H_3A^+ = H_2A + H^+$		
20	(2.20)[n]		
25	1.9 ± 0.1	3.2 ± 0.2	2.1 ± 0.7
	(1.6)[e,s]	(1.0)[e]	(−4)[e]
		(0.99)[k]	
25.2	(2.17)[t]	(2.22)[t]	(−2.5)[t]
	Guanosine, N_1H–C_6O, pK_2, $H_2A = HA^- + H^+$		
20	(9.24)[n]		
	(9.31)[u]		
25	9.25 ± 0.01	7.65 ± 0.04	−16.7 ± 0.1
	(9.24)[e]	(8.8)[e]	(−13.0)[e]
	(9.16)[s]		
	Guanosine, ribose OH, pK_3, $HA^- = A^{2-} + H^+$		
10	12.83 ± 0.07	11.04 ± 0.10	−19.7 ± 0.4
25	12.33 ± 0.02	10.85 ± 0.03	−20.0 ± 0.1
40	11.60 ± 0.04	10.86 ± 0.04	−18.4 ± 0.2
	Hypoxanthine, N_7H^+, pK_1, $H_3A^+ = H_2A + H^+$		
20	(1.98)[b]		
25	1.79 ± 0.02	2.95 ± 0.07	1.7 ± 0.3
	(1.9)[v]	(2.5)[v]	(0.3)[v]
	Hypoxanthine, N_1H–C_6O, pK_2, $H_2A = HA^- + H^+$		
20	(8.94)[b]		
25	8.91 ± 0.02	7.88 ± 0.05	−14.4 ± 0.2
	(8.8)[e]	(7.2)[e]	(−16.1)[e]
	(8.7)[j]		
	(8.88)[h]		
	(8.8)[v]	(8.0)[v]	(−13.4)[v]
	Hypoxanthine, N_9H, pK_3, $HA^- = A^{2-} + H^+$		
10	12.64 ± 0.01	9.81 ± 0.04	−23.2 ± 0.1
20	(12.10)[w]		
25	12.07 ± 0.01	9.53 ± 0.02	−23.3 ± 0.1
	(12.0)[v]	(10.0)[v]	(−21.5)[v]
40	11.81 ± 0.01	9.00 ± 0.04	−25.3 ± 0.1
	Inosine, N_1H–C_6O, pK_1, $H_2A = HA^- + H^+$		
20	(8.82)[x]		
25	8.96 ± 0.01	6.50 ± 0.07	−19.2 ± 0.2
	(8.9)[e]	(7.2)[e]	(−16.4)[e]
	(8.7)[j]		
	(8.72)[s]		
	Inosine, ribose OH, pK_2, $HA^- = A^{2-} + H^+$		
10	12.99 ± 0.07	10.4 ± 0.2	−22.8 ± 0.8
25	12.36 ± 0.02	10.65 ± 0.03	−20.9 ± 0.1
	(12.33)[r]		
40	11.84 ± 0.05	10.60 ± 0.05	−20.3 ± 0.2
	Xanthine, N_1H–C_6O, pK_1 $H_2A = HA^- + H^+$		
20	(7.44)[s]		
	(7.70)[a,a]		
25	7.53 ± 0.01	6.33 ± 0.10	−13.2 ± 0.2
	(7.7)[y]		
25 (?)	(7.53)[b,b]		
	Xanthine, N_9H, pK_2, $HA^- = A^{2-} + H^+$		
10	12.36 ± 0.03	10.18 ± 0.06	−20.6 ± 0.2
20	(11.12)[s]		
	(11.94)[a,a]		
25	11.84 ± 0.01	9.61 ± 0.03	−22.0 ± 0.1
25 (?)	(11.63)[c,c]		
40	11.51 ± 0.03	9.16 ± 0.05	−23.4 ± 0.1
	Xanthosine, N_1H–C_6O, pK_1, $H_2A = HA^- + H^+$		
20	(5.67)[u]		
25	5.67 ± 0.01	3.74 ± 0.03	−13.4 ± 0.1
	(6.0)[y]		
25 (?)	(5.50)[b,b]		
	Xanthosine, ribose OH, pK_2, $HA^- = A^{2-} + H^+$		
10	12.85 ± 0.03	11.02 ± 0.05	−19.9 ± 0.2
25	12.00 ± 0.05	10.86 ± 0.08	−18.9 ± 0.3
25 (?)	(13)[c,c]		
40	11.76 ± 0.08	10.75 ± 0.06	−19.5 ± 0.2

