^{14}N and ^{2}H Quadrupole Double Resonance in Salts of Cytosine and Adenine

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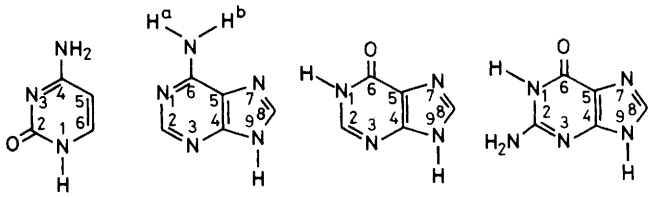
^{14}N and ^{2}H quadrupole resonance frequencies have been measured at room temperature by double resonance techniques in four salts of cytosine and the dihydrobromide of adenine. By means of careful Zeeman measurements, consistent quadrupole coupling constants and asymmetry parameters have been deduced and assigned to specific nuclei in the molecule. The approximate orientation of the ^{14}N electric field gradient tensor has been deduced by a comparison of the results with those for solid imidazole discussed in the previous paper. Protonation of the cytosine molecule is shown to increase the $2p_{\pi}$ conjugation of the exocyclic NH_2 group with the ring, so that much of the positive charge of the cation may reside on the amino group.

In previous papers [1,2] we have discussed some of the difficulties associated with the assignment of ^{14}N quadrupole double resonance signals to NH_2, NH, and N groups in a number of purines and substituted imidazoles and their salts. Similar problems arise in the nucleotide bases and their salts, which in the case of guanine have been a source of confusion in the literature.[3,4] In this paper, we report a study of four hydrohalide salts of cytosine and one of adenine in which a combination of ^{14}N and ^{2}H quadrupole double resonance spectra has produced reliable assignments of the observed lines. In the case of cytosine hydrobromide, the work has led to a revision of our previously published assignment.[2] A new assignment of the ^{14}N quadrupole resonance frequencies in guanine at room temperature is also reported, which correlates better with the results from other nucleotide bases.



(I) (II) (III) (IV)

Experimental

Cytosine (Sigma chemicals pur.), adenine (Koch-Light pur.), guanine (Sigma chemicals pur.), and hypoxanthine (B.D.H. biochemicals) were used without further purification. The cytosine salts were prepared by recrystallization from 4M-solutions of the AnalaR acid in pure or partially deuteriated water, as we have previously described.[2] In the case of the hydroiodide, simultaneous crystallization of the tri-iodide also occurred, but the difficulty was overcome by the use of ethanol–water solutions, the pure tri-iodide being obtained from more concentrated aqueous solutions of HI. With the exception of the latter, all the cytosine salts appear to exist in two distinct crystalline forms at room temperature, the α-form being favoured by rapid recrystallization from the hot solution and the β by crystallization from a 50 : 50 H_2O–D_2O mixture. Under normal crystallization conditions, a mixture is usually obtained. The β-form of cytosine hydrochloride was prepared on only one occasion; analytical data are not available and the sample may have been hydrated. Adenine dihydrobromide and guanine monohydrochloride and dihydrobromide were recrystallized from 4—6M-aqueous solutions of the AnalaR acid. The composition of these compounds (apart from β-cytosine hydrochloride) was checked by C, N, H, and halogen analysis.

The experimental techniques used in the quadrupole double resonance experiments at room temperature have been described in previous papers.[1,2] Zeeman studies in fields ranging from 0.1 to 3 mT were extensively used to assist in the ^{14}N and ^{2}H assignments, particularly in the detection of low-frequency ν_z signals, as we have discussed in the preceding paper.

^{79}Br Quadrupole resonance signals were detected in the hydrobromide of cytosine (α-form) and the dihydrobromides of adenine and quanine on a Decca super-regenerative oscillator spectrometer. In both α- and β-cytosine hydrochlorides signals tentatively assigned to ^{35}Cl were detected by double resonance methods with 80 V across the Q coil, although in no case were the corresponding ^{37}Cl signals seen. The ^{35}Cl signals broadened considerably, even in a weak Zeeman field, irrespective of its relative orientation with aspect to the r.f. field, behaviour very different from that of ^{14}N spectra.

Results

The room temperature ^{14}N results for the nucleotide bases cytosine (I), adenine (II), hypoxanthine (III), and guanine (IV) are discussed first. For cytosine, assignments at 77[3] and 291 K[4] have already been published.

