Photochemical Reactions and Analyses. All irradiations were carried out using a Halos 300-W high-pressure mercury lamp with a water-cooled quartz jacket. In a typical procedure, 0.005 mmol of the diazo compound was added to 2.0 mL of the appropriate alcohol in Pyrex tubes. The sample was then degassed, sealed, and suspended in a transparent Pyrex Dewar filled with coolant. Coolants were water (20 °C), ice–water (~0 °C), dry ice–ethanol (-78 °C), liquid nitrogen–isopentane (-110 and -155 °C), and liquid nitrogen (-196 °C). Irradiation was generally continued until all the diazo compound was destroyed. When irradiation was performed in the solid phase, a second Pyrex tube was inserted into the sample tube in order to maximize exposure and utilization of radiation. The method greatly reduced the irradiation period especially in solid-phase experiments. Since control experiments confirmed that insertion ratios were relatively sensitive to the period of irradiation–thaw cycles, the irradiations outlined in Tables I–III were conducted over 4-h intervals without thawing the matrix in the dark, unless otherwise indicated. Sensitized experiments were performed under conditions similar to those described above. Usually a 50-molar excess of benzophenone to the diazo compound was added to ensure that >95% of the incident light was absorbed by the sensitizer. That addition of benzophenone greatly accelerates the rates of decomposition was noted even in the solid-phase experiment. No products from the reactions of carbene with benzophenone, e.g., oxirane were detected. Control experiments exclude possible photoconversion of the product during the irradiation period and also demonstrate that no reaction occurs in the absence of light over the temperature range studied.

Product identifications were established either by GC as well as GC–MS comparisons using authentic samples synthesized as described above or by isolating individual components followed by spectroscopic identification. Product distributions were conveniently determined by standard GC technique.

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Registry No. 1, 766-91-6; **7**, 883-40-9; **15** (X = *p*-MeO), 23304-25-8; **15** (X = *p*-Me), 23304-24-7; **15** (X = *p*-Cl), 19277-54-4; **15** (X = *m*-MeO), 65864-99-5; **15** (X = *p*-Br), 73900-14-8; **15** (X = *p*-CO_2Me), 73900-18-2; **15** (X = *p*-CN), 70135-28-3; MeOH, 67-56-1; EtOH, 64-17-5; *i*-PrOH, 67-63-0.

Studies of the Tautomerism of Purine and the Protonation of Purine and Its 7- and 9-Methyl Derivatives by Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy[1]

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Abstract: The nitrogen-15 NMR shifts of purine and its 7- and 9-methyl derivatives were measured at the natural-abundance level as a function of pH. The results made it possible for both the sites and magnitudes of protonation of purine and its derivatives to be determined. A semiquantitative determination was made of the position of the N7H–N9H tautomeric equilibria of purine in both water and dimethyl sulfoxide.

Nitrogen-15 NMR has proved very valuable for investigating the protonation of the bases which are components of the nucleic acids, nucleosides, nucleotides, and related compounds.[2,3] Because of the biological importance of nucleic acids, proton magnetic resonance ^{1}H (NMR) has been used extensively to study the stabilization and enzymatic replication of such compounds.[4,5] However, attempts to elucidate the course of protonation of purine by ^{1}H NMR were not conclusive.[6] The additional ambiguity associated with the tautomeric equilibrium of its N7H and N9H forms suggested that ^{15}N NMR spectroscopy might be especially effective for investigating the protonation and tautomeric behavior of the nitrogens of purine.

We describe here the results of an ^{15}N NMR study of purine and its 7-methyl and 9-methyl derivatives in aqueous solution over the pH range 0 to 9. The course of protonation of purine in dimethyl sulfoxide attendant to titration with trifluoroacetic acid was also examined. The results indicate essentially exclusive protonation of purine and its derivatives on the N1 nitrogen.

