Carbon-13 Magnetic Resonance. XIX.[1] Benzimidazole, Purine, and Their Anionic and Cationic Species

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Abstract: Carbon-13 chemical shifts are reported for benzimidazole and its deprotonated and protonated derivatives as well as the purine anionic and cationic species. The data obtained from these fused-ring heterocycles indicate that the protonation parameters derived from simple five- and six-membered heterocycles can be used to predict chemical shift changes resulting from nitrogen protonation and deprotonation in more complex molecules. Protonation parameters are used to establish the relative contributions of the H-7 and H-9 tautomeric forms of aqueous purine and also the protonation site associated with cation formation. The CNDO–SCF–MO theory is used to rationalize the observed chemical shift data.

The relationship of purine to some of the bases found in ribonucleic acids has resulted in extensive theoretical investigation of the former compounds in an effort to correlate the electronic structure with the known chemical properties.[2] The information thus obtained from purine and other model compounds provides a basis for extrapolation to the more complex molecular species in order to understand their chemical characteristics. Of particular importance to theorists is information on the apportionment of the labile proton between positions N-7 or N-9 in purine. In general the proton has been assigned at position N-9, the site of glycosidic linkage in adenine, but in aqueous solution no direct evidence has been presented to exclude the assignment at N-7. Pullman and Pullman[3] and Coller[4] have considered both of the two alternate assignments in which the labile hydrogen in purine is placed at N-7 and N-9, respectively, while Pullman and Pullman[3] also treated the case in which the effect of the proton is averaged between the tautomeric positions by assuming free exchange with the solvent. Both of these earlier studies indicated that N-9 was energetically favored over N-7. More recently, however, Pullman *et al.*,[5,6] using refined Hückel and CNDO–SCF[7–9] calculations have reported that the two tautomeric forms of purine have essentially equivalent energies. The problem has been further complicated by the results of X-ray studies on purine[10] which place the labile proton at N-7 in the crystalline state.

Carbon-13 magnetic resonance has proven useful as a tool to investigate molecular conformation and steric interactions[11–14] as well as electronic charge distribution.[15–18] The initial investigations of purine by ^{13}C techniques[19] were an attempt to obtain an experimental preference for the various π-electron charge-density calculations appearing in the literature. Although a crude correlation was demonstrated between carbon-13 chemical shift data and the various molecular orbital approximations, no preference could be found for one approximation over another. The experimental data provided no information regarding the position of the labile proton in aqueous solution. In order to understand the effects of fused heterocyclic ring systems and nitrogen protonation and proton tautomerism on carbon-13 chemical shifts, Pugmire and Grant have investigated the aza analogs of napthalene[18] as well as the five- and six-membered[16] nitrogen heterocycles. In the former work significant long-range benzenoid substituent parameters due to nitrogen substitution into the ring system were noted while, in the latter two sets of compounds, changes were observed in the chemical shifts associated with protonation of neutral and negatively charged nitrogen atoms. These so-called protonation parameters were found to be highly reproducible among the different molecular species in the five- and six-membered nitrogen heterocycles and also reflect the averaging effects produced by a proton exchanging between its various tautomeric sites.

In the present work, the carbon-13 chemical shifts have been obtained for benzimidazole anion, benzimidazole, benzimidazole cation, purine anion, and purine cation. The chemical shift values thus obtained are compared with the protonation parameters observed previously,[16] and the positions of the labile proton in neutral purine as well as the protonated site in the cationic derivative are obtained. The carbon-13 chemical shift data confirm the conclusions reached by pmr techniques[20,21] that purine protonates at N-1 to form the cationic species.

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Table I. Proton and Carbon-13 Chemical Shift Values for Imidazole, Benzimidazole, Purine, and Their Anionic and Cationic Species

Compound	Position	Γ_i	δ_H[a]	$\beta_{^{13}C}$[a]	$\delta_{^{13}C}$[c]
Imidazole anion	2	3.976385943	−0.42	−16.65	145.09
	4,5	3.976456175	0.02	1.78	126.76
Imidazole	2	3.976421305	−0.68	−7.69	136.23
	4,5	3.976473941	−0.02	6.21	122.33
Imidazole cation	2	3.976434099	−2.26	−6.05	134.59
	4,5	3.976485938	−0.81	6.44	122.10
Benzimidazole anion	2	3.976365611	−0.90	−21.91	150.45
	4,7	3.976499852	−0.56	12.13	116.41
	5,6	3.976482683	0.01	8.44	120.10
	8,9			−15.34	143.88
Benzimidazole	2	3.976491870	−1.03	−12.92	141.46
	4,7	3.976503169	−0.50	13.13	115.41
	5,6	3.976472410	−0.18	5.67	122.87
	8,9			−9.38	137.92
Benzimidazole cation	2	3.976415061	−2.46	−11.04	139.58
	4,7	3.976507671	~−0.6[b]	14.10	114.44
	5,6	3.976456560	~−0.6[b]	1.25	127.29
	8,9			−1.25	129.79
Purine anion	2	3.976373102	−1.85	−20.98	149.52
	4			−32.16	160.70
	5			−5.93	134.47
	6	3.976396968	−2.00	−15.13	143.67
	8	3.976342940	−1.66	−28.37	156.91
Purine	2	3.976363714	−1.98	−23.46	152.00
	4			−26.32	154.86
	5			0.14	128.40
	6	3.976392693	−2.10	−16.30	144.84
	8	3.976379499	−1.81	−19.33	147.87
Purine cation	2	3.976380788	−2.50	−19.79	148.33
	4			−29.56	158.10
	5			0.12	128.42
	6	3.976415194	−2.78	−11.32	139.86
	8	3.976360901	−2.25	−24.45	152.99

[a] Taken with respect to benzene. [b] The proton spectra could not be resolved at 60 MHz. [c] Taken with respect to TMS, which is 128.54 ppm upfield from benzene.