Those for adenine at 291 K are given in Table 1; assignments of the spectrum at 77 K have already been reported.[3–5] At 291 K only the ν_x signals from N(1) and N(3) in the six-membered ring were observed, although both ν_x and ν_z were observed for N(7) in the five-membered ring. Similar problems were encountered in hypoxanthine and guanine (Table 2); in the case of guanine, previous studies at room temperature [4] are inconsistent with our present results. The guanine signals are weak because of the unexpectedly short proton T_1 values,[6] but signals assignable to all the nitrogen atoms can be detected (Figure 1), and the quadrupole coupling constants and asymmetry parameters deduced therefrom (Table 2) are in reasonable agreement with those for hypoxanthine, which are in turn consistent with results obtained at 77 K.[3] Some of the discrepancies could be explained if the previous ^{14}N signals had been obtained in a weak magnetic field of about 2.4 mT, although there are still serious differences between the spectra in the frequency range from 1 to 2 MHz which we are unable to explain. It is of interest to note that with the exception of $^{14}N(9)$–H in adenine, all the other ^{14}N

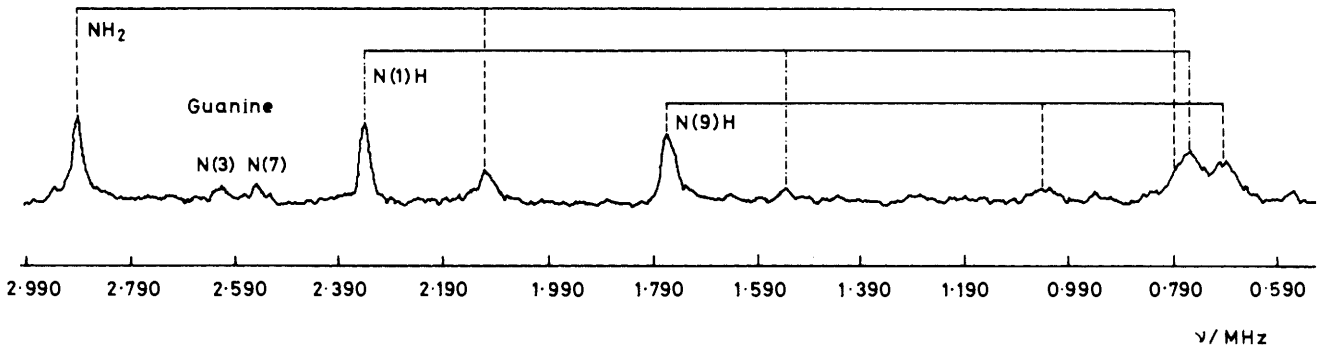


Figure 1. Part of the ^{14}N quadrupole double resonance spectrum of guanine at 291 K

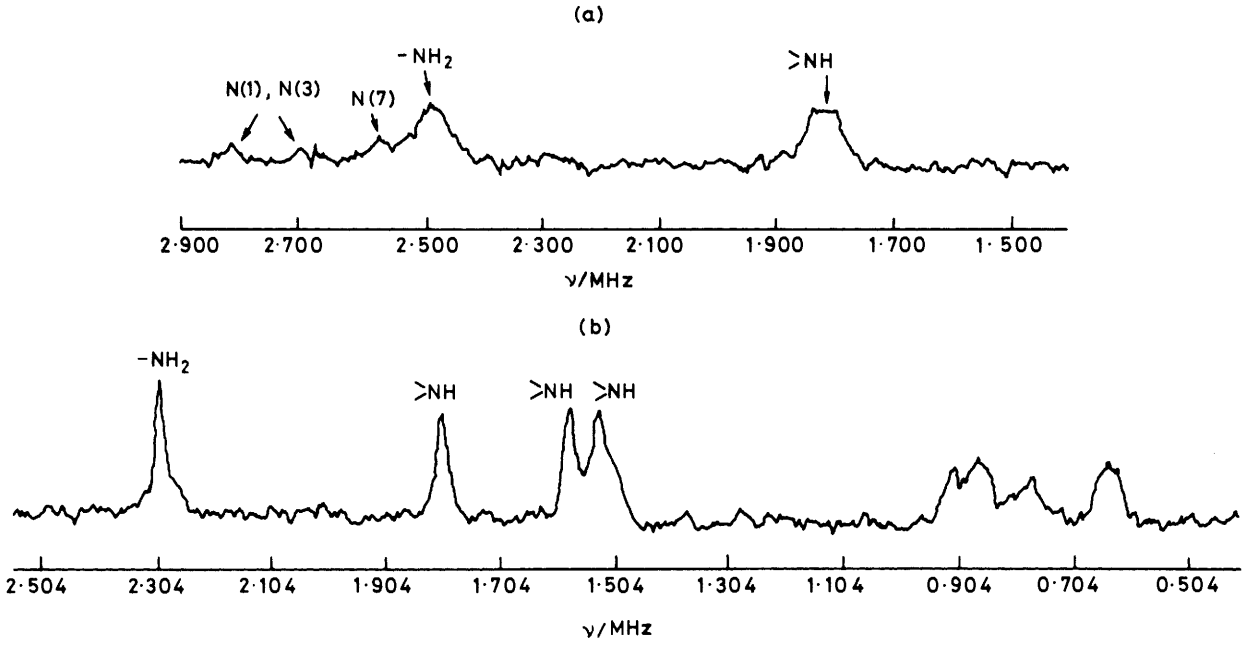


Figure 2. Part of the ^{14}N quadrupole double resonance spectra of (a) adenine and (b) its dihydrobromide at 291 K: τ_P 5 s, τ_Q 0.5 s, 2 kHz step, 5 kHz frequency modulation width, V_Q 80 V peak-to-peak

