$^{13}C,^{1}H$ Spin–Spin Coupling. 9. Purine[1]

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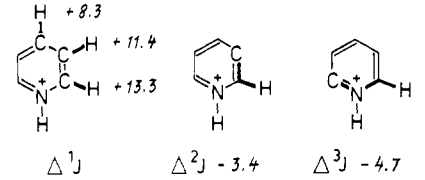
Abstract: The $^{13}C,^{1}H$ spin–spin coupling constants of purine, its anion, its cation, and its dication are reported for aqueous solution, 5% NaOD, and D_2SO_4 of varying concentrations. In addition, such data for purine and its cations are given for solutions in dimethyl sulfoxide, trifluoroacetic acid (TFA), and fluorosulfonic acid. With the help of various model systems, N-1 protonation for the monocation and subsequent N-7/N-9 protonation for the dication were detected. From the pD dependence of the $^{1}J(^{13}C,^{1}H)$ data, the second pK_a value of purine is calculated (−1.5). In TFA, besides N-1 protonation, 33% dication formation is found. It is shown that the N-7H/N-9H tautomerism of purine can be studied on the basis of the vicinal interactions $^{3}J(4,8)$ and $^{3}J(5,8)$. For neutral solution in D_2O and for the monocation in aqueous mineral acid, the tautomer ratio is 1:1. In Me_2SO the N-9H tautomer is favored by a factor of 2, and in TFA the monocation shows an $(N\text{-}7H)^+/(N\text{-}9H)^+$ tautomer ratio of 3:7. With the use of FP-INDO calculations, we show that earlier difficulties in predicting the correct protonation effect for $^{1}J(2,\alpha)$ in pyridine can be solved by using the MNDO-optimized geometry for pyridinium ion. Similar calculations for purine and its ions allow the determination of tautomer ratios on the basis of the calculated ^{3}J values. This procedure is also tested successfully for adenine.

Information about protonation sites in polyaza heterocycles is of interest to organic as well as bioorganic chemistry, since the chemical behavior of these compounds is strongly influenced by the basic centers. We have shown that long-range $^{13}C,^{1}H$ spin–spin coupling constants are useful parameters for the detection of such protonation sites and for structure elucidations of ionic species.[2-4] In addition, information about tautomeric equilibria may be available from these quantities.[5] In a recent paper[6] the protonation effects on $^{13}C,^{1}H$ spin–spin coupling constants for the parent system pyridine have been determined, and the present study extends these investigations to purine.

The biological importance of purine and its derivatives has initiated a number of proton[7,8] and carbon-13[9-13] as well as nitrogen-15[14] NMR studies. Most of them dealt, however, with chemical shift measurements, using coupling information only scarcely. For purine itself, Read and Goldstein[7] followed the pH dependence of $^{1}J(^{13}C,^{1}H)$ on the basis of the ^{13}C satellite spectra in ^{1}H NMR, whereas Thorpe et al.[12] and, more recently, Ts'o et al.[13] reported coupling data in neutral media. Our investigation concentrates on the changes observed for the complete set of $J(^{13}C,^{1}H)$ data over a large pD region and in media of differing acidities such as anhydrous trifluoroacetic acid (TFA) and fluorosulfonic acid (FSO_3H). In addition, the question of purine tautomerism will be considered on the basis of the vicinal interactions $^{3}J(4,8)$ and $^{3}J(5,8)$ in the imidazole substructure.

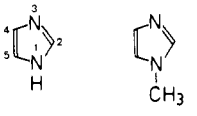
Results and Discussion

Model Compounds. For the system pyridine/pyridinium ion, the following changes of $^{13}C,^{1}H$ spin–spin coupling constants (in Hz) have been recognized to be the most sensitive indicators for the detection of protonation sites:[6] (a) increase of the $^{1}J(^{13}C,^{1}H)$ data in the order $\alpha > \beta > \gamma$ with respect to the protonated nitrogen (**1**), (b) decrease of $^{2}J(^{13}C,^{1}H)$ in fragment **2**, and (c) decrease of $^{3}J(^{13}C,^{1}H)$ in fragment **3**.



1 **2** **3**

With respect to the present investigation it was necessary to supplement these data by the changes expected for the spin–spin coupling constants in the five-membered ring partial structure of purine. We, therefore, have determined the $J(^{13}C,^{1}H)$ values for the model compound imidazole (**4**). Measurements were made in 50% NaOH, dimethyl sulfoxide (Me_2SO), and TFA to complement earlier results for aqueous solutions at pH 4.5, 9.0, and 10.0.[5,15] Since the observed values for **4** are affected by the rapid proton exchange between N-1 and N-3, additional measurements for *N*-methylimidazole (**5**) were indicated. From the results



4 **5**

summarized in Table I the following conclusions can be drawn: Protonation of the anion $\mathbf{4}^-$ (50% NaOH) leads to strong increases for the one-bond coupling constants in positions 2 and 4 and, in accord with the rules b and c above, to a decrease for the ^{2}J and ^{3}J values. The same trend continues if we go from **4** to $\mathbf{4}^+$ (solvent change from Me_2SO or D_2O to CF_3COOH). The last step is more clearly analyzed by using the pair $\mathbf{5}/\mathbf{5}^+$, which yields as the most

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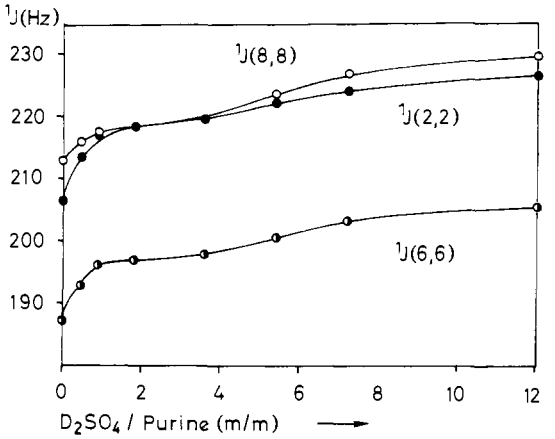


Figure 1. Behavior of one-bond $^{13}C,^1H$ coupling constants of purine with increasing concentration of D_2SO_4.