TABLE I (*Footnotes*)

[a] Reported values are valid at $\mu = 0$ and are the averages of several runs in each case with the uncertainties expressed as standard deviations among runs. Of previous $\Delta H°$ values, only those from *i*, *k*, *q*, and *v* were determined calorimetrically. See Discussion for information concerning the assignment of ionization sites. [b] Albert (1953), M = 0.005. [c] Lewin and Tann (1962), $\mu \sim 0$. [d] Izatt and Christensen (1962), $\mu = 0$. [e] Suchorukow *et al.* (1964), $\mu = 0.1$. The $\Delta H_2°$ value for guanosine is calculated from the pK_2 and $\Delta S_2°$ values reported in this reference because the $\Delta H_2°$ value reported in the reference, 3.6 kcal/mole, appears to be in error. [f] Alberty *et al.* (1951), $\mu = 0.15$ (NaCl). [g] Harkins and Freiser (1958), $\mu = 0.005$. [h] Reinhert and Weiss (1969a), $\mu = 0.05$ ($NaClO_4$). [i] Christensen and Izatt (1962), $\mu = 0$. [j] Ogston, (1936). [k] Rawitscher and Sturtevant (1960), $\mu = 0.1$ (NaCl). [l] Taylor (1948), $\mu \sim 0.005$. [m] Lewin and Barnes (1966). The pK value listed first was determined potentiometrically at 20° and $\mu = 0.0135$; the second pK value was determined spectrophotometrically at 20° and $\mu = 0.1$. The order of listing of the ΔH and ΔS values is the same as that of the set of pK values from which they were calculated. [n] Fiskin and Beer (1965), $\mu = 1$ ($NaNO_3$). [o] Levene and Simms (1925), M = 0.0500. [p] Reinert and Weiss (1969b), $\mu = 0.05$ ($NaClO_4$). [q] Izatt *et al.* (1965, 1966), $\mu = 0$. [r] Levene *et al.* (1926). [s] Levene and Simms (1925), $\mu = 0.0250$. [t] Bunville and Schwalbe (1966), $\mu = 0.1$. [u] Albert (1953), M = 0.002. [v] Wooley *et al.* (1970). [w] Albert and Brown (1954), M = 0.02. [x] Albert (1953), M = 0.1. [y] Ogston (1935), M ~ 0.001. [z] Footnote *v*, M = 0.001. [a,a] Pfleiderer and Nübel (1961), μ not specified. [b,b] Cavalieri *et al.* (1954), $\mu = 0.05$. Temperature not specified; assumed to be 25°. [c,c] Calvalieri *et al.* (1954), $\mu = 1$. Temperature not specified; assumed to be 25°.

compound. These sites are (a) the N_7H^+ group in all compounds, (b) the N_9H group in adenine, guanine, hypoxanthine, and xanthine, and (c) the ribose group ionization in the nucleosides. On the other hand, neighboring groups might be expected to influence markedly the $\Delta H°$ values for proton ionization from the pyrimidine moieties of the compounds studied. The $\Delta H°$ values in Table I generally confirm these observations and are summarized together with the corresponding pK and $\Delta S°$ values in Table II.

The only available examples of case (a) are protonated guanosine and protonated hypoxanthine. The thermodynamic quantities for the first ionization from the protonated forms of these compounds are similar as seen in Table IIa. Ionization from protonated guanosine is known to be from the N_7H^+ group (Miles *et al.*, 1963b; Shapiro, 1968) and the similarity of the $\Delta H°$ values in these structurally similar substances suggests that ionization from protonated hypoxanthine is also from the N_7H^+ group. Furthermore, the $\Delta S°$ values are approximately equal because of the similar charge types involved leading to nearly equal pK values for the two species. We attempted to protonate protonated adenine, using a calorimetric titration, but were unsuccessful. We conclude from this experiment that the basicity of the N_7 atom in adenine is less than that of either guanosine or hypoxanthine.