Table 1. ^{14}N Frequencies, quadrupole coupling constants, and asymmetry parameters [a] in adenine and its dihydrobromide at 291 K

		N(1)	N(3)	NH_2	N(7)	N(9)
Adenine (A)	ν_x, ν_y, ν_z/MHz	2.816, —, —	2.708, —, —	2.476, 1.816 (0.660)	2.565, —, 0.321	1.791, —, 0.647
	e^2qQ/h/MHz, η			2.861, 0.461	3.206, 0.200	1.957, 0.661
Adenine·2HBr	ν_x, ν_y, ν_z/MHz	1.825, 0.939, 0.889		2.318, 1.522, 0.797	1.554, 0.889, 0.664	1.603, 0.939, 0.664
(AB)	e^2qQ/h/MHz, η	1.841, 0.966		2.559, 0.623	1.629, 0.815	1.695, 0.784

[a] Errors of ±0.003 MHz in e^2qQ/h and ±0.005 in η.

quadrupole coupling constants increase on going from 77 to 291 K, although the changes are small.

Turning now to the cytosine salts, we have been able by the use of careful Zeeman studies to detect ν_x, ν_y, and ν_z signals from all three ^{14}N nuclei in all but two of the compounds prepared. The ^{14}N frequencies and quadrupole coupling constants and asymmetry parameters derived therefrom are listed in Tables 3, 4, and 5, together with previously reported values for pure and partially deuteriated cytosine.[4] The values determined for α-cytosine hydrochloride are in reasonable agreement with recently published values [7] of N(3)H 2.514 MHz (0.771), N(1)H 2.414 MHz (0.506), and NH_2 2.559 MHz (0.508) determined at 77 K. In adenine dihydrobromide (Table 1), signals from N(1), N(7), N(9), and the NH_2 group can be assigned on the basis that protonation has occurred at N(1) and N(7), which is consistent with the *X*-ray crystal structure analysis of the dihydrochloride; [8] the three well resolved NH ν_x signals between 1.5 and 1.9 MHz (Figure 2) show clearly that diprotonation has occurred. Unfortunately, all observed signals from both the monohydrochloride and dihydrobromide of guanine were too weak to be assigned satisfactorily, owing largely to the short proton T_1 value. In the case of ^2H, ν_z signals were not observed. Nevertheless, by careful use of Zeeman shifts, we were able to distinguish between ν_x and ν_y lines; [1] Figure 3 shows spectra for α-cytosine hydrobromide (100% ^2H) in which lines 1,2,5,6

Table 2. ^{14}N Frequencies, quadrupole coupling constants, and asymmetry parameters [a] in hypoxanthine and guanine at 291 K

		>N(1)H	=N(3)–	NH_2	=N(7)–	>N(9)H
Hypoxanthine (H)	ν_x, ν_y, ν_z/MHz	1.628, 0.852, 0.779	2.858, —, —		2.587, —, 0.372	1.752, 1.067, 0.681
	e^2qQ/h/MHz, η	1.651, 0.944			3.201, 0.232	1.879, 0.729
Guanine (G)	ν_x,ν_y,ν_z/MHz	2.368, 1.566, 0.794	2.652, —, —	2.920, 2.138 (0.782)	2.578, —, 0.258	1.790, 1.069, 0.717
	e^2qQ/h/MHz, η	2.628, 0.604		3.372, 0.464	3.265, 0.158	1.909, 0.751

[a] Errors of $\pm$0.003 MHz in e^2qQ/h and $\pm$0.005 in η.

Table 3. ^{14}N Frequencies, quadrupole coupling constants, and asymmetry parameters [a] for the NH_2 group of cytosine and its hydrohalides at 291 K

Compound	ν_x/MHz	ν_y/MHz	ν_z/MHz	$\frac{e^2qQ/h}{\text{MHz}}$	η
Cytosine	2.490	1.941	0.539	2.961	0.364
Cytosine (50% ^{2}H) [b]	2.493	1.942	0.548	2.959	0.370
α-Cytosine·HCl	2.222	1.567	0.653	2.527	0.517
α-Cytosine·HCl (50% ^{2}H) [b]	2.225	1.566	0.656	2.529	0.519
α-Cytosine·HBr	2.202	1.541	0.663	2.494	0.532
α-Cytosine·HBr (50% ^{2}H) [b]	2.206	1.536	0.662	2.500	0.530
α-Cytosine·HI	2.262	1.595	0.673	2.567	0.524
α-Cytosine·HI_3	2.230	1.552	0.690 (10)	2.521 (7)	0.538 (11)
β-Cytosine·HCl (50% ^{2}H) [b]	2.046	1.280	0.760	2.221	0.684
β-Cytosine·HBr (50% ^{2}H) [b]	2.138	1.482	0.650	2.417	0.538
β-Cytosine·HI	2.278	1.590	0.683	2.582	0.529
β-Cytosine·HI (50% ^{2}H) [b]	2.282	1.626		2.605	0.504

[a] Errors of $\pm$0.003 MHz in e^2qQ/h and $\pm$0.005 in η, except for α-cytosine·HI_3. [b] Deuteriation percentages refer to exchangeable hydrogen only.