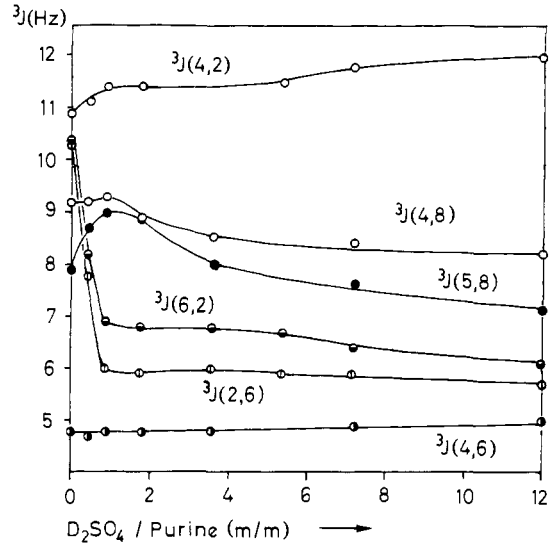
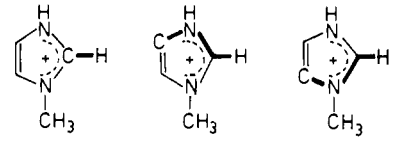


Figure 2. Behavior of vicinal $^{13}C,^1H$ coupling constants of purine with increasing concentration of D_2SO_4.

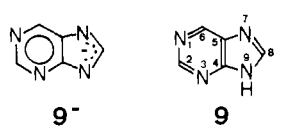
significant protonation effects (in Hz) relevant to purine those indicated by diagrams **6–8**.



6 **7** **8**

Purine. To minimize additional medium effects, we recorded data for purine in aqueous NaOD and D_2SO_4. The changes observed for the $^nJ(^{13}C,^1H)$ values over a large concentration range under acidic conditions are shown graphically in Figures 1 and 2. In Table II, the results for 5% NaOD (ca. 1.25 N), D_2O, 20% D_2SO_4 (ca. 2 N), and 90% D_2SO_4 (ca. 10 N) are collected. Under these conditions we are dealing with the anion, the neutral system, the monocation, and the dication, respectively.[8]

Starting with the anion $\mathbf{9}^-$, the most pronounced coupling constant changes ΔJ for the transition to the neutral parent compound **9** (D_2O) are as follows:

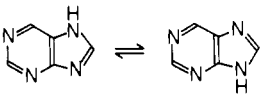


$\mathbf{9}^-$ **9**

Δ^1J	(2,2)	+5.1	(6,6)	+7.7	(8,8)	+14.8
Δ^3J	(4,2)	+0.7	(4,8)	−1.0	(5,8)	−1.2

Significant for the protonation of the five-membered ring fragment are the strong increase found for $^1J(8,8)$ and the decrease observed for the two vicinal interactions $^3J(4,8)$ and $^3J(5,8)$. The fact that $^1J(2,2)$ and $^1J(6,6)$ are also affected, even if less strongly, is due to conjugation between the pyrimidine and imidazole partial structures in purine, an explanation already cited by Grant[9a] to rationalize the ^{13}C chemical shift changes observed for the pair $\mathbf{9}^-/\mathbf{9}$.

For the structure of the neutral system **9**, the observation that $^3J(4,8)$ as well as $^3J(5,8)$ decreases upon protonation is of importance since it points to the tautomeric equilibrium **9a** ⇌ **9b** present in purine.[16] Due to this tautomerism both N-7 and N-9

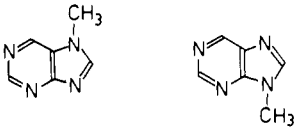


9a **9b**

are formally protonated. The question then arises whether from the magnitude of $^3J(4,8)$ and $^3J(5,8)$ the equilibrium position of **9a** ⇌ **9b**, that is, the concentration of the individual tautomers, can be determined. This aspect will be discussed in the next section.

Tautomerism of Purine in D_2O. The findings of Wasylishen and Tomlinson[5] that imidazole tautomerism can be studied on the basis of $^3J(^{13}C,^1H)$ values supported the idea that similar investigations are possible in the purine field. This approach seemed of interest since coupling parameters are normally far less sensitive to medium and concentration effects than chemical shifts and, therefore, might yield more reliable estimates of tautomer concentrations than chemical shift measurements do. Grant et al.[10] had shown that the latter parameters can be used to estimate the concentration of **9a** and **9b** in solution but were not able, at that time, to determine the tautomer populations in H_2O, due to the solvent dependence of the chemical shift parameter.

For the present purpose, the two methylpurines **10** and **11** served



10 **11**

as model compounds and their $^{13}C,^1H$ coupling constants were measured (Table III). From these results and eq 1 where n =

$$\%\text{N-7H}(\mathbf{9a}) = 100[^3J(n,8)(\mathbf{9b}) - {}^3J(n,8)_{\text{expll}}]/[^3J(n,8)(\mathbf{9b}) - {}^3J(n,8)(\mathbf{9a})] \quad (1)$$

4 or 5 for **10** and **11**, respectively, the percentage of the N-7H tautomer can be calculated on the basis of the $^3J(^{13}C,^1H)$ values measured for purine (Table II) if a correction for the pure substituent effect of the methyl group in **10** and **11** is applied that allows the use of $^3J(n,8)$ in **10** and **11** for $^3J(n,8)$ in **9a** and **9b**, respectively.