Data are given in Table IIb for three examples of case (b), *viz.*, adenine, hypoxanthine, and xanthine. The $\Delta H°$ values in the cases of the three compounds are seen to be nearly identical indicating that the same type of nitrogen atom is involved in each ionization, and the $\Delta S°$ values are consistent with the charge types of the two reactions involved. The much higher pK values for hypoxanthine and xanthine compared with that of adenine are thus a result of the more negative $\Delta S°$ values in these cases.

Four examples of case (c) are given in Table IIc, *viz.*, adenosine, guanosine, inosine, and xanthosine. The $\Delta H°$ values for the last three compounds are nearly identical with that for adenosine being somewhat lower.

In reactions 3–7 the ionizations from the pyrimidine moieties have been assigned to the N_1H group. This assignment is supported by Miles (Miles *et al.*, 1963b) in the case of the pK_2 value in guanosine (reaction 3). The $\Delta H°$ values for ionization from the N_1H group might be expected to be similar to those for the N_9H ionization in adenine, hypoxanthine, and xanthosine. However, in Table IId a decrease is seen in the $\Delta H°$ values in both the base (hypoxanthine, xanthine) and nucleoside (guanosine, inosine, and xanthosine) series, and all values are much less than expected for ionization from this group. The lower than expected $\Delta H°$ values

TABLE II: Selected pK, $\Delta H°$, and $\Delta S°$ Values at 25° (Taken from Table I) for the Indicated Reaction Types.

Compound	Ionization Site	pK	$\Delta H°$ (kcal/mole)	$\Delta S°$ (cal/deg mole)
	Part a			
	$H_2A^+ = HA + H^+$			
Guanosine	N_7H^+	1.9	3.2	2.1
Hypoxanthine	N_7H^+	1.8	3.0	1.7
	Part b			
	$HA = A^- + H^+$			
Adenine	N_9-H	9.87	9.65	−12.9
	$HA^- = A^{2-} + H^+$			
Hypoxanthine	N_9-H	12.07	9.53	−23.3
Xanthine	N_9-H	11.84	9.61	−22.0
	Part c			
	$HA^- = A^{2-} + H^+$			
Adenosine	Ribose OH	12.35	9.7	−24.0
Guanosine	Ribose OH	12.33	10.85	−20.0
Inosine	Ribose OH	12.36	10.65	−20.9
Xanthosine	Ribose OH	12.00	10.86	−18.9
	Part d			
	$H_2A = HA^- + H^+$			
	Bases			
Hypoxanthine	N_1H-C_6O	8.91	7.88	−14.4
Xanthine	N_1H-C_6O	7.53	6.33	−13.2
	Nucleosides			
Guanosine	N_1H-C_6O	9.25	7.65	−16.7
Inosine	N_1H-C_6O	8.96	6.50	−19.2
Xanthosine	N_1H-C_6O	5.67	3.74	−13.4

TABLE III: Heat Capacity Values Valid over the Temperature Range 10–40° for Reaction $HA^- = A^{2-} + H^+$.

Acid	Ionization Site	$\Delta C_p°$ (cal/deg mole)
	Bases	
Hypoxanthine	N_9H	−27
Xanthine	N_9H	−34
	Nucleosides	
Guanosine	Ribose OH	−6
Inosine	Ribose OH	7
Xanthosine	Ribose OH	−9

for these ionizations could result from the involvement of neighboring (C=O, CNH_2) groups in the ionization process through the formation of microspecies. No evidence appears to have been reported for or against microspecies in these compounds. As seen in Table IId, the $\Delta H°$ trends are primarily responsible for the appreciable acidity increases in both the base and nucleoside series; the $\Delta S°$ values remaining nearly constant in each series as would be expected since the reaction charge type is the same in all ionizations. The large difference seen in Table I in the $\Delta H°$ values for proton ionization from the N_1H group of guanosine (7.65) and protonated adenosine (3.91) confirms that the electronic environment of the nitrogen atoms are quite different in the two cases. Some involvement of the ribose group in the 9 position on proton ionization from the pyrimidine moiety appears likely as is evidenced by the decrease in the $\Delta H°$ values (Table I) in going from adenine to adenosine, hypoxanthine to inosine and xanthine to xanthosine, $\Delta(\Delta H°) = 0.90$, 1.38, and 2.59 kcal per mole, respectively.