Experimental Section

A. Equipment. Initially, a Varian DP-60 high-resolution spectrometer equipped with a V-4311 transmitter operating at 15.085 MHz was used to observe the carbon-13 magnetic resonance spectra. Proton decoupling was accomplished with a Varian V-4320 spin decoupler operating at 60 MHz in the manner described previously.[16] The study was concluded with another Varian high-resolution spectrometer (AFS-60) with equipment and proton decoupling techniques as previously described.[18]

B. Spectroscopic Details. The decoupled carbon-13 resonance peaks were obtained under conditions of adiabatic rapid passage to determine the approximate decoupler frequency, and then a precise determination was made under slow-sweep conditions with sample spinning. The chemical shift in parts per million (ppm) from benzene is determined from Γ_i, the ratio of the decoupler frequency to the transmitter frequency (f_i/ν_i) and the corresponding proton chemical shift in accordance with

$$\delta_{^{13}C} = \frac{\Gamma_t - \Gamma_0}{\Gamma_t} + \frac{\Gamma_0}{\Gamma_t}\delta_H \cong \frac{\Gamma_t - \Gamma_0}{\Gamma_t} + \delta_H \qquad (1)$$

C. Sample Preparation. All samples were degassed under vacuum by the normal freeze–thaw method in a methanol and Dry Ice bath and then sealed in 12-mm tubes. Samples for proton analysis were prepared in a similar manner in 5-mm tubes. The sodium salt of 3-trimethylsilylpropane-1-sulfonic acid was used as an internal standard.

Solid benzimidazole was dissolved in absolute alcohol and run as a saturated solution. The potassium salt of benzimidazole was prepared in a similar manner by a simple acid–base reaction and run as a saturated alcoholic solution, while the hydrochloride salt was similarly prepared and run in saturated aqueous solution. The potassium and hydrogen sulfate salts of purine were also prepared by acid–base reactions and run in concentrated aqueous solutions.

Results

A. Proton Chemical Shifts. The benzimidazole spectrum has been interpreted by Black and Heffernan[22] on the basis of the work by Dischler and Englert[23] on similar molecules. The resonance position of H-2, which has unit intensity, occurs at low field for benzimidazole and its cationic and anionic species. Positions H-4,7 in benzimidazole occur at lower field than H-5,6 with an assumed identical relative ordering of chemical shifts for the benzenoid protons in the anionic species. On the other hand, formation of the hydrochloride salt results in the coalescence of protons H-4,7 and H-5,6, and the chemical shift values could not be resolved at 60 MHz. The proton chemical shift values obtained in this study are presented in Table I.

The proton chemical shifts in purine have been established by Schweizer, *et al.*,[24] who report a relative ordering of H-6 < H-2 < H-8, in order of increasing magnetic field strength. Purine studies at pH 1 (0.1 *M* DCl in D_2O) and 14 (1 *M* NaOD in D_2O) indicate that all three proton resonance positions are shifted downfield with decreasing pH and that the relative positions of the peaks remain unchanged.[24] However, Chan, *et al.*,[20] report that the proton shifts in purine and its charged species exhibit a marked dependence on both concentration and solvent The proton chemical shifts obtained for the purine species in this study are given in Table I where the concentration of all solutions is approximately 6 mol % water.

B. Carbon-13 Chemical Shifts. The chemical shift values obtained in this study are presented in Table I

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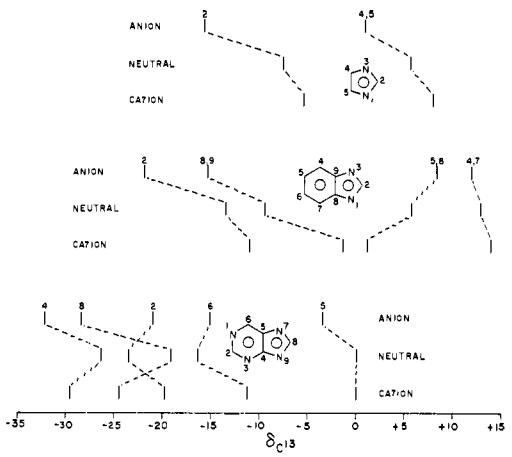

Figure 1. Movement of carbon-13 resonance lines in imidazole, benzimidazole, and purine associated with successive protonation of the anionic species.

along with the carbon-13 chemical shifts of imidazole and its two charged species for purposes of comparison. In the body of the text all chemical shifts are given relative to benzene, as with past practice in this laboratory. Because of the recent interest in a TMS chemical shift scale, however, Table I also contains for convenience the shifts relative to TMS with increasing positive values indicating lower field strength.

1. Benzimidazole. In the case of benzimidazole and its two charged species, the resonance position of C-2 is readily identified in each case as the only peak having unit intensity in the spectra. The values thus obtained for C-2 are -21.9_1, -12.9_2, and -11.0_4 ppm for the anionic, neutral, and cationic species, respectively. It is significant to note that the movement of C-2 in imidazole to higher field with decreasing pH is also paralleled by the pH-induced change in the resonance position of C-2 in the benzimidazole species (see Figure 1).