To evaluate this effect for $^3J(4,8)$ and $^3J(5,8)$ we used the following procedures: For methylimidazole in D_2O the two couplings amount to 10.4 and 3.3 Hz, respectively (cf. Table I). The sum (13.7 Hz) is slightly smaller than twice the coupling measured in imidazole (7.13 × 2 = 14.26 Hz), where a degenerate equilibrium exists. With the assumption that the observed difference is totally due to the methyl effect on $^3J(5,2)$ in **5**, the methyl group should reduce the $^3J(^{13}C,^1H)$ coupling through the

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Table I. $^{13}C,^{1}H$ Spin-Spin Coupling Constants for Imidazole (4) and *N*-Methylimidazole (5) in Different Media (Hz)[a]

A. Imidazole (4)

solvent	$^{1}J(2,2)$	$[^{1}J(4,4) + {}^{1}J(5,5)]/2$	$[^{2}J(4,5) + {}^{2}J(5,4)]/2$	$[^{3}J(2,4) + {}^{3}J(2,5)]/2$	$[^{3}J(4,2) + {}^{3}J(5,2)]/2$
50% NaOH	193.5	179.2	14.7	11.6	8.3
Me_2SO-d_6	205.8	187.6	13.4	9.4	7.5
D_2O, pH 10[b]	208.8	190.8			
D_2O[c]			13.26	8.91	7.13
TFA	220.6	201.7	12.2	6.3	6.0

B. *N*-Methylimidazole (5)

solvent	$^{1}J(2,2)$	$^{1}J(4,4)$	$^{1}J(5,5)$	$^{2}J(4,5)$	$^{2}J(5,4)$	$^{3}J(2,4)$	$^{3}J(2,5)$	$^{3}J(4,2)$	$^{3}J(5,2)$
Me_2SO-d_6	206.2	187.0	188.6	10.3	16.5	10.7	6.7	11.2	3.4
D_2O[c]				10.4	15.6	9.7	6.5	10.4	3.3
TFA	220.6	202.8	201.8	11.6	11.9	5.7	5.7	6.0	5.2

[a] For earlier and related data see ref 45. [b] Reference 15. [c] Reference 5.

Table II. $^{13}C,^{1}H$ Spin-Spin Coupling Constants of Purine in Different Media (Hz)[a]

solvent	$^{1}J(2,2)$	$^{3}J(2,6)$	$^{3}J(4,2)$	$^{3}J(4,6)$	$^{3}J(4,8)$	$^{4}J(5,2)$	$^{2}J(5,6)$	$^{3}J(5,8)$	$^{3}J(6,2)$	$^{1}J(6,6)$	$^{4}J(6,8)$	$^{1}J(8,8)$
5% NaOD	201.5	10.2	10.2	4.6	10.2	1.2	5.6	9.3	10.2	180.1	0.7	198.2
D_2O	206.6	10.4	10.9	4.8	9.2	1.4	5.8	8.1	10.3	187.2	0.6	213.0
20% D_2SO_4	218.2	6.1	11.2	4.8	9.2	1.2	2.2	9.1	6.8	196.1	0.8	217.7
90% D_2SO_4	227.2	5.7	12.0	5.0	8.2			7.1	6.1	205.7		230.0
Me_2SO-d_6[b]	203.3	10.8	11.2	4.9	8.2	1.3	6.5	9.3	10.5	184.0	0.5	210.8
TFA	221.5	5.9	11.5	4.9	7.8	1.6	1.6	9.8	6.9	199.3		221.1
FSO_3H	228.9	5.7	12.2	5.1	8.3	1.1	1.8	7.1	6.4	206.8		231.0

[a] For concentrations, see Experimental Section. [b] $^{5}J(2,8) = 0.5$ Hz.

Table III. $^{13}C,^{1}H$ Spin-Spin Coupling Constants of 7- and 9-Methylpurine (10, 11) in Different Media[a]

solvent	$^{1}J(2,2)$	$^{3}J(2,6)$	$^{3}J(4,2)$	$^{3}J(4,6)$	$^{3}J(4,8)$	$^{4}J(5,2)$	$^{2}J(5,6)$	$^{3}J(5,8)$	$^{3}J(6,2)$	$^{1}J(6,6)$	$^{4}J(6,8)$	$^{1}J(8,8)$
(A) 7-Methylpurine (10)												
Me_2SO-d_6	202.9	10.8	10.9	4.2	13.0	1.3	5.7	4.3	10.8	186.5	1.0	210.9
D_2O	205.9	10.4	10.8	4.2	12.5		4.9	4.9	10.6	188.2		211.6
20% D_2SO_4	217.3	6.2	11.2	4.1	12.3			5.5	7.3	196.2		216.9
TFA	220.8	6.3	11.1	4.2	11.1			5.7	6.9	197.8		220.2
90% D_2SO_4	226.6	5.9	11.9	4.9	8.2			6.9	5.8	203.4		229.0
(B) 9-Methylpurine (11)												
Me_2SO-d_6	204.3	10.9	11.0	5.5	5.5	1.4	6.7	11.5	10.4	183.3	0.7	212.4
D_2O	207.0	10.5	11.0	5.5	5.5	1.2	5.9	11.0	10.0	185.7		213.5
20% D_2SO_4	218.3	6.1	11.4	5.7	5.7	1.2	2.5	11.7	6.8	194.9		218.3
TFA	221.4	5.8	11.5	5.8	5.8			11.2	6.4	198.7		220.1
90% D_2SO_4	227.6	6.5	11.7	4.6	7.3			7.5	6.1	205.1		228.7

[a] For concentrations, see Experimental Section.

methylated nitrogen additionally by 0.6 Hz (correction A). This correction is smaller than the one used by Wasylishen and Tomlinson[5] (1 Hz), which was derived from the comparison of the respective vicinal interactions in benzene and toluene. An independent estimate of the methyl effect was, however, possible with the results of $\mathbf{5}^{+}$, where the two coupling constants $^{3}J(4,2)$ and $^{3}J(5,2)$ amount to 6.0 and 5.2 Hz, respectively (Table I). The methyl group thus decreases the coupling through the pyrrole-like nitrogen by 0.8 Hz (correction B). It also becomes clear from a comparison with the results obtained for $\mathbf{4}^{+}$ that this additional substituent effect of the methyl group is confined to the coupling through the substituted nitrogen.

As it turned out, the selection of a specific correction was not critical since the results obtained by either of the two procedures A and B were virtually identical within experimental error (±0.3 Hz for the *J* values, ±5% for the tautomer populations). In the following we cite, therefore, only results based on correction A.