Table I. Nitrogen-15 Chemical Shifts of Purine and Derivatives[a]

compound	N1	N3	N7	N9
purine	109.7	124.8	181.6	185.7
	(14.3)	(14.0)	(10.3)	(9.1)
purine (dimethyl sulfoxide)	98.4	116.0	166.8	187.3
	(16.5)	(15.2)	(9.7)	(9.7)
9-methylpurine	109.3	132.7	145.0	244.1
	(14.6)	(14.6)	(11.1)	(8.5)
7-methylpurine	108.5	116.7	229.4	143.0
	(13.8)	(13.7)	(9.4)	(11.7)

[a] In ppm upfield from external D^{15}NO$_3$. The spectra were taken in aqueous solution unless otherwise indicated. The $^2J_{^{15}N-H}$ couplings Hz, values (in parentheses) reported here are regarded as being uncertain by at most ±1.5 Hz.

Experimental Section

Purine was commercially available and was used without further purification.

A suspension of purine (10 g, 0.083 mol) and sodium hydride (57%; 0.46 g, 0.083 mol) in *N*,*N*-dimethylformamide (20 ml) was stirred under argon at 0 °C for 20 min. Methyl iodide (11.7 g, 0.083 mol) was added and the resulting mixture was stirred for 2 h. The *N*,*N*-dimethylformamide was removed under reduced pressure, and dry-column chromatography with 95% ethanol afforded 1.66 g (15%) of 7-methylpurine, mp 180 °C (lit.[7] mp 183 °C), and 4.45 g (40%) of 9-methylpurine, mp 160 °C (lit.[7] mp 165 °C). An alternative preparation of 7-methylpurine has

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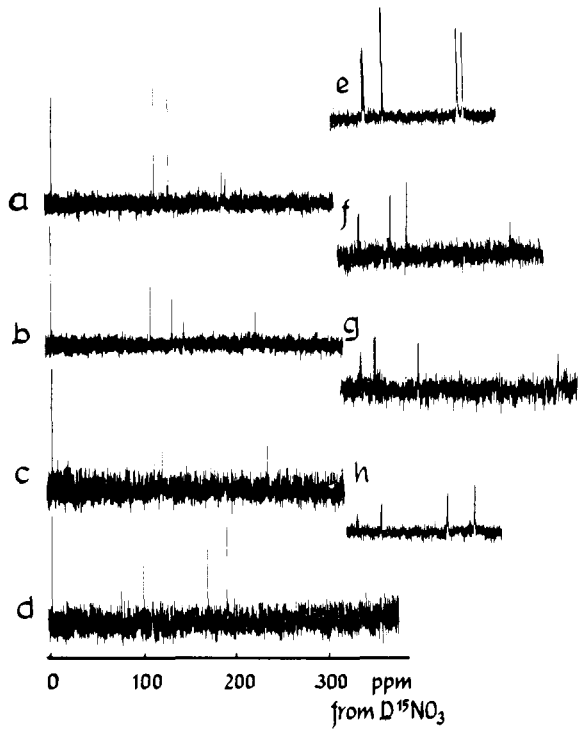

Figure 1. The ^{15}N NMR proton-decoupled spectra of aqueous solutions of (a) purine, (b) 9-methylpurine, (c) 7-methylpurine, (d) purine in dimethyl sulfoxide, and their proton-coupled spectra e, f, g, and h, respectively.

been reported by Bredereck et al.[8]

The ^{15}N NMR spectra of purine were taken in D_2O and dimethyl sulfoxide, and those of the 7-methyl- and 9-methylpurine derivatives in neutral aqueous solution, except where noted otherwise. The concentrations were 1–2 M. The pH values of the aqueous solutions were determined by means of a Radiometer pH meter.

The ^{15}N spectra were obtained at a frequency of 18.25 MHz with a Bruker WH-180 pulse spectrometer.[9] A capillary containing 1 M solution of $D^{15}NO_3$ in D_2O provided the reference standard, while D_2O or deuteriodimethyl sulfoxide was used as an internal field-frequency lock. The reported chemical shifts are in parts per million, upfield from the resonance of external $D^{15}NO_3$. The normal operating conditions employed pulse widths of 9.7–12.9 μs (35–45° flip angle) and pulse delays of 15–21 s. With these conditions, useful spectra could be obtained with accumulation times of 3–8 h, depending on the concentration. The samples were run at about 30 °C using gated proton decoupling or no proton decoupling. To determine changes in nitrogen shifts on protonation, trifluoroacetic acid was added to the dimethyl sulfoxide solutions and concentrated hydrochloric acid to the aqueous solutions.