The bridgehead carbons C-8,9 are readily identified in each benzimidazole species, since a quaternary carbon does not exhibit the proton splitting pattern characteristic of a carbon atom with directly bonded protons. The chemical shifts of C-8,9 have values of -15.3_4, -9.3_8, and -1.2_5 ppm for the anionic, neutral, and cationic species, respectively, and follow a trend similar to that observed at C-2.

The carbon-13 chemical shifts at positions C-4,7 and C-5,6 in the anion and parent were readily assigned by the selective decoupling technique, but an alternate means for assigning the peaks in the cation is required, since the proton resonance positions of H-4,7 and H-5,6 coalesce. The data indicate that C-4,7 moves upfield $+1.0_0$ ppm when the anion is protonated to form the parent while C-5,6 moves downfield -2.7_7 ppm. The resonance positions in question for the cation appear at 1.25 and 14.1_0 ppm and represent shifts with respect to the parent of either -11.8_2 or $+ 0.97$ ppm at C-4,7 and either $+ 8.4_3$ or -4.4_2 ppm at C-5,6. As the chemical shift data in Table I for the various imidazole species display consistent trends with successive protonation, there is little justification for not assuming that consistent trends also occur with successive protonation of benzimidazole anion. Hence, if C-4,7 is assigned at 14.1_0 and C-5,6 at 1.25 ppm, protonation shifts of $+ 0.9_7$ and -4.4_2 are realized, which are consistent with the parameters obtained with the protonation of the anionic species. The alternate assignment would require protonation parameters of -11.88 and $+ 8.43$ ppm at C-4,7 and C-5,6, respectively, and the sign and magnitude of these shift parameters are inconsistent with those noted for anion protonation. Hence, the assignment of C-4,7 and C-5,6 at 14.1_0 and 1.25 ppm, respectively, is considered to be preferable.

2. Purine Anion. The carbon-13 chemical shifts in purine have been reported elsewhere[19] but are presented in Table I for the sake of completeness and for comparison purposes. The bridgehead carbon atoms in the purine anion are readily identified as previously discussed and appear at -32.1_6 and -5.9_3 ppm. The former value is assigned to C-4, as the relative separation of the bridgehead carbons in the anionic and parent species are nearly identical (26.23 ppm in the anion *vs.* 26.46 ppm in the parent) and there is no justification for suggesting that C-4 and C-5 in purine would exchange their relative positions under strongly basic conditions. The net result is downfield shifts of -5.8_4 ppm at C-4 and -6.0_7 at C-5 when purine is deprotonated, and these values compare favorably with a -4.4_3-ppm downfield shift at C-4,5 when imidazole is deprotonated. The remaining carbons, C-2, C-6, and C-8, were assigned by means of the following statistical analysis of the decoupling information.[25] In order to calculate the carbon-13 chemical shifts at positions C-2, C-6, and C-8 in purine with eq 1, it is necessary to know the correct combinations of $\delta_{D_i} = (\Gamma_t - \Gamma_0)/\Gamma_t$, the decoupling shift, and δ_{H_i}, the proton shift. For the three peaks in question, six possible permutations exist in assigning the three proton values to the three decoupling shifts. The choice of the correct permutation is facilitated by a statistical approach using linear regression techniques to obtain the best possible agreement between decoupling data and sweep calibration data.

The chemical shift at carbon C_t is the sum of decoupling and proton shifts as follows

$$\delta_{C_i} = \delta_{D_i} + \delta_{H_i} \tag{2}$$

where the sum of the chemical shifts of all n nuclei is obtained from

$$\sum_i^n \delta_{C_i} = \sum_i^n \delta_{D_i} + \sum_i^n \delta_{H_i} \tag{3}$$

the mean position or center of gravity for the spectrum, $\bar{\delta}_{C_i}$, is given by

$$\bar{\delta}_C = \frac{1}{n} \sum_i^n \delta_{C_i} \tag{4}$$

In the slow-sweep spectrum, this position may be readily found graphically. The distance, x_t, of each individual peak from the mean position is used to calculate the value of δ_C, with respect to $\bar{\delta}_C$ by the relation

$$y_t = \delta_{D_i} + \delta_{H_i} - \bar{\delta}_C = bx_t \tag{5}$$

(25) The statistical analysis was employed to analyze the data prior to acquisition of the AFS-60 spectrometer. As the purine anion and cation appeared to decompose with standing in the sample tubes, the samples were not rerun on the field-frequency-lock spectrometer. The details of the statistical analysis of the data obtained on the field-sweep spectrometer are presented here as they may be useful in laboratories where field-sweep spectrometers are still in use. Confidence is gained in the results of this analysis as the assignments can be independently verified by arguments based on nitrogen protonation parameters.