Using the corrected coupling constants for D_2O from Table II, we calculate with eq 1 N-7H/N9-H tautomer ratios of 48:52 and 53:47, based on $^{3}J(4,8)$ and $^{3}J(5,8)$, respectively. The energy of **9a** and **9b** in D_2O is, therefore, practically identical, a conclusion supported also by the results of earlier investigations.[16]

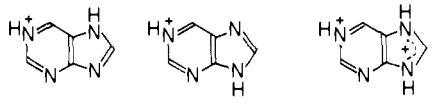
Mono- and Dication. Turning now to the observations made for acidic solutions we note from Figures 1 and 2 a strong change for all coupling constants at a D_2SO_4/purine molar ratio of 1:1 (20% D_2SO_4 in Table II). The most significant ΔJ values are the following:

$\Delta^{1}J$	(2,2)	+11.6	(6,6)	+8.9	(8,8)	+4.7
$\Delta^{2}J$	(5,6)	−3.6				
$\Delta^{3}J$	(2,6)	−4.3	(5,8)	+1.0	(6,2)	−3.5

Again, all ^{1}J values are affected, but the stronger increase for $^{1}J(2,2)$ and $^{1}J(6,6)$ points to dominant protonation in the pyrimidine fragment. That N-1 is protonated is clearly indicated by the large decrease observed for $^{2}J(5,6)$ and the vicinal interactions $^{3}J(2,6)$ and $^{3}J(6,2)$. In contrast, the change for $^{3}J(4,2)$ is only +0.3 Hz. These results are therefore in accord with earlier findings from ^{1}H NMR studies in TFA[8] and chemical shift measurements.[9] However, on the basis of the pH dependence of the $^{1}J(^{13}C,^{1}H)$ values, Read and Goldstein[7] suggested that a considerable amount of N-3 as well as N-7/N-9 protonated tautomers may be present at pH ~0 (17 ± 12% and 29 ± 7%, respectively). From our data we find no indication of the presence of such cations with different structures. Even if one recognizes that the sensitivity of chemical shifts as well as $^{13}C,^{1}H$ coupling constants for the detection of small amounts of other structures is difficult to estimate, we feel that the presence of concentrations in the order of those cited above can be excluded. In addition, as will be shown subsequently, the $^{1}J(^{13}C,^{1}H)$ values are those coupling constants that are most sensitive to medium effects. They increase steadily with increasing

acidity of the medium, a fact that can also be recognized from Figure 1. Considerations based solely on these parameters are, therefore, not without ambiguity. The majority of the experimental data, therefore, support the (N-1)-protonated structure for the monocation of purine.

It is also of interest to check if the tautomer population is affected by N-1 protonation. Using once again the methylpurines as model compounds and the coupling constants measured under the same experimental conditions (20% D_2SO_4, Table III), we derive with the corrected ^3J values N-7H/N-9H ratios of 48:52 and 46:54 on the basis of $^3J(4,8)$ and $^3J(5,8)$, respectively. The average ratio is again practically 1:1. In aqueous mineral acid the (N-1)-protonated tautomers **9a**$^+$ and **9b**$^+$ have, therefore, within experimental error, the same energy.



9a$^+$ **9b**$^+$ **9**$^{2+}$

Finally, the transition to the dication in 90% D_2SO_4 yields the following diagnostic ΔJ values:

Δ^1J	(2,2)	+9.0	(6,6)	+9.6	(8,8)	+12.3
Δ^3J	(4,8)	−1.0	(5,8)	−2.0		

Here again, all ^1J values increase strongly. The order $\Delta^1J(8,8) > \Delta^1J(2,2) > \Delta^1J(6,6)$ and, more important, the reduction of the two vicinal interactions $^3J(4,8)$ and $^3J(5,8)$ indicate protonation in the imidazole part (**9**$^{2+}$). That both ^3J values decrease clearly establishes the protonation of a tautomeric mixture **9a**$^+ \rightleftharpoons$ **9b**$^+$.[17]

It is of interest here to follow the behavior of the ^1J data in the region above a molar ratio D_2SO_4/purine ~ 2 (Figure 1). The plot clearly shows the characteristics of a "titration curve", even if the changes are less pronounced than in the region of a molar ratio of 1:1, where the first protonation occurs. With Hammett's H_0 function for the acidity of the solution studied,[19] apparent pK_a values (H_0 values at "half-diprotonation") are found from the point of inflection, using a third-order polynomial to fit the experimental curve. The results are −2.50, −2.79, and −2.76 on the basis of $^1J(2,2)$, $^1J(6,6)$, and $^1J(8,8)$, respectively, leading to an average of −2.7. Since we worked in rather concentrated solution, a fair amount of D_2SO_4 was already used up by purine protonation. Considering this, the concentration of the solution was actually 26%, corresponding to a corrected pK_a value of −1.5 for the second protonation of purine in deuterated media.

In this context we note a linear relationship between the pK_a values for the first and second protonation step for pyrazines.[20] In the purine field pK_a values of mono- and diprotonation have been obtained for adenine and guanine[21] (4.1, −0.33 and 3.2, −1.04, respectively), and on the basis of these results one finds a linear relationship of the following form: $pK_a^{II} = 0.79\ pK_a^{I} - 3.56$. With $pK_a^{I} = 2.4$ for purine,[22] we obtain $pK_a^{II} = -1.7$, in good agreement with our experimental value.

It is further illuminating to inspect the curvature found for the different vicinal coupling constants (Figure 2) more closely. The dramatic decrease for $^3J(6,2)$ and $^3J(2,6)$ at the beginning of the curve clearly indicates that N-1 is directly involved in the first protonation step. In contrast, $^3J(4,2)$ shows a behavior similar to that found for the one-bond interactions (Figure 1), thus demonstrating that N-3 is only indirectly affected by protonation. Quite naturally, $^3J(4,6)$ is hardly affected at all, whereas the unsteady behavior observed for $^3J(4,8)$ and $^3J(5,8)$ is undoubtedly due to the N-7H/N-9H tautomerism. For these constants only weighted averages of the respective interactions in **9a** and **9b** or **9a**$^+$ and **9b**$^+$, respectively, are observed.

Solvent Effects on the $^nJ(^{13}C,^1H)$ Data in Purine. In the following section we shall discuss the data found for different solvents such as Me_2SO, TFA, and FSO_3H. The respective coupling constants are given in Table II. As was shown by Wagner and v. Philipsborn,[8] under these conditions we are dealing with the neutral species **9**, the (N-1)-protonated monocation (**9a**$^+ \rightleftharpoons$ **9b**$^+$), and the dication **9**$^{2+}$. The coupling constant data are, therefore, in general agreement with the results derived for aqueous solution; there are, however, a number of interesting differences indicating structural alterations.