Table 4. ^{14}N Frequencies, quadrupole coupling constants, and asymmetry parameters [a] for N(1)H in cytosine and its hydrohalides at 291 K

Compound	ν_x/MHz	ν_y/MHz	ν_z/MHz	$\frac{e^2qQ/h}{\text{MHz}}$	η
Cytosine (C)	2.016	1.247	0.762	2.180	0.699
Cytosine (50% ^{2}H) [b]	2.017	1.246	0.764	2.180	0.701
α-Cytosine HCl (1)	2.215	1.458	0.758	2.448	0.619
α-Cytosine·HCl (50% ^{2}H) [b]	2.215	1.462	0.757	2.449	0.618
α-Cytosine·HBr (2)	2.229	1.441	0.784	2.449	0.640
α-Cytosine·HBr (50% ^{2}H) [b]	2.227	1.443	0.773	2.454	0.630
α-Cytosine·HI (3)	2.226	1.578	0.643	2.539	0.506
α-Cytosine·HI_3 (4)	2.230	1.487	0.737	2.482	0.594
β-Cytosine·HCl (50% ^{2}H) [b]	2.284				
β-Cytosine·HBr (50% ^{2}H) [b] (6)	2.296	1.593	0.709	2.589	0.547
β-Cytosine·HI (7)	2.189	1.503	0.683	2.463	0.555
β-Cytosine·HI (50% ^{2}H) [b]	2.189	1.495	0.686	2.461	0.557

[a] Errors of $\pm$0.003 MHz in e^2qQ/h and $\pm$0.005 in η. [b] Deuteriation percentages refer to exchangeable hydrogen only.

Table 5. ^{14}N Frequencies, quadrupole coupling constants, and asymmetry parameters [a] for N(3) in cytosine and its hydrohalides at 291 K

Compound	ν_x/MHz	ν_y/MHz	ν_z/MHz	$\frac{e^2qQ/h}{\text{MHz}}$	η
Cytosine (C)	2.712		1.126	2.865	0.786
Cytosine (50% ^{2}H) [b]	2.711		1.129	2.862	0.789
α-Cytosine·HCl (1′)	2.354	1.402	0.948	2.507	0.756
α-Cytosine·HCl (50% ^{2}H) [b]	2.357	1.414	0.949	2.510	0.756
α-Cytosine·HBr (2′)	2.386	1.517	0.870	2.601	0.669
α-Cytosine·HBr (50% ^{2}H) [b]	2.383	1.506	0.867	2.599	0.667
α-Cytosine·HI (3′)	2.411	(1.690)	0.712	2.740	0.520
α-Cytosine·HI_3 (4′)	2.460	1.682	0.779	2.761	0.564
β-Cytosine·HCl (50% ^{2}H) [b] (5′)	2.414	(1.592)	0.798 (6)	2.687 (4)	0.594 (5)
β-Cytosine·HBr (50% ^{2}H) [b] (6′)	2.467	1.703	0.759	2.783	0.545
β-Cytosine·HI (7′)	2.412	1.658	0.750	2.716	0.552
β-Cytosine·HI (50% ^{2}H) [b]	2.414	1.660	0.754	2.717	0.554

[a] Errors of $\pm$0.003 MHz in e^2qQ/h and $\pm$0.005 in η except for β-cytosine·HCl (50% ^{2}H). [b] Deuteriation percentages refer to exchangeable hydrogen only.

Table 6. ^2H Frequencies, quadrupole coupling constants, and asymmetry parameters [a] in cytosine and its hydrohalides at 291 K

	NH_2				NH			
Compound	ν_x/kHz	ν_y/kHz	e^2qQ/h kHz	η	ν_x/kHz	ν_y/kHz	e^2qQ/h kHz	η
Cytosine	162.2	144.5	204.5	0.173	129.6	111.8	160.9	0.221
	157.3	140.3	198.4	0.171				
α-Cytosine·HCl	166.3	152.3	212.4	0.132	138.8	122.2	174.0	0.191
	156.2	142.0	198.8	0.143	120.5	107.2	151.8	0.175
α-Cytosine·HBr	164.0	149.1	208.7	0.143	137.5	119.7	171.8	0.213
	156.7	142.0	199.1	0.148	130.9	114.6	163.7	0.199
α-Cytosine·HI	167.0	152.0	212.7	0.141	139.1	121.9	174.0	0.198
	158.0	143.2	200.8	0.147	150.3	134.0	189.5	0.172
β-Cytosine·HBr	158.1 [b]	143.4 [b]	201.0 [b]	0.146 [b]	137.5	121.1	172.4	0.191
					141.4	122.9	176.2	0.210
β-Cytosine·HI	164.9	150.9	210.5	0.133	138.2	120.8	172.7	0.202
	159.6	147.9	205.0	0.114	145.0	128.9	182.6	0.176

[a] Errors of ±0.9 kHz in e^2qQ/h and ±0.010 in η. [b] Average values.