where b is the sweep calibration factor. Since the calculated chemical shift values of the peaks change with the various combinations of δ_{D_i} and δ_{H_i}, the value of b will vary for each of the $n!$ different permutations. The best value of b corresponding to the correct permutation is obtained when the error in the spectral fit is minimized with respect to b. This is accomplished by differentiating the error squared, ϵ^2, relative to b. The square of ϵ is used to give equal weight to both positive and negative error terms and thereby eliminates cancellation of positive and negative deviations. Thus

$$\frac{\partial \epsilon^2}{\partial b_i} = \frac{\partial}{\partial b_i} \sum_i (y_i - b_i x_i)^2 = 0 \qquad (6)$$

and

$$b_i = \sum_i y_i x_i / \sum_i x_i^2 \qquad (7)$$

Once the value of b is known for a particular permutation, the chemical shift of each peak can be calculated with respect to the mean chemical shift value by

$$k_i = b x_i \qquad (8)$$

The variance in the fit for each permutation then becomes

$$\sum_i \frac{\epsilon_i^2}{n-1} = \sum_i \frac{(y_i - b x_i)^2}{n-1} = \sum_i \frac{(y_i - k_i)^2}{n-1} \qquad (9)$$

The results of the above analysis are given in Table II, where the variance is given for each permutation. Of the six possible permutations of proton chemical shifts and carbon decoupling parameters only the combinations (628) and (826) are not significantly different at the 95% confidence level. The analysis assigns the chemical shift at C-2 as −20.98 ppm. If the neutral purine molecule is considered as the product of protonation of the anion and it is assumed that protonation is at N-7 or N-9, C-8 can be assigned on the basis of the chemical shift changes at the corresponding positions in imidazole and benzimidazole. As is noted in Figure 1 and Table IIIA, deprotonation of imidazole and benzimidazole moves the chemical shift of C-2 (note this position corresponds to C-8 in purine) downfield −8.86 and −8.99 ppm, respectively. Only the permutation which assigns C-8 at −28.37 ppm provides a reasonable deprotonation change (−9.04 ppm, compared to neutral purine), and hence C-6 is assigned at −15.13 ppm by default. It is interesting to note that deprotonation of purine produces an upfield shift of +2.48 ppm at C-2, which compares favorably with the change of +2.77 at the corresponding position (C-5,6) in benzimidazole. On the other hand, C-6 in purine and C-4,7 in benzimidazole experience opposing deprotonation shifts (+1.15, and −1.00 ppm, respectively), but the magnitude of the values is such that the discrepancy is not serious considering the differences in the structures of the six-membered rings.

Table II. Statistical Analysis of the Permutations of δ_{H_i} and $(\Gamma_i - \Gamma_0)/\Gamma_i$ in the Purine Anion and Cation

Permutation[a]	Variance × 10⁴
Anion	
268	227.0
286	235.0
628	9.0
682	210.0
826	2.5
862	178.0
Cation	
268	586.5
286	316.5
628	4.5
682	456.5
826	34.5
862	784.0

[a] The permutation designation represents the various combinations of H-2, H-6, and H-8 proton shifts with the various values of decoupling parameters $(\Gamma_i - \Gamma_0)/\Gamma_i$ as given by eq 1. The notation represents a constant ordering of decoupling parameters running from low- to high-field values.

Table III

Imidazole Position	Imidazole $\Delta\delta^{13}C$,[a] ppm	Benzimidazole Position	Benzimidazole $\Delta\delta^{13}C$,[a] ppm	Purine Position	Purine $\Delta\delta^{13}C$,[a] ppm
A. Chemical Shifts Occurring at Corresponding Positions When Imidazole, Benzimidazole, and Purine Anions Are Protonated					
2	+8.86	2	+8.99	8	+9.04
4,5	+4.43	8,9	+5.96	4	+5.84
				5	+6.07
		5,6	−2.77	2	−2.48
		4,7	+1.00	6	−1.15
B. Changes in Carbon-13 Chemical Shifts Occuring at Corresponding Positions When Imidazole, Benzimidazole, and Purine Are Protonated to Form the Cationic Species					
2	+1.64	2	+1.88	8	−5.12
4,5	+2.23	8,9	+8.43	4	−3.24
				5	−0.02
		5,6	−4.42	2	+3.76
		4,7	+0.97	6	+4.98

[a] Taken relative to (A) anion or (B) neutral parent.

3. Purine Cation. In purine cation the bridgehead carbons are observed at −29.56 and +0.12 ppm and are assigned to C-4 and C-5, respectively, by analogy with the relative ordering in the neutral parent species. The previously described statistical analysis was applied to the six different combinations of decoupling parameters and proton chemical shifts given by eq 1 for C-2, C-6, and C-8. The results of this analysis are presented in Table II and indicate that only two permutations of proton shifts and decoupler shifts are significant at the 95% confidence level. However, both statistically significant permutations assign C-2 at −19.70 ppm. This chemical shift value represents an upfield shift of +3.76 ppm relative to the neutral parent species and is consistent in sign, if not in absolute magnitude, with the protonation data obtained in simple six-membered heterocycles.[16] The data indicate that protonation occurs at N-1, and one would expect[16] that C-6 would also move upfield with respect to the neutral parent. If C-6 is assigned at −11.32 ppm, a positive protonation parameter is in fact realized (4.98 ppm), while the alternate assignment (−24.45) would require a large negative protonation parameter (−8.15 ppm) and would not be consistent with previous findings.[16] Confidence in the assignment is provided by noting that assignment of C-8 at −11.32 ppm would indicate an upfield shift of +8.99 ppm, which is totally inconsistent with the experimental data previously noted, even considering the possibility of purine protonating in the five-membered

ring.[26] Hence, C-8 is assigned at −24.45 ppm and experiences a −5.12-ppm protonation shift relative to the corresponding line position in the purine parent.