In comparing the data obtained in D_2O and Me_2SO, respectively, we note that all $^1J(^{13}C,^1H)$ values are smaller in Me_2SO by about 3 Hz. This finding must be attributed to the change from a protic to an aprotic solvent, since it is expected that hydrogen bonding between the nitrogen lone pairs and the solvent affects the $J(^{13}C,^1H)$ data much in the same way as protonation does, however, less strongly. This is also true for the ^{15}N chemical shifts in purine nucleotides.[14a]

The above conclusions are supported further by the smaller, equally significant changes observed for the vicinal interactions $^3J(2,6)$, $^3J(6,2)$, and $^3J(4,2)$. In all cases an increase is found in Me_2SO that amounts to 0.4, 0.2, and 0.4 Hz, in the order given above. Since protonation of the free electron pair reduces vicinal spin–spin interactions across the nitrogen atom (cf. **3**), the lone pairs of N-1 and N-3 both take part in hydrogen bonding in D_2O.

In addition, a significant difference exists for the two vicinal interactions $^3J(4,8)$ and $^3J(5,8)$ in both solvents. In D_2O the relation $^3J(4,8) > {}^3J(5,8)$ holds, whereas in Me_2SO the reverse is true. This finding evidently points to a different tautomer ratio in both solvents. Using eq 1 and the data for our model compounds, the methylpurines, we find from Table II after correction N-7H/N-9H ratios of 30:70 and 33:67, in good agreement. The N-9H tautomer is thus favored in Me_2SO by a factor of 2. From chemical shift measurements, Grant et al.[10] found a ratio of 40:60. As judged from their table, the experimental error using chemical shift increments may be as large as 10%. For our considerations an experimental error of 0.3 Hz for the coupling constants as an upper limit yields an error in the tautomer population of 5% only.

The suggestion made recently by Pullman et al.[23] that the N-7H/N-9H tautomer ratios in D_2O and Me_2SO are not very different is thus not supported by the NMR data.

Turning now to the situation in TFA, we consider besides monoprotonation the partial formation of a dication in light of the pK_a^{II} value determined above and an H_0 value for TFA of −4.4 or −3.0.[24,25] Comparing the ^1J data in TFA with those obtained for the sulfuric acid experiments, we find good agreement with the data set recorded for 40% D_2SO_4, which corresponds to a medium of $H_0 = -2.4$.[19] If we compare this value, which is not corrected for the amount of D_2SO_4 used up by purine protonation, with the uncorrected pK_a^{II} value (−2.7), the difference amounts to 0.3 pK_a unit. Accordingly, ca. 33% dication should be present.

To evaluate the **9a**$^+$/**9b**$^+$ molar ratio we again use the methylpurines **10** and **11** as model systems. Since their pK_a values are identical with that of purine,[22] here, too, 33% dication formation can be assumed. The observed coupling constants are thus given by

$$^3J_{exp\,II} = \frac{1}{3}\,{}^3J^{2+} + \frac{2}{3}\,{}^3J^{+} \qquad (2)$$

where $^3J^+$ and $^3J^{2+}$ are the coupling constants in the mono- and dication, respectively. Since the contribution of $^3J^{2+}$ to the observed coupling constants for purine and the methyl compounds in TFA is the same, the differences in the experimental values

(17) It is, of course, not clear if N-7 and N-9 are protonated with equal probability or if the dication arises through N-7 protonation via the equilibrium **9a**$^+ \rightleftharpoons$ **9b**$^+$ as suggested.[18]

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are exclusively the result of the different tautomer populations. The data for $\mathbf{10}^+$ and $\mathbf{11}^+$ (Table III), corrected for the methyl effect, can thus be used as reference values for eq 1, which allows the percentage of the $(N\text{-}7H)^+$ tautomer to be calculated from the measured ^3J values of purine (Table II). We find values of 30% and 29%, based on $^3J(4,8)$ and $^3J(5,8)$, respectively. The tautomer ratio $\mathbf{9a}^+/\mathbf{9b}^+$ is thus approximately 3:7, and the ratio of all species present in TFA, including the dication $\mathbf{9}^{2+}$, amounts to $\mathbf{9a}^+/\mathbf{9b}^+/\mathbf{9}^{2+}$ = 20:47:33, or roughly 2:5:3.[26]

Finally, in FSO_3H we find the largest values for all ^1J data. The remaining constants are, within experimental error, the same as those in 90% D_2SO_4.

Concentration Dependence of $^nJ(^{13}C,^1H)$ Values in D_2O. Since it is well-known that the ^{13}C chemical shifts of purine in D_2O are concentration dependent due to vertical stacking,[13,27] it was of interest to investigate this aspect also for the $^{13}C,^1H$ coupling constants. Measurements made at four different concentrations from 0.52 to 2.38 *m* revealed no change in the coupling constants that exceeded the experimental error, supporting our suggestion that coupling constants are far less sensitive to long-range effects than chemical shifts. In turn, our observation also means that the tautomer ratio did not change more than 5% in the concentration range studied. It is at present not clear if this will be enough to account for the discrepancies between the calculated and experimental dimerization shift $\Delta\delta_2$ for C-4 and C-5 observed by Ts'o et al.[13] More accurate measurements at lower concentration would be necessary to answer this question.

Calculations. Recently,[6] we have tried to predict protonation effects on $J(^{13}C,^1H)$ data in pyridine by theoretical calculations. Both the simple SOS-CNDO/2 method and the more advanced FP-INDO treatment, in particular, failed to reproduce the large increase found experimentally for $^1J(2,\alpha)$. The possibility that this result may have originated from the use of standard geometrical parameters and that protonation might induce structural changes not accounted for by our treatment was suggested. On the other hand, in the literature it was stated that to predict protonation effects on $J(^{13}C,^1H)$ values correctly, in addition to the Fermi contact term, orbital dipole and magnetic spin dipolar terms should perhaps be included,[23,28] a treatment that is usually necessary only for coupling constants involving two heavy nuclei.[29] It was, therefore, of interest to investigate this problem more deeply, and we felt that an improvement of the geometries used for the calculations might be rewarding.