Table 7. ^2H Frequencies, quadrupole coupling constants, and asymmetry parameters [a] in hypoxanthine, adenine, and adenine dihydrobromide at 291 K

	NH_2				NH			
Compound	ν_x/kHz	ν_y/kHz	e^2qQ/h kHz	η	ν_x/kHz	ν_y/kHz	e^2qQ/h kHz	η
Hypoxanthine					138.0	118.0	170.7	0.234
					133.1	116.5	166.4	0.200
Adenine	155.6	139.5	196.7	0.164				
Adenine·2HBr	159.0	146.1	203.4	0.127	143.0	128.6	181.1	0.159
	147.7	134.1	187.9	0.145	125.8	114.9	160.5	0.136
					125.8	109.4	156.8	0.209

[a] Errors of ±0.9 kHz in e^2qQ/h and ±0.010 in η.

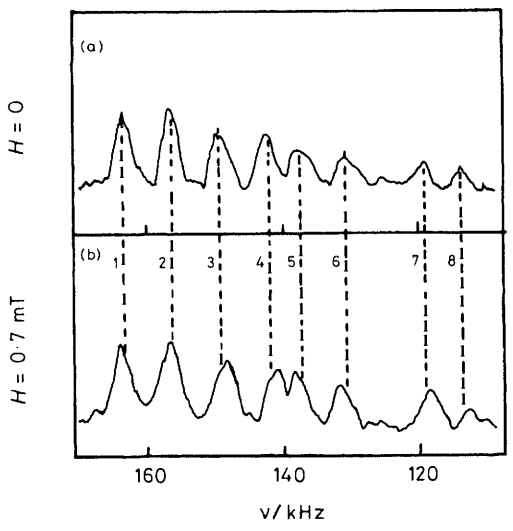


Figure 3. ^2H Quadrupole double resonance spectra of deuteriated α-cytosine hydrobromide at 291 K (a) in zero field, (b) in a magnetic field of 0.7 mT at the Q coil

Table 8. Halogen quadrupole resonance frequencies

Compound	Nucleus	ν_Q/MHz	Temp./K
α-Cytosine·HCl	^{35}Cl	2.702	291
α-Cytosine·HCl (*ca.* 100% ^2H) [a]	^{35}Cl	2.584	291
α-Cytosine·HCl (*ca.* 50% ^2H) [a]	^{35}Cl	2.640	291
α-Cytosine·HBr	^{79}Br	18.717	294
		18.831	276
		19.142	195
		19.468	77
α-Cytosine·HBr (*ca.* 100% ^2H) [a]	^{79}Br	17.959	294
		18.800	77
Adenine·2HBr	^{81}Br	17.298	301
		18.492	
		17.489	276
		18.560	
		18.022	195
		18.650	
		18.881 [b]	77
	^{79}Br	20.741	303
		22.148	
		22.614 [b]	77
Guanine·2HBr	^{81}Br	16.685	298
		17.407	77

[a] Deuteriation percentages refer to exchangeable hydrogen only. [b] Average values.

are clearly ν_x and lines 3,4,7,8 ν_y. Table 6 lists ^2H quadrupole resonance frequencies and coupling constants and asymmetry parameters derived therefrom for cytosine and some of its hydrohalides at 291 K, and Table 7 the same parameters for the observed signals in hypoxanthine, adenine, and adenine dihydrobromide at the same temperature. No ^2H signals were detected in guanine at 291 K, nor in any of its salts.

The ^{35}Cl and $^{79,81}Br$ quadrupole resonance signals observed at various temperatures are reported in Table 8. Efforts to detect ^{127}I quadrupole resonance lines in the α-hydroiodide proved unsuccessful. The partially deuteriated hydrobromides showed a complex pattern of lines on a super-regenerative oscillator spectrometer, owing presumably to deuteriation effects on the hydrogen-bonded Br^-; the patterns were not

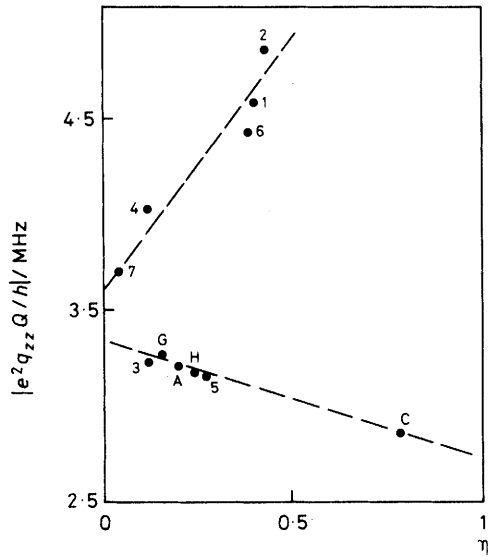


Figure 4. Plot of $|e^2qQ/h|$ against η for the imino (−N=) group in several nucleotide bases with additional points for other heterocyclic systems taken from the literature. C = cytosine, A = adenine, H = hypoxanthine, G = guanine; 1 = pyridine [9,10] (solid), 2 = pyridine [11] (gaseous), 3 = imidazole [1] (solid), 4 = imidazole [12] (gaseous), 5 = benzimidazole [1] (solid), 6 = pyrimidine,[10] 7 = 1-benzylimidazole [13]

interpreted and Br frequencies for the partially deuteriated hydrohalides have therefore been omitted from Table 8.