Discussion

Figure 1 graphically illustrates the variations in position of the carbon-13 resonance lines induced by successive protonation of the anions of imidazole, benzimidazole, and purine. In imidazole C-2 and C-4,5 are found upfield as the molecule is successively protonated. Likewise positions C-2, C-4,7, and C-8,9 in benzimidazole consistently move upfield while C-5,6 moves downfield as protonation occurs. In the case of purine, C-5 moves to higher field for the first protonation step but is unchanged in the second step. Positions C-4 and C-8 initially are found upfield, while the second protonation step reverses the trend and the lines move downfield to positions which are only slightly higher than those found in the anion. Positions C-2 and C-6 move downfield initially, but the second protonation displaces the resonance lines upfield with the resulting cationic chemical shift values greater than the corresponding values in the purine anion. The movement of the spectral lines in purine reflects the greater complexity of the perturbation effects for this species as compared with the two imidazole molecules studied in this work and suggests considerable electronic reorganization with each successive protonation step.

A. Protonation Site in Purine. The data in Table IIIA summarize the magnitude of the carbon-13 chemical shift changes which occur on protonation of imidazole, benzimidazole, and purine anions. It is noted that position C-2 in imidazole and benzimidazole anions move upfield +8.86 and +8.99 ppm following protonation, and these values agree well with the +9.04-ppm upfield shift observed for purine at C-8. For imidazole, C-4,5 moves upfield 4.43 ppm with respect to the anion, and this reduced value relative to C-2 reflects the averaging effects produced by equilibration of the proton between the two equivalent tautomeric forms. In benzimidazole the bridgehead carbons C-8,9 move upfield +5.96 ppm with respect to the anionic species in accordance with the same general protonation characteristics observed at C-2 and at other α-carbon positions in the simple heterocyclic species.[16] As in the case with imidazole, the protonation shift is lower than at C-2 due to tautomeric averaging of the two equivalent forms. The increased magnitude of the shift at C-8,9 (+5.96 ppm)[27] in benzimidazole over that observed at C-4,5 in imidazole (+4.43 ppm) probably reflects the effects produced by fusing the benzene ring to a simple heterocycle with concomitant changes in the electronic structure of the individual ring systems taken separately. The chemical shift changes in the six-membered ring (+1.00 and −2.77 ppm for C-4,7 and C-5,6, respectively) indicate that protonation in the five-membered ring produces smaller but significant long-range protonation shifts. This is particularly true at C-5,6, which is further removed than C-4,7 from the protonation site but exhibits the larger effect.

The benzimidazole data in Table IIIA are used as a guide in determining the site for protonation in purine. As expected from the preceding data, C-8 in purine is displaced 9.04 ppm to higher field than in the anionic species, and this value is in excellent agreement with the imidazole and benzimidazole data (see Table IIIA). If protonation of the anion were to occur primarily at N-9, C-4 would be expected to move upfield 8–10 ppm and C-5 downfield slightly,[28] with the reverse being expected if protonation occurred at N-7. As both bridgehead carbons in purine anion move upfield with protonation (C-4, +5.84 ppm; C-5, +6.07 ppm, which is to be compared with the +5.96-ppm shift change noted at C-8,9 in benzimidazole, where tautomeric averaging occurs), one can conclude that tautomeric averaging is occurring in purine and the nearly equal magnitude of the shift values suggests little distinction for either N-7 or N-9 as the preferred site for protonation. In aqueous solution the labile proton probably spends almost equal time at N-7 and N-9. It is also worth noting at this point that the protonation effects are transmitted to the six-membered ring in purine, with the largest effect (2.48 ppm) noted at C-2, which is nearly identical with the −2.77-ppm shift at the same relative position (C-5,6) in benzimidazole. Hence, the long-range perturbation effects noted for the benzimidazole system are preserved in the more complex purine structure.

B. Protonation Site for Purine Cation. Examination of the chemical shift changes in Table IIIB provides further data as to the magnitude of inter-ring perturbation effects. Protonation of neutral benzimidazole produces an upfield shift at C-4,7 (+0.97) nearly identical with that noted previously for protonation of the anionic species (+1.00) while the resonance position of C-5,6 is shifted downfield an additional −4.42 ppm with respect to the neutral species, which is of even greater magnitude than the shift change noted for anion protonation (−2.77 ppm). Thus, large, long-range perturbation effects are associated with further protonation in the five-membered ring in benzimidazole.

With the preceding results on the protonation of benzimidazole in mind, the protonation data in Table IIIB can be used to confirm the conclusions of Chan, *et al.*,[20,21] that protonation occurs in the six-membered ring of purine. The fact that C-8 moves to lower field (by −5.12 ppm) eliminates the possibility of significant further protonation at N-7 or N-9. If protonation occurs in the six-membered ring at N-3, one would expect C-2 and C-4 to exhibit the α-protonation effect and move upfield while C-6, being in a para position to the site of protonation, would experience a downfield γ shift.[29] On the other hand, protonation at N-1 would move both C-2 and C-6 to higher resonance positions while C-4 moved downfield. The data given in Table IIIB and Figure 1 indicate that the latter case is the more logical choice since C-2 and C-6 do, in fact, move

(26) Cation formation in imidazole moves the C-2 (corresponds to C-8 in the purines) resonance line upfield +1.64 ppm with respect to the neutral species. Protonation in the five-membered ring of benzimidazole produces a +1.88-ppm upfield shift at the same position. If protonation occurred at N-7,9 in purine, C-8 would be expected to experience a +1.5- to +2.0-ppm upfield shift.