Using experimental structures was believed to be a less successful approach, since the two different experimental results available—one for pyridine hydrogen chloride[30] and the other for pyridine hydrogen nitrate[31]—disagree with regard to bond length as well as bond angles. Even more important, none of them gives the position of the hydrogen atoms. In addition, in the structure determination for the pyridine hydrogen nitrate, the question of whether or not full protonation has occurred or if only a hydrogen bond is present was not completely settled. Finally, the use of experimental geometries would be an approach limited to those systems already studied by diffraction methods and would prevent possible applications in other fields.

We, therefore, decided to use theoretical methods throughout, not only for the calculation of the spin–spin coupling constants but also for geometry optimization. Accordingly, the MNDO procedure,[32] which was shown to be particularly useful for compounds containing heteroatoms,[33] was employed to derive optimized structural parameters as a basis for the FP-INDO calculation[34] of the coupling constants. Bond length as well as bond angles were optimized, the only requirement being planarity and C_{2v} symmetry of the structure. The optimized geometry for pyridinium ion is given in Figure 3, and the $^1J(^{13}C,^1H)$ coupling constants are collected in Table IV. It is found that our procedure now leads to a correct order of protonation effects for the $^1J(^{13}C,^1H)$ values, certainly a considerable improvement. On comparison of the calculated geometrical parameters of pyridinium ion (Figure 3) with those of pyridine,[33] the main change at C-2,6 is the reduction of the internal CCN angle by 3.8°, contrary to earlier suggestions of constant ring geometry.[28] Consequently, at least part of the increase found experimentally for $^1J(2,\alpha)$ upon protonation must be due to enhanced s character in the C-2H_α bond. This is also borne out by calculations that show a substantial increase for the 2s–1s bond order (pyridine: 0.5789; pyridinium ion: 0.5919). Since the coupling is proportional to the square of the bond order,[29] the experimental observation can be rationalized by the geometrical change.

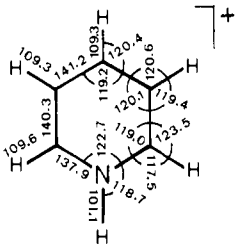

Figure 3. MNDO-optimized geometry of pyridinium ion; bond lengths in pm, bond angles in degrees.

Table IV. Calculated and Observed $^1J(^{13}C,^1H)$ Data (Hz) of Pyridine and Pyridinium Ion

		$^1J(2,\alpha)$	$^1J(3,\beta)$	$^1J(4,\gamma)$
pyridine	calcd	163.94	145.98	145.31
	obsd[a]	177.37	162.11	158.15
pyridinium ion	calcd	182.93	162.11	158.15
	obsd[b]	190.7	173.95	169.43
protonation effects, ΔJ	calcd	+19.0	+16.2	+12.8
	obsd	+13.3	+11.4	+8.3

[a] Reference 46. [b] Reference 6.

The other ΔJ values calculated are not much altered if compared with the earlier results[6] and are in general agreement with experiment,[6] with the exception of the increments for $^3J(5,\beta)$ and $^4J(6,\beta)$. Both are of opposite sign to those found experimentally.[35]

Calculations similar to those described for pyridinium ion were then performed for purine, its anion, and its mono- and dication. The optimized parameters for the planar systems are given in Table V. In agreement with results from X-ray investigations,[36] we find that protonation of N-1 lengthens the adjacent CN bonds and widens the CNC angle. The same is true for N-7 or N-9 protonation.

From the calculated coupling constants (Table VI) it is seen that the experimental trends are well reproduced. In particular, the sensitivity of $^3J(4,8)$ and $^3J(5,8)$ for the N-7H/N-9H tautomerism is demonstrated, a fact that was recently derived also from calculated spin–spin coupling constants of imidazole.[23]

(26) This result differs from the conclusions drawn earlier by Wagner and v. Philipsborn[8] from ^1H NMR studies. These authors assumed exclusively monoprotonation of purine in anhydrous TFA in analogy to investigations made in aqueous mineral acids. The pK_a value cited by them for TFA (+0.5) corresponds, however, to aqueous TFA and is, therefore, not valid for their NMR experiments, where more acidic conditions prevailed.

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(35) We have also tried to further improve the calculated $^1J(^{13}C,^1H)$ values by taking into account charge density effects, following the procedure of Pople et al.[34] Using MNDO, INDO, as well as CNDO charge densities, however, we found no uniform improvement, and it is difficult to decide if the charge density effect is significant. Nevertheless, the correct order of the protonation effects was retained, the result for the INDO densities being $\Delta J(2,\alpha)$ = 36.9, $\Delta J(3,\beta)$ = 27.2, and $\Delta J(4,\gamma)$ = 26.6 Hz.

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Table V. MNDO-Optimized Geometry for Purine Anion (9^-), the Neutral N-7H- and N-9H Tautomers (9a, 9b), Their Monoprotonated Forms ($9a^+$, $9b^+$), and Purine Dication (9^{2+})[a]

A. Bond Lengths, pm

	1,2	2,3	3,4	4,5	5,6	6,1	5,7	7,8	8,9	9,4	2,2'	6,6'	8,8'	1,1'	7,7'	9,9'
anion	137.4	134.2	136.7	146.4	140.7	134.6	138.1	136.5	137.8	137.1	109.8	109.4	108.8			
neutral N7-H	137.3	134.8	136.0	144.2	141.1	134.1	139.6	140.0	134.0	140.0	109.9	109.3	108.8		99.4	
neutral N9-H	137.2	135.0	135.8	143.8	141.6	134.4	140.1	133.5	141.1	138.7	109.9	109.3	108.7			99.5
cation N7-H	140.5	132.9	136.6	146.5	139.0	138.3	139.9	139.5	135.6	137.7	110.1	109.5	109.2	101.0	100.1	
cation N9-H	140.1	133.3	136.6	145.6	139.4	138.4	140.4	133.2	142.4	136.9	110.1	109.5	109.1	101.1		100.3
dication	140.6	134.2	135.5	144.3	140.8	137.8	141.3	136.9	138.5	139.6	110.7	109.9	110.0	102.1	101.2	101.7