Results and Discussion

The ^{15}N chemical shifts for purine and its 7-methyl and 9-methyl derivatives in water and for purine in dimethyl sulfoxide are listed in Table I. The nitrogen–hydrogen coupling constants can be used to assign the chemical shifts of N1 and N3 of these compounds. The N3 resonance of purine in water appears as a doublet (J = 14.0 Hz), while the N1 resonance is split into a doublet of doublets (J = 14.3 Hz) by the H6 and H2 protons. The resonances at 181.6 and 185.7 ppm have coupling constants of 10.37 and 9.5 Hz, respectively. These coupling constants permit the upfield resonances to be identified as imidazole nitrogens by comparison with couplings determined from the proton spectra of ^{15}N-labeled adenine derivatives, which show nitrogen–hydrogen couplings of about 10 Hz for the imidazole rings.[10] Similarly, the ^{15}N NMR shifts for 7-methyl and 9-methylpurine were also assigned on the basis of coupling constants, as shown in Figure 1. The small separation of 4 ppm between the N7 and N9

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Table II. Dependence of ^{15}N Shifts of Purine on Trifluoroacetic Acid Concentration in Dimethyl Sulfoxide[a]

mol equiv of acid	N1	N3	N7	N9
0	98.4	116.0	166.8	187.3
0.25	114.9	116.2	167.1	186.8
0.5	127.5	116.3	166.8	186.4
0.75	135.3	116.4	166.7	186.2
1.0	142.6	116.6	167.1	186.1
2.0	158.9	116.9	165.5	186.6

[a] In ppm upfield from external $D^{15}NO_3$.

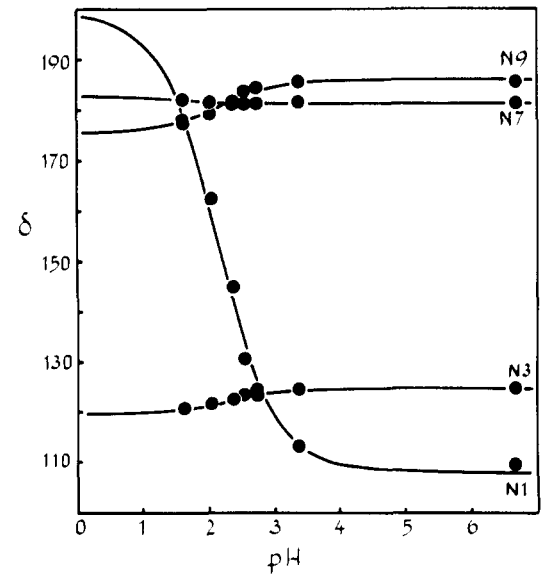
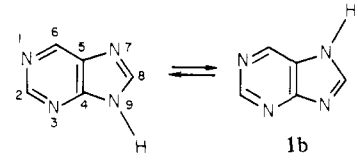


Figure 2. Dependence on the ^{15}N shifts of purine on pH. The curves for this and succeeding figures were calculated using a nonlinear least-squares fit to the change of shift with pH in which pk_a and the extremes of chemical shifts at low and high pH were varied to obtain the best fit to the experimental points.

resonances for purine in neutral water indicates that the N7H and N9H tautomers are present in nearly equal amounts. This is not the case for dimethyl sulfoxide as solvent, wherein the chemical shifts for N7 and N9 are 166.8 and 187.3 ppm, respectively. In dimethyl sulfoxide, tautomer **1a** is somewhat more



1a **1b**

favored. Such a shift in equilibrium was also observed for adenine in dimethyl sulfoxide, possibly as the result of specific solvent–solute interactions, or possibly because of unfavorable repulsions between the unshared electron pairs of **1b** which would be reduced by hydrogen bonding with water.[3]