(27) Using the α and β protonation parameters of the simple five-membered heterocycles taken from ref 16, one can predict an upfield shift of +3.71 ppm for C-8,9 under these conditions. However, these changes fail to consider alterations introduced by the benzene ring.

(28) The downfield shift would be due to the negative β effect noted in reference for the simple heterocycles.

(29) In the simple six-membered heterocycles (ref 16), the following protonation parameters were obtained: $\alpha = +7.8 \pm 0.7$, $\beta = -4.4 \pm 0.7$, and $\gamma = -12.7 \pm 0.9$ ppm.

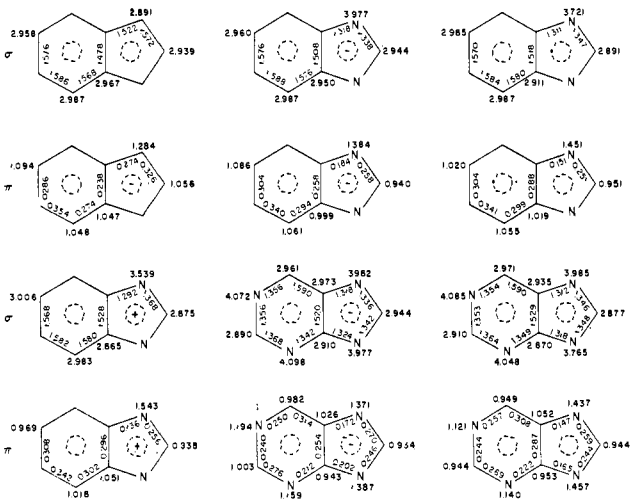

Figure 2. Molecular orbital parameters obtained from the CNDO/2 program include both σ and π charge densities (outside of ring) and bond orders (inside of rings). Idealized geometries were used in all calculations reported in this work. For neutral benzimidazole and purine, equivalent tautomeric averages were assumed.

upfield while C-4 moves downfield (+3.76, +4.98, and −3.24 ppm, respectively). Although the magnitudes of the protonation parameters are less than expected, the relative order is correct. The decreased magnitude of the protonation shifts may be the result of the proton spending a small fraction of its time on the other unoccupied nitrogen positions. On the other hand, the molecular species in question is a fused-ring system and the ring structure may also produce perturbations for which there is no basis to evaluate the data by comparison with simpler ring systems. It is also observed that protonation in the six-membered ring produces a large (−5.12 ppm) downfield shift at C-8 and thus preserves the long-range inter-ring shifts noted previously in protonation effects. (*I.e.*, C-2 moves 2.48 ppm to lower field when protonation occurs in the five-membered ring and this decreased magnitude suggests that inter-ring perturbation effects are more readily transferred from the six- to five-membered rings than are the reverse effects.)

C. Theoretical Considerations. The CNDO–SCF[7-9] molecular orbital formalism was used to compute charge densities and bond orders, and these parameters were used to calculate the chemical shifts in the manner previously described.[16] The electronic structure calculations are presented in Figure 2, where the contributions due to σ and π electrons have been separated for purposes of comparison among the various molecular species. The experimental and theoretical chemical shifts with respect to benzene are shown in Figure 3 for indenyl anion and the various heterocyclic species studied. While the gross correlation of the data with theory is evident, the scatter in the plot indicates that important features such as ring structure and probable variations in the effective average energy approximation have not been properly taken into account.

Previous work in this series[16,18] has demonstrated the utility of using an isoelectronic compound for referencing both theoretical and experimental data. It is recognized that present theoretical treatments do not always adequately account for effects due to ring size, fusion, bridgehead carbons, various ΔE values, etc.

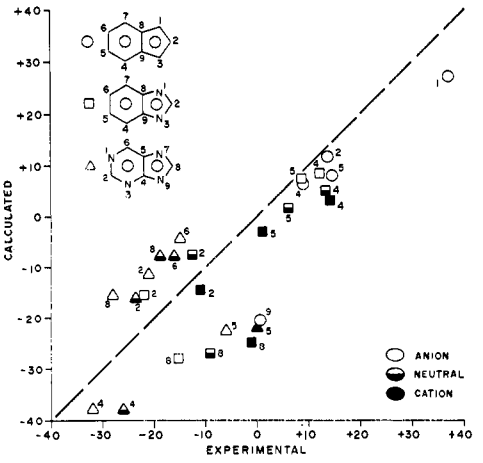

Figure 3. CNDO-predicted chemical shifts are plotted *vs.* experimental values for the compounds indicated. Both predicted and experimental values are referenced to benzene.