B. Bond Angles, deg

	2,1,6	1,2,3	2,3,4	3,4,5	4,5,6	5,6,1	4,5,7	5,7,8	7,8,9	8,9,4	9,4,5	1,2,2'	1,6,6'	7,8,8'	6,1,1'	8,7,7'	8,9,9'
anion	118.8	127.6	114.4	122.2	117.4	119.6	107.9	103.6	116.5	103.1	108.9	115.5	117.1	122.0			
neutral N7-H	119.8	126.9	114.2	122.4	118.7	118.0	104.5	107.4	112.4	105.3	110.3	115.7	119.9	122.3		126.6	
neutral N9-H	120.0	126.7	113.1	124.9	116.6	118.7	109.6	105.7	112.3	106.7	105.6	116.2	117.7	125.8			126.7
cation N7-H	124.0	122.9	116.2	122.6	120.1	114.4	103.9	107.7	112.3	105.6	110.5	117.3	118.9	122.9	117.9	126.6	
cation N9-H	124.1	122.8	115.3	124.1	119.0	114.6	108.9	106.1	112.1	106.9	106.0	117.7	119.1	126.2	118.2		126.5
dication	124.8	121.7	115.8	124.7	118.9	114.2	105.8	109.4	108.8	108.9	107.1	118.6	119.5	126.0	117.8	125.4	125.3

[a] Hydrogen positions are primed.

Table VI. Calculated $^{13}C,^1H$ Spin-Spin Coupling Constants for Purine Anion (9^-), the Neutral (N-7)H and N-9H Tautomers (9a, 9b), Their Monoprotonated Forms ($9a^+$, $9b^+$), and Purine Dication (9^{2+}) (Hz)

cmpd	$^1J(2,2)$	$^3J(2,6)$	$^3J(4,2)$	$^3J(4,6)$	$^3J(4,8)$	$^4J(5,2)$	$^2J(5,6)$	$^3J(5,8)$	$^3J(6,2)$	$^1J(6,6)$	$^4J(6,8)$	$^1J(8,8)$
9^-	167.2	14.4	11.7	9.8	16.6	−2.0	−1.5	14.8	13.2	135.1	1.4	146.7
9a	189.2	13.7	14.3	7.9	15.3	−2.4	−0.5	4.9	13.3	165.3	1.6	189.9
9b	189.3	14.6	15.9	11.6	6.2	−2.6	+1.6	13.0	12.8	162.2	0.2	191.8
$9a^+$	216.7	2.9	11.6	6.1	15.0	−1.0	−5.3	7.4	5.5	179.4	1.0	201.7
$9b^+$	216.7	3.5	12.9	9.4	7.5	−1.2	−3.0	14.7	5.2	179.6	0.5	209.1
9^{2+}	229.0	4.2	13.5	7.8	6.8	−1.6	−3.1	6.5	5.8	191.7	0.6	220.9

Table VII. Calculated $^{13}C,^1H$ Spin-Spin Coupling Constants for the Tautomers of Adenine (12) (Hz)

	$^1J(2,2)$	$^1J(8,8)$	$^3J(4,2)$	$^3J(4,8)$	$^3J(5,8)$
N-7H	183.8	189.0	15.2	14.5	5.1
N-9H	184.1	192.4	16.6	5.9	13.2

As for the $^1J(^{13}C,^1H)$ data, it is interesting to note that relatively large deviations from the experimental values are found in the case of the anion (9^-). Whereas for the neutral and positively charged species the values calculated are too small by ca. 5–20 Hz, the differences between calculated and observed quantities amount to 35–50 Hz for 9^-. This seems to indicate that, in aqueous solution, the anion is strongly stabilized by hydrogen bonds, a fact that is not taken into account by our calculations. In line with this argument are the calculated vicinal interactions $^3J(4,8)$ and $^3J(5,8)$, which are in turn considerably larger than those observed experimentally.

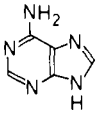
Predictions of Tautomer Populations. It is now possible to derive tautomer populations also on the basis of the calculated coupling constants, if one uses the ratios $^3J(4,8)/^3J(5,8)$ as a measure for the tautomer populations. In this way, possible systematic errors in the calculated coupling constants are largely eliminated. With the general equation

$$\frac{x[^3J(4,8)\text{N-7H}] + (1-x)[^3J(4,8)\text{N-9H}]}{x[^3J(5,8)\text{N-7H}] + (1-x)[^3J(5,8)\text{N-9H}]} = \frac{^3J(4,8)_{\text{expl}}}{^3J(5,8)_{\text{expl}}} = R \quad (3)$$

and the calculated values from Table VI we derive for x, the mole fraction of the N-7H tautomer, the following expressions for the neutral system and the monocation, respectively: $x = (13.0R - 6.2)/(9.1 + 8.1R)$ and $x = (14.7R - 7.5)/(7.5 + 7.3R)$.

The experimentally determined R values ($J(^{13}C,^1H)$ data from Table II) yield for the neutral system $x = 0.47$ (D_2O) and $x = 0.32$ (Me_2SO). For the monocation in 20% D_2SO_4 we find $x = 0.50$. These results may now be compared with those derived empirically with the methylpurines: $x = 0.51$, 0.32, and 0.47, in the order given above. The close agreement certainly lends support to our approach.

As a further test we chose adenine (**12**), where theoretical and



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experimental results[16] showed that the N-9H tautomer is strongly favored. The geometry was again optimized, and the coupling constants collected in Table VII were calculated. From the measured constants $^3J(4,8) = 6.5$ Hz and $^3J(5,8) = 9.9$ Hz (in Me_2SO), we calculate with eq 3 $x = 0.19$. Under similar conditions Grant et al.[10] found $x \approx 0.15$ and Dreyfus et al.[37] $x = 0.22$. It seems, therefore, that the determination of tautomer ratios based on theoretical coupling constants as derived from MNDO-optimized geometries yields reliable results.

Conclusion

The present investigation has shown that $^{13}C,^1H$ coupling constants are sensitive parameters for tautomerism and protonation of purine, and similar results may be expected for other heterocycles. The potential behind such studies so far has only rarely been used,[38] and we believe that a number of interesting problems exist that can be solved with the help of the $^{13}C,^1H$ coupling constants. As pointed out above, these parameters are primarily influenced by structural features and are in this respect superior to chemical shifts, where medium effects and intramolecular interactions play a more important role. Furthermore, studies employing ^{15}N-enriched systems[14c,39] show that additional information of interest is available through $^{15}N,^1H$ and $^{15}N,^{13}C$

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coupling constants, and these data will become more important as natural-abundance studies become feasible.