Earlier efforts to determine the protonation site(s) of purine suggest that N1 is the principal protonation site, although the possibility of protonation at N3 and one of the imidazole nitrogen atoms was not ruled out.[6] Although pyrimidine itself is a weaker base than imidazole, protonation could be expected to occur on the pyrimidine ring of purine because the imidazole NH nitrogen is strongly electron-donating to the pyrimidine ring.[11]

The ^{15}N NMR shift changes on protonation are strongly indicative of essentially exclusive protonation of purine on the N1 nitrogen in both dimethyl sulfoxide and water. In dimethyl sulfoxide, the N1 resonance moves upfield with increasing trifluoroacetic acid concentration, while the N3, N7, and N9 chemical shifts remain almost unchanged (Table II). In water,

(11) Lister, J. H. "Fused Pyrimidines", New York, 1971; p 444.

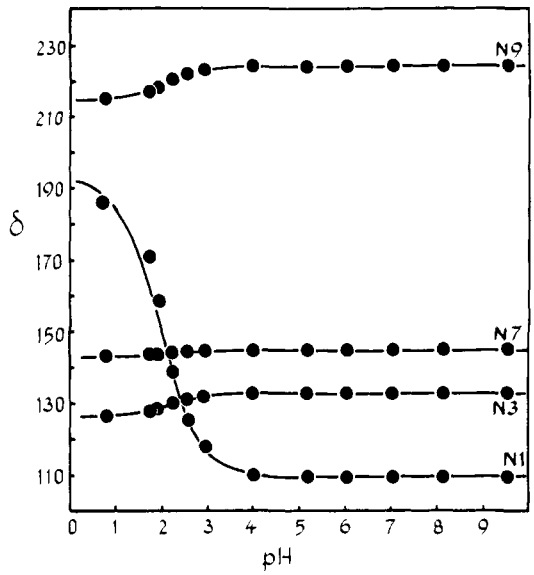


Figure 3. Dependence of the ^{15}N shifts of 9-methylpurine on pH.

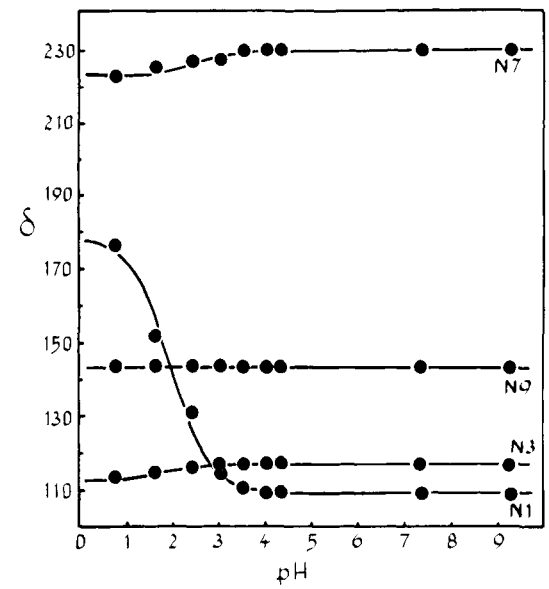
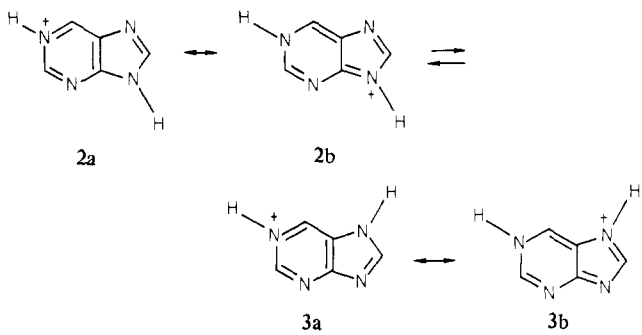


Figure 4. Dependence of the ^{15}N shifts of 7-methylpurine on pH.