Discussion

Although the ^{14}N quadrupole interactions in the nucleotide bases have already been the subject of some discussion in the literature [3,4] it is worth comparing the ^{14}N quadrupole parameters at the imino (−N=) sites with those in other heterocyclic systems such as have been discussed in the previous paper.[1]

Figure 4 does this by a plot of $|e^2qQ/h|(^{14}N)$ against η. Despite the differences in ring size, the two quadrupole parameters show a reasonable and almost linear correlation, which may be understood if it is assumed that the electric field gradient (e.f.g.) axes at the N atom remain fixed in the molecular framework, the individual components changing in accordance with variation in the $2p$ electron density.[13] The planar or near-planar symmetry of most of these molecules requires one component to lie perpendicular to this plane, which in both gaseous pyridine [11] and imidazole [12] is q_{yy} (where $|q_{zz}| \geqslant |q_{yy}| \geqslant |q_{xx}|$, with $q_{zz} = q$); q_{zz} is inferred to lie nearly parallel to the $2p_\sigma$ orbital with e^2qQ/h negative as expected theoretically.[14,15] The change in slope at the $\eta = 0$ axis near 3.50 MHz may then be interpreted as an interchange in the x and y axes, such as is assumed to occur in imidazole on going from the gaseous (point 4) to the solid (point 3) state. If this interpretation is correct, then the orientation of the ^{14}N e.f.g. axes at those −N= sites which give detectable signals in cytosine, adenine, guanine, and hypothanthine is as shown in Figure 5, just as in solid imidazole and benzimidazole, presumably a consequence of weak hydrogen bonding in all these compounds (which may be the reason why the −N= signals observed in the nucleotide bases at room temperature are detected at all). The assumption of a fixed e.f.g. axial orientation, with q_{zz} bisecting the CNC angle, is certainly an approximation; a deviation of 4° has been noted in gaseous imidazole.[12] The failure of the plot in Figure 4 to reveal any marked dependence on ring size is also found in the analysis of the results for the amino group to be discussed next.

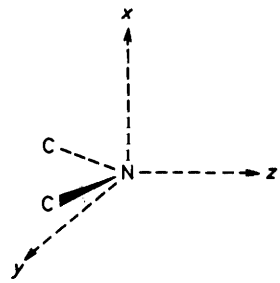


Figure 5. Proposed orientation of the principal components of the ^{14}N electric field gradient tensor at the imino site (−N=) in solid cytosine, adenine [N(7)], guanine [N(7)], and hypoxanthine [N(7)]

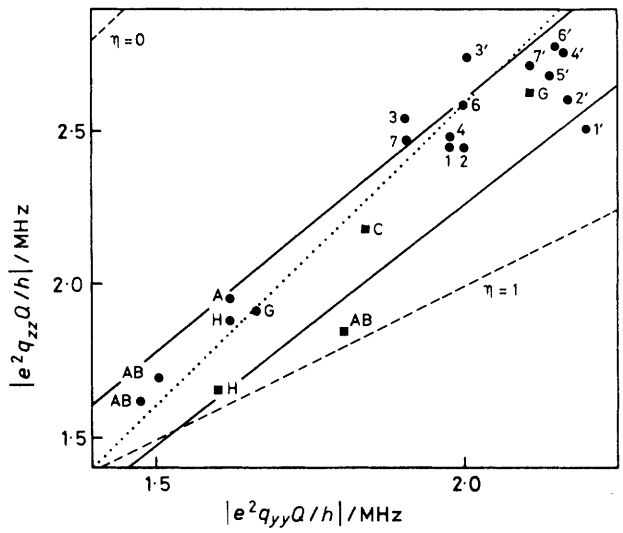


Figure 6. Plot of $|e^2q_{zz}Q/h|$ against $|e^2q_{yy}Q/h|$ for the NH groups in several nucleotide bases and their salts. The labelling of the former is the same as in Figure 4 and in the latter is taken from Tables 1, 4, and 5. The closed circles refer to NH groups in five-membered rings and the closed squares to those in six-membered rings. The dotted line has a slope of 2

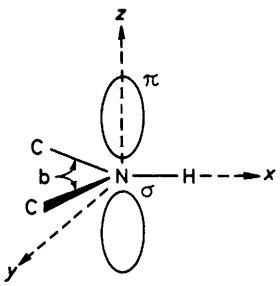


Figure 7. Proposed orientation of the principal components of the ^{14}N electric field gradient tensor at the amino sites (>NH) in cytosine and the other nucleotide bases and their salts

As in our previous analysis of the N(1) quadrupole parameters in substituted imidazoles,[1] we have plotted in Figure 6 $|e^2q_{zz}Q/h|$ against $|e^2q_{yy}Q/h|$ for the NH groups in both five- and six-membered rings. The results lie close to an almost linear band shown by the continuous lines in which also fall the same parameters for a number of *C*-substituted imidazoles.