We have shown (Izatt *et al.*, 1966; Christensen *et al.*, 1967a,b) that the $\Delta S°$ value associated with a particular proton ionization step is generally, but not always, characteristic for given product and reactant charge types. In the present study, the $\Delta S°$ values for proton ionization from positively charged species, *i.e.*, $H_2A^+ = HA + H^+$ and $H_3A^+ = H_2A + H^+$, are consistent with those expected for the involved product and reactant charges. Where proton ionization is from a neutral molecule, *i.e.*, $HA = A^- + H^+$, the range of $\Delta S°$ values (cal/deg mole), except in the cases of adenosine (−24.0) and inosine (−19.2), is from −13 to −17 which is somewhat less negative than would be expected (Christensen *et al.*, 1967a) for ionization involving these charge types. These less negative values are reminiscent of those for proton ionization from formic acid and some amino acids which were attributed to considerable interaction between the neutral acid and the solvent (Christensen *et al.*, 1967a).

There are five ionizations in Table I of the type $HA^- = A^{2-} + H^+$. The entropy changes for these reactions at 25° (−18 to −23) are similar to those typically observed for reactions where the proton ionizes from a neutral molecule (Christensen *et al.*, 1967a) rather than like those found for proton ionization from mononegative ions where the ionization is from the immediate vicinity of the charge as in $H_2PO_4^-$ (Christensen *et al.*, 1967a) and adenosine monophosphate where $\Delta S°$ values are −30 and −36, respectively (Christensen and Izatt, 1962). The $\Delta S°$ values in Table I for reactions of this type suggest that in these cases the proton ionizes from a site on the ion which is relatively unaffected by the mononegative charge. Thus, the $\Delta S°$ values are consistent with the assigned ionization sites. Similar behavior has been observed previously for proton ionization from uridine (Christensen *et al.*, 1967b), thymidine (Christensen *et al.*, 1967b), glucose 6-phosphate (Izatt *et al.*, 1966), and 1,12-$B_{12}H_{10}^{2-}$ (Hansen *et al.*, 1966).

The ionization with $pK \sim 12$ were studied at 10 and 40° and $\Delta C_p°$ values (cal/deg mole) calculated from the variations of the $\Delta H°$ values with temperature are given in Table III. The uncertainties of the $\Delta C_p°$ values are estimated to be ±5 cal/deg mole. The $\Delta C_p°$ values for the nucleosides and bases are seen to be nearly the same within each group (~0 and −30, respectively) but the two groups are clearly different from each other. All five ionizations are of the reaction type $HA^- = A^{2-} + H^+$. Values of $\Delta C_p°$ for this reaction type in the cases of simple acids have been found to range from approximately −45 to −54 (Izatt and Christensen, 1968; Larson and Hepler, 1969), *e.g.*, $H_2PO_4^-$, −54; HSO_4^-, −50; $(CH_2)_n(COO^-)(COOH)$, −45 to −54. The $\Delta C_p°$ values for both the purine bases and nucleosides are seen to be significantly lower than these. If unaffected by charge the $\Delta C_p°$ values would be expected to fall in the range of approximately −23 to −38 which range of values is characteristic of reactions of the type $HA = H^+ + A^-$ (Christensen *et al.*, 1968; Izatt and Christensen, 1968; Larson and Hepler, 1969; Christensen *et al.*, 1970). The $\Delta C_p°$ values for the bases hypoxanthine and xanthine do fall within this range indicating that the proton ionization in these cases is from a site relatively unaffected by the mononegative charge. Furthermore, this explanation is consistent with the lower than expected $\Delta S_2°$ values for these compounds and with the spatial separation of their individual ionization sites as seen in reactions 4 and 6. The $\Delta C_p°$ values for the nucleosides guanosine, inosine, and xanthosine, however, are much less negative than would be expected even for proton ionization from a mononegative ion. Previous work with adenosine has shown that both the 2′- and 3′-hydroxyl groups are required for the acidic character found in the $pK \sim 12$ range (Izatt *et al.*, 1965, 1966). The unusually low $\Delta C_p°$ values for the nucleosides could result from less than expected ordering of the solvent due to intramolecular hydrogen bonding between the 2′- and 3′-hydroxyl groups upon ionization.

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