The use of a common ring system (in this case idenyl anion, which is isoelectronic to the molecular species considered) to reference experimental and theoretical data essentially subtracts out the effects in question, as one is then only concerned with perturbations induced by molecular changes which are superimposed on a common ring structure. Hence, to a first approximation, those effects due to the basic geometric system are held constant, and only the effects of induced perturbations are observed. Table IV contains the appropriately referenced data which is also graphically portrayed in Figure 4. The latter figure shows a significant improvement in the correlation of the data and demonstrates the problem of comparing indenyl sys-

Table IV. Theoretical Estimates of ^{13}C Chemical Shifts in Benzimidazole, Purine, and Their Charged Species

Compound	Position[a]	σ (tot), ppm	f	σ (tot), ppm	δ^{13} (exptl),[b] ppm
Indenyl anion		0	1.000	0	0
Benzimidazole	2	−29.9	0.982	−36.9	−35.5
anion	4, 7	+2.1		−4.5	+3.0
	5, 6	−0.6		−7.2	−6.2
	8, 9	−7.2		−14.4	−15.8
Benzimidazole	2	−21.9	0.993	−24.5	−26.5
	4, 7	−1.0		−3.5	+4.0
	5, 6	−6.5		−9.0	−8.8
	8, 9	−6.1		−8.8	−9.8
Benzimidazole	2	−28.9	1.015	−23.4	−24.6
cation	4, 7	−2.9		+2.3	+4.9
	5, 6	−10.9		−5.6	−13.4
	8, 9	−4.1		+1.5	−1.7
Purine anion	2	−18.7		−33.6	−35.6
	4	−17.4	0.956	−32.2	−32.6
	5	−1.8		−16.1	−6.4
	6	−10.7		−24.3	−24.3
	8	−30.1		−44.1	−41.9
Purine	2	−23.7		−34.1	−38.1
	4	−17.7		−28.7	−26.8
	5	−1.2	0.973	−11.8	−0.4
	6	−14.3		−24.5	−25.4
	8	−22.7		−32.9	−32.9

[a] See Figure 3 for numbering scheme. [b] Values reported relative to the corresponding positions in indenyl anion (A. J. Jones, private communication: C-5,6, 14.65; C-4,7, 9.15; C-8,9, 0.47; C-1,3, 36.71; C-2, 13.56 ppm with respect to benzene).

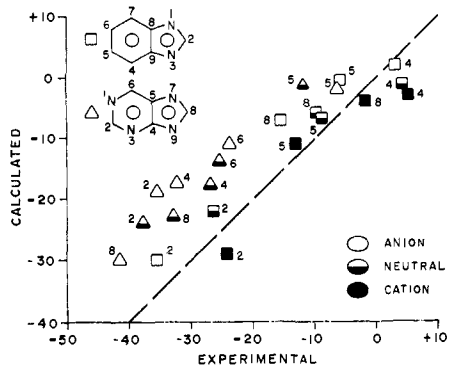

Figure 4. CNDO-predicted chemical shifts are plotted *vs.* experimental values. All values are referenced to the corresponding positions in indenyl anion. An average excitation energy of 10 eV is assumed for each species.

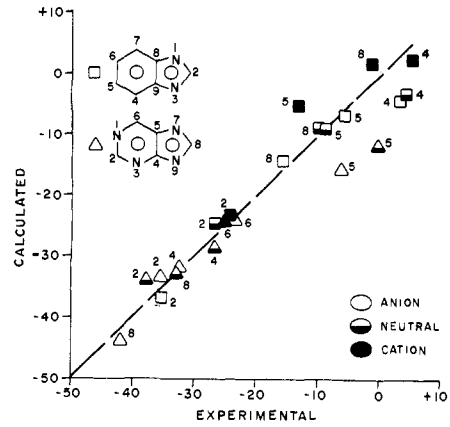

Figure 5. Theoretical chemical shifts predicted by a modified CNDO–SCF treatment are plotted against the experimental values. The treatment is modified by taking $\Delta E = f \times 10$ eV. The f values, given in Table IV, reflect changes in the electronic energy levels as a result of nitrogen protonation.

tems directly with benzene. Even though the correlation is displaced slightly from the perfect correlation line, the difference in slope may be due to a poor value chosen for ΔE (in the present case, all calculations assumed a ΔE value of 10 eV).

In order to partially compensate for the errors introduced by assuming a constant ΔE in the calculations, f ratios are calculated for the various species of interest using the average electronic energy of the occupied and unoccupied molecular orbitals in indenyl anion as reference.[30] The theoretical results obtained from this parameterization are given in Table IV and are displayed in Figure 5. In Figure 5 one realizes only a minor improvement in the relative ordering of the points when compared to Figure 4, but the differences in the two plots do stress the importance of the ΔE term. This result suggests the need for a more sophisticated method than the present ΔE approximations for treating the admixture of excited states into the ground-state wave function. Even though there is some deviation of five data points (at positions C-5 in purine and its anion, C-5,6 in benzimidazole cation, and C-4,7 in benzimidazole at its anion), it is noted that almost all carbon resonance positions are predicted to move in accordance with the protonation parameters discussed above.

A close examination of the data in Table IV and Figure 2 indicates that protonation of benzimidazole anion actually increases the π charge density at C-2 and, together with a decrease[31] in the C–N mobile bond order, chemical shift theory[16] predicts the upfield shift (+12.4 ppm *vs.* experimental value of +8.98 ppm) in the resonance position. Likewise, theory predicts an increase in π charge density at C-8,9 and an upfield shift of +5.6 ppm is predicted (experimental value is +5.96 ppm). The downfield shift at C-5,6 is preserved by theory (−1.8 ppm *vs.* −2.77 ppm experimentally) as is the +1.0-ppm upfield shift predicted at C-4,7 (experimentally +1.00 ppm). The predicted shifts associated with cation formation are also preserved except at C-5,6, where an upfield shift is predicted (the experimental shift is −4.42).