Presumably therefore the NH e.f.g. tensor has the same sign and a similar orientation in both cases, *viz.* (e^2qQ/h) is negative and q_{zz} lies perpendicular to the molecular plane, with q_{xx} roughly parallel to N–H [15] (Figure 7), which implies an interchange of the x and y axes relative to their position in gaseous imidazole [12] and pyrrole.[16] The variation of the points within this band has been ascribed to shifts of charge within imidazolinic NH groups in a rather similar N–H · · · N hydrogen-bonding environment.[1] The nucleotide bases, however, show a considerably wider range of hydrogen-bonding interactions with each other; [17] nevertheless, within the six-membered rings, N(1) in cytosine [18] and N(1) in guanine [19] both form N–H · · · N hydrogen bonds, which is consistent with their position in Figure 6. As regards the nature of the electronic charge shifts, the Townes–Dailey equations for the NH group in the orientation of Figure 7 are [1] as in equations (1—3) with $\cot^2\theta = ca.\ 2/7$ ($2\theta = \angle$CNC). Many of the

$$q_{xx} = q_0[\sigma(1 - \cot^2\theta) - \pi/2 + b(\cot^2\theta - \tfrac{1}{2})] \quad (1)$$

$$q_{yy} = q_0[-(\sigma/2)(1 - \cot^2\theta) - \pi/2 + (b/2)(2 - \cot^2\theta)] \quad (2)$$

$$q_{zz} = q_0[-(\sigma/2)(1 - \cot^2\theta) + \pi - (b/2)(1 + \cot^2\theta)] \quad (3)$$

points in Figure 6 lie close to a line of slope −2 (assuming $e^2q_{zz}Q/h$ to be negative), which is consistent with changes in the $2p_\pi$ orbital population at the N atom (π) being the dominant factor, the ratio of the coefficients for this term in q_{zz} with respect to q_{yy} being precisely this value. Changes in the N–H (σ) and N–C (b) populations are also likely [9] but if the slope were exactly −2 they would have to be equal in magnitude. The lack of sensitivity to small changes in θ is explained by equations (1—3) because a ratio $q_{zz}/q_{yy} = -2$ imposes no conditions on the value of θ.

We next discuss the changes that occur on protonation. For cytosine, both hydrochloride and hydrobromide display one halogen quadrupole resonance frequency (Table 8) with a $^{79}Br/^{35}Cl$ frequency ratio of 6.93 in the undeuteriated samples and 6.95 in the deuteriated ones, close to the ratio (7.04) of the atomic quadrupole coupling constants. These results strongly support the contention that the two salts are isostructural, with one molecule of salt in the asymmetric unit. The crystal structure of one form of cytosine hydrochloride has been published [20] and is consistent with the latter conclusion; we show later that it is probably what we have called the α-form. It may however be concluded that in both α- and β-forms of the hydrochloride and hydrobromide, only one set of ^{14}N quadrupole resonance frequencies should be observed. The results presented in Tables 3—6 have been interpreted on this assumption, which has been made for all the other cytosine salts studied.

Table 3 shows clearly that one obvious effect of protonation is a decrease in magnitude of the $^{14}NH_2$ quadrupole coupling constant and an increase in the asymmetry parameter, the most likely explanation of which is an increase in conjugation of the N $2p_\pi$ orbital with the π-system of the ring. In agreement with this, the *X*-ray structure analyses of a number of cytosine salts [17,20–22] reveal shorter C–NH_2 bonds then in cytosine.[18] A further consequence is the existence of restricted rotation about the C–NH_2 bond, as has already been observed in solution,[23] and predicted in theoretical calculations of the energy barrier.[24] In the solid salts, Table 6 clearly shows that the two C–NH_2 deuterons are not equivalent; except in β-cytosine hydrobromide, two sets of ^2H quadrupole coupling constants and asymmetry parameters are clearly resolved and in some of the spectra the multiplet structure expected from dipolar coupling between the two ND_2 deuterons [25] was also observed.