the N1 resonance again moves upfield with added acid, while the N3 and N9 resonances move downfield. The shift of the other imidazole nitrogen remains virtually unchanged. Because the ^{15}N protonation shifts of nitrogens in pyridine or pyrimidine are strongly in the *upfield* direction,[2,12] the change in shift of N3 is not consistent with significant protonation of this nitrogen of purine. The changes in shift for complete protonation of purine from the shift vs. pH curves in water are: N1, 91.2 ppm (upfield); N3, 5.0 ppm (downfield); N7, 1.3 ppm (upfield); N9, 10.8 ppm (downfield). The pk_a value calculated from the titration curve is 2.20 ± 0.08 (see Figure 2); the literature reports pk_a 2.39[13] and 2.52.[14]

Two resonance structures with positive charges on nitrogen can be written for the N1 conjugate-acid tautomers of purine (**2a–2b**, **3a–3b**). The mix of the isomeric N1 conjugate acid tautomers (**2** and **3**) need not necessarily correspond to the mix for neutral purine. We have postulated for adenine that the favorableness of the N9H tautomer may well be because the lone-pair electron repulsions involving the N3 and N9 nitrogens, and/or the electron-withdrawing inductive effect of N3 would lead to a more stable structure with N9 being an NH nitrogen rather than an azine nitrogen.[3] The electron-withdrawing effect should tend to shift the N9H ⇌ N7H equilibrium toward the N7H side because **2b** corresponds to having more positive charge closer to N3 than does **3b**, while the electron-repulsion effect would be relatively unchanged on going from **1** to **3a** and **3b**. The rather small changes in N7 and N9 shifts which occur on protonation in either water or dimethyl sulfoxide do not indicate any large changes in the **2** ⇌ **3** equilibrium constant. An increase in the proportion of **3** would surely cause a marked downfield shift of N9. For this reason, the electron-repulsion effect may be the more important.



The shift changes which occur on protonation of 7- and 9-methylpurine in water are shown in Figures 3 and 4. On complete protonation of the 9-methyl derivative, the resonance of N1 is calculated to be shifted upfield by 83.9 ppm, while the N3, N7, and N9 resonances are shifted downfield by 6.4, 1.6, and 9.8 ppm, respectively, as shown in Figure 3. The results indicate essentially exclusive protonation of N1 for the 9-methylpurine. The pK_a derived from the shift changes of N1 is 2.02 ± 0.07 as compared with the reported values of 2.36[13] and 2.48.[14]

Protonation of 7-methylpurine in water also appears to occur essentially exclusively at N1 with a calculated upfield shift of 77.0 ppm (see Figure 4). The N3 and N7 resonances here move downfield by 4.0 and 7.0 ppm, respectively, while the N9 resonance changes very little. The pk_a for the 7-methyl derivative is calculated to be 1.81 ± 0.04 from the ^{15}N NMR shift changes as against the reported value of 2.29.[14]

It has been suggested from ultraviolet absorption spectra,[13] that 7-alkylated purines may protonate on nitrogens different from those found for purine or the 9-alkylated analogues. There is no evidence for this from the ^{15}N NMR spectra.

The shifts reported here for purine nitrogens may be influenced to some degree by base stacking.[15] The difficulties associated with obtaining natural-abundance ^{15}N spectra at low concentrations render studies of changes in shift over a wide range of concentrations impractical. However, attempts to improve the fits of the pH dependence of the shifts of N1 of purine, 7-methylpurine and 9-methylpurine, as in Figures 2–4, by inclusion of Hill coefficients[16] gave disparate values of the coefficients and essentially no improvement of the correlation coefficients. To the degree that base-stacking changes chemical shifts through ring-current effects, stacking–destacking is not expected to influence nitrogen shifts much more than proton shifts (±2 ppm).[15]

Registry No. 1a, 51953-03-8; **1b**, 120-73-0; **2a**, 81064-21-3; **3a**, 18348-60-2; **3b**, 18348-60-2; 9-methylpurine, 20427-22-9; 9-methylpurine conjugate acid, 81064-22-4; 7-methylpurine, 18346-04-8; 7-methylpurine conjugate acid, 81064-23-5.

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