The CNDO calculations are also relatively successful in predicting the direction and relative magnitudes of chemical shift variations between the purine anion and the neutral parent. Experimentally C-8, C-4, and C-5 in purine move upfield (+9.04, +6.07, and +5.84 ppm, respectively) with respect to purine anion and C-2 and C-6 move downfield (−2.48 and −1.15 ppm). The upfield experimental shift changes at C-8, C-4, and C-5 are to be compared to the predicted values of +11.2, +3.5, and +4.3 ppm, respectively, while the theoretical shifts at C-2 and C-6 move downfield −0.5 and −0.2 ppm, respectively. It was assumed that the tautomeric hydrogen averaged equally between N-7 and N-9 and the predicted values are the averages for these two forms.

Attempts were made to calculate the chemical shifts in purine cation by placing the two protons on N-1,7 and N-1,9 as well as on N-3,7 and N-3,9, but the same degree of success was not enjoyed as in the other molecular species studied. Although a gross correlation is obtained, even when the proton is averaged between N-7 and N-9 the theory fails to preserve many of the trends, as there is no way to adequately estimate the effect of tautomeric averaging of many possible species. Thus, no attempt was made to exhaustively treat the purine cationic species.

The data presented in this work demonstrate the sensitivity of carbon-13 magnetic resonance parameters to structural changes and its utility in delineating such problems in complex molecular systems. In addition, the CNDO calculations are successful in predicting many of the protonation trends observed for these species, including the long-range inter-ring effects found experimentally. Such chemical shift variations are due to inductive effects which are operative between rings. For instance, comparison of the π charge densities in purine anion and purine indicates that significant charge polarization occurs at the most remote positions N-1, N-3, C-2, and C-6 as a result of protonation in the five-membered ring. A similar observation can be made at C-5,6 and C-4,7 in the benzimidazole species. The experimental results, which support the

(30) This same procedure was employed in ref 16, and credit for the approach is due to W. Adam.

(31) A decrease in spin pairing associated with a decrease in the bond order reduces the carbon-13 paramagnetic shielding parameter and shifts the resonance position to higher field, while an increase in spin pairing enhances the paramagnetic term. Reference 16 contains a discussion of the effects of charge-density and bond-order terms on the paramagnetic shielding tensor.

theoretical calculations, suggest the presence of interring polarization effects of significant magnitudes.

Admittedly, the theoretical calculations are somewhat crude, but the correlation with experiment nevertheless demonstrates the essential reliability of the MO results. Even though perfect correlation is not possible at all positions in view of the many simplying assumptions required to theoretically assess the rather complex data obtained herein, the results are encouraging and illustrate the usefulness of approximate all-valence-electron methods such as the CNDO approach, and justify those workers interested in obtaining even better computer routines for treating molecules of this general size.

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Carbon-13 Magnetic Resonance. XX.[1] 4-Azaindene (Pyrrocoline) and Related Bridgehead Nitrogen Heterocycles

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Abstract: The carbon-13 chemical shifts for pyrrocoline and a number of related azaindenes have been measured in order to determine the extent that the free pair on the bridgehead nitrogen contributes to the delocalized electronic structures. The data indicate that a nitrogen atom at a bridgehead position produces only relatively minor perturbations as compared with the isoelectronic indenyl anion and that a high degree of aromaticity occurs in this class of compounds. The electronic structure is calculated by means of the CNDO–SCF–MO formalism and is used to rationalize the experimental results.

Although considerable advancement has been made in understanding the electronic structure and concomitant properties of benzenoid and nonbenzenoid aromatic carboxylic systems, a much less clearly defined status exists in the area of aromatic heterocyclic chemistry. This is especially true in nitrogen heterocycles when one departs from simple systems such as pyrrole and pyridine. The heteroaromatic properties of these two ring systems have been studied extensively.[2,3] Extension of these structural elements into fused-ring systems of two or more nitrogens, however, leads to rather widely variable theoretical predictions in several instances, and the available information becomes largely empirical for individual examples.

A particular case of interest, and presently among the least understood in detail, is that class of heterocycles containing the familiar aromatic ring systems fused through a common bridgehead nitrogen. The question which arises is how the charge distribution in one ring system will affect the electronic structure of another heterocyclic ring fused in this manner. Of special concern is the role of the two unshared electrons on the bridgehead nitrogen atom in the electronic distribution and bonding in this molecular system.

From previous studies[4–8] based on chemical properties as well as proton magnetic resonance (pmr) and ultraviolet spectroscopy, it has been concluded that 1,4-diazaindene (imidazo[1,2-*a*]pyridine) is a ten-π-electron aromatic system with considerable delocalization of π electrons and the ability to sustain a ring current in the presence of an external magnetic field. Similar conclusions have been reached from studies of such other ten-π-electron compounds as 4-azaindene (pyrrocoline),[9] 3,4-diazaindene (pyrazolo[2,3-*a*]pyridine),[10] and 1,4,8-triazaindene (imidazo[1,2-*a*]pyrimidine).[11]

Carbon-13 magnetic resonance offers a powerful method for investigating the aromatic properties of hydrocarbons and hydrocarbon derivatives and the effects of ring currents.[12,13] Pugmire, *et al.*,[14,15] as well as several other authors[16–18] have extended the use of carbon-13 chemical shifts to the study of the charge densities and bond orders in the five- and six-membered nitrogen heterocycles[14,17,18] as well as a number of polycyclic heteroaromatic compounds.[1,15,16,18] The carbon-13 chemical shift data allow one to investigate those molecular properties which give rise to magnetic shielding effects, and the technique provides a useful means to assess various theoretical treatments of molecular bonding schemes.

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