Experimental Section

Samples of purine, imidazole, and *N*-methylimidazole (99%) were commercially available (Sigma Chemicals, EGA-Chemie) and used without further purification.

The synthesis of the methylpurines followed literature procedures; 7-methylpurine was synthesized from 2,6-dichloro-7-methylpurine according to the procedure given by Bullock and Jardetzky.[40] For the synthesis of 9-methylpurine, 5-amino-4-(methylamino)pyrimidine[41] was made from 3,5-dichloro-4-aminopyrimidine and cyclized as described.[42]

For unequivocal assignment of several $^{13}C,^{1}H$ spin–spin coupling constants, specifically deuterated derivatives of the various systems were prepared. Following the general procedure of Schweizer et al.,[43] 8-deuteriopurine, 2-deuterio-1-methylimidazole, 8-deuterio-7-methylpurine, and 8-deuterio-9-methylpurine were prepared. 6-Deuteriopurine was prepared from 6-chloropurine according to the procedure of Bullock and Jardetzky.[40]

Solvents. Anhydrous TFA (purissimum) was obtained from Ferak and FSO_3H from EGA-Chemie. Deuterated solvents [(D_2O (99.75% D), 40% NaOD, Me_2SO-d_6 (99% D)] were purchased from Merck and EGA-Chemie; concentrated D_2SO_4 was purchased from Merck Sharp and Dohme (99% D).

Spectra were measured with a Bruker WP-80 and WH-400 spectrometers equipped with a ^{2}H lock channel an ^{1}H decoupler, and a Bruker Aspect 2000 computer. The ^{13}C resonance frequency was 20.15 and 100.61 MHz, respectively. According to the spectral parameters used, the digital resolution was 0.1–0.3 Hz. The probe temperature was ca. 30 °C. The concentrations were as follows: imidazole (Table I) 50% NaOH 1.6 *m*, Me_2SO-d_6 2.3 *m*, TFA 1.7 *m* (all ca. 2.9 M); N-methylimidazole (Table I) Me_2SO-d_6 2.2 *m*, TFA 1.7 *m* (all ca. 2.9 M); purine (Table II) 5% NaOD 0.86 *m*, D_2O, 20% and 90% D_2SO_4 1.0 *m*, Me_2SO-d_6 0.8 *m*, TFA 0.6 *m*, FSO_3H 0.5 *m* (all ca. 1.0 M); 7- and 9-methylpurine (Table III) Me_2SO-d_6, D_2O 0.7 *m*; 20% and 90% D_2SO_4 0.74 *m*; TFA 0.6 *m* (all ca. 0.9 M).

Coupling constants could be measured by using first-order analysis in all cases. For the imidazole anion, the validity of first-order rules was checked by comparing the results from a 20.15- and a 100.61-MHz analysis corresponding to ^{1}H frequencies of 80 and 400 MHz, respectively. Both results agreed closely. With the deuterated derivatives, the assignment of all J values was unambiguous. For purine in D_2O, our results agree with those of Ts'o et al.;[13] the assignment for $^{3}J(4,2)$ and $^{3}J(4,8)$ in Me_2SO-d_6, given by Thorpe et al.,[12] however, has to be revised (cf. Table II). In the case of the methyl derivatives **5**, **10**, and **11**, selective methyl ^{1}H decoupling had to be used to resolve the splittings of interest.

The chemical shift data of our measurements agreed closely with those already published, except for $\mathbf{4}^-$. Here we found $\delta(2)$ 147.41 and $\delta(4,5)$ 127.98, relative to external dioxane with δ 67.4. These values are ~2 ppm downfield from those reported in the literature,[44] where, according to the experimental conditions and the pK_a value of 14.5 for imidazole anion,[22] deprotonation was most probably not completed.

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Registry No. 1, 16969-45-2; **4**, 288-32-4; **5**, 616-47-7; **9**, 120-73-0; $\mathbf{9}^-$, 32074-46-7; $\mathbf{9}^+$, 18348-60-2; $\mathbf{9}^{2+}$, 81814-98-4; **10**, 18346-04-8; **11**, 20427-22-9; **12**, 73-24-5; pyridine, 110-86-1.

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Electronic Effects on Triplet and Singlet Excited-State Carbonyl Formation in the Thermolysis of 3-Aryl-3-methyl-1,2-dioxetanes

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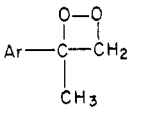
Abstract: A series of para- and meta-substituted 3-aryl-3-methyl-1,2-dioxetanes (**1**) was studied in order to evaluate the electronic effect of substituents on the efficiencies of excited-state carbonyl production. All substituents reduced the efficiency of triplet carbonyl production. Several correlations point to the formation of a triplet carbonyl exciplex, originating from a triplet biradical, in the thermolysis of **1**. It also appears that substituent variation in the proacetophenone portion of **1** results in triplet efficiency changes primarily in formaldehyde, which can be rationalized in terms of a triplet exciplex. Substituent effects on singlet (S_1) efficiency are markedly different from those observed with triplet efficiencies. The possibility of heavy-atom effects in **1** was pursued with *p*-Br and *m*-Br substituents. No detectable heavy-atom effect was observed with singlet (S_1) efficiencies, but the *p*-Br substituent appeared to decrease the triplet efficiency. This suggests that a *p*-Br heavy-atom effect may operate from the triplet exciplex, providing the approximations used in the evaluations of the heavy-atom effect are valid. The effect of substituents on rate of thermolysis of **1** provides further evidence for a biradical mechanism.

The effect of dioxetane structure on the efficiency of producing excited-state carbonyl products during thermolysis is an intriguing problem that is not resolved. Some evidence has been presented that suggests both electronic and steric effects influence efficiencies.[1] Increasing steric effects appeared to increase the triplet efficiency, while electron-releasing substituents appeared to decrease the triplet efficiency. The available dioxetanes did not allow a systematic study of these effects. Both steric and electronic effects were simultaneously varied in most instances, and the effect of electron-withdrawing groups was not evaluated.

In order to isolate electronic effects vs. efficiency of excited-state carbonyl production, we have now studied a series of para- and meta-substituted dioxetanes (**1**). Included in the substituents are



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