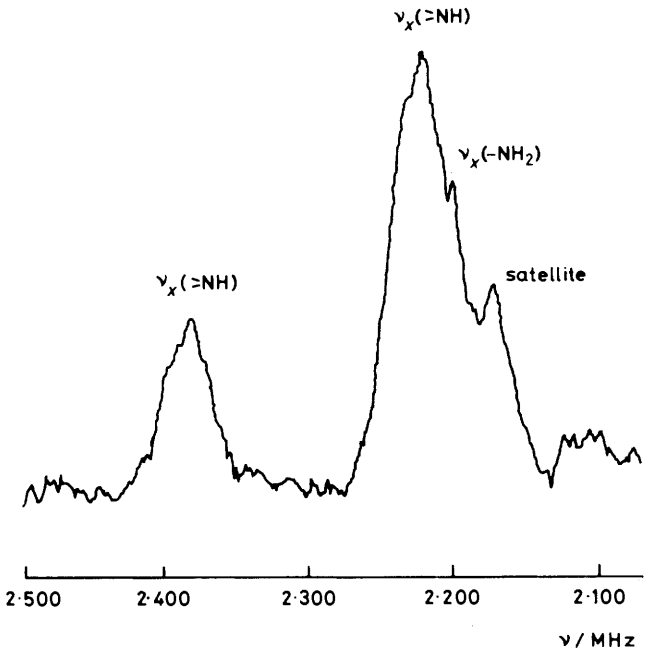


Figure 8. ν_x Lines of α-cytosine hydrobromide at 291 K with a mu-metal can surrounding the Q-coil. τ_P 7 s, τ_Q 1 s, 1 kHz step, V_Q 80 V peak-to-peak

The relationship of C–NH_2 double-bond character to the ^{14}N quadrupole parameters has been discussed by Schempp and Chao.[9] The details of the analysis depend on the degree of planarity of the group and the bond angles; for sp^2-type orbitals and $\angle$HNH = 120°, q_{zz} lies along or close to the lobes of the $2p_\pi$ orbital, e^2qQ/h is negative, and its product with $(1 - \eta/3)$ is proportional to the difference between the $2p_\pi$ populations and those of the N–H orbitals. The product $(e^2qQ/h)\eta$ should remain constant if the σ populations and bond angles remain unchanged, which is reasonably consistent with the results in Table 3 (at least within the serious approximations of this model). The changes in e^2qQ/h and η are therefore dominated by the $2p_\pi$ populations, which are required to decrease on going from cytosine to its cation, a transfer which is possibly enhanced by the proximity of the negatively charged halide ion to which the NH_2 group is hydrogen bonded. Thus it is of interest to note that similar changes in the quadrupole parameters do not occur in the nucleotide base pair 1-methylcytosine hemihydroiodide hemihydrate,[7] in which one hydrogen atom of the NH_2 group hydrogen bonds to oxygen, as distinct from cytosine in which both bond to oxygen,[26] and from the cytosine salts discussed here in which the same hydrogen atoms hydrogen bond to the halide ion.

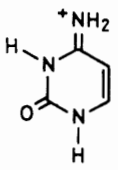
We next discuss the ring ^{14}NH quadrupole parameters, of which two are measured, N(1)H and N(3)H, the latter being the protonation site. In our original assignments,[2] the $^{14}NH_2$ and ^{14}N(3)H frequencies were interchanged, largely on the basis of poorly resolved triplet structure in the 2.4 MHz line in α-cytosine hydrobromide, which was attributed to the ' solid effect '.[5] More careful measurements with a mu-metal shield surrounding the sample just resolved the broad band near 2.2 MHz into three lines (Figure (8), two of which were assigned to ν_x ($^{14}NH_2$) and its low-frequency ' solid-effect ' satellite, the high-frequency one being concealed by the strong ν_x (^{14}NH) line. With these new assignments, a better correlation of the ^{14}N and ^2H quadrupole parameters with the expected hydrogen-bonding scheme in the α-form is obtained.

To justify this conclusion, we note that of the two NH ^{2}H quadrupole coupling constants in Table 6, the lower value varies by not more than 3 kHz from salt to salt whereas the higher value varies over a range of 38 kHz, in the order $I^- > Br^- > Cl^-$. Now in the crystal structure of the hydrochloride,[20] N(1)H forms a ring hydrogen-bonded system with the C=O oxygen atom of a neighbouring cytosine cation whereas the protonated N(3)H hydrogen bonds to Cl^-. If the same structure holds for the bromide (and iodide), then we expect to see one roughly constant set of ^{2}H quadrupole coupling constants, assigned to N(1)H, and another highly variable in the order $I^- > Br^- > Cl^-$,[17] assigned to N(3)H. Values of NH ^{2}H quadrupole coupling constants published in the literature have been correlated with the H···X hydrogen-bonding distance; [26,27] from these relationships for N–H···O and N–H···Cl^- hydrogen bonds and H···O and H···Cl^- distances taken from the *X*-ray crystal structure analysis of the hydrochloride, we would deduce values of 156 kHz at N(3)H, 199 and 214 kHz at NH_2, and 175 kHz at N(1)H, in remarkably close agreement with the values observed at 291 K in the so-called α-form of 152, 199, 212, and 174 kHz respectively; this evidence supports the contention that the *X*-ray structure analysis relates to what we have called the α-form.

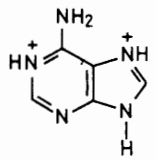
The values of q_{zz} and q_{yy} for all the NH groups within both five- and six-membered rings of the protonated species reported here fall within or close to the results for the neutral bases in Figure 6, so we may assume that with the possible exception of N(1)H in adenine dihydrobromide the electric field gradient tensor has the orientation shown in Figure 7. In the cytosine cation, both NH sites have higher $|e^2qQ/h|$ values relative to the single NH site in the base, which according to our interpretation of Figure 6 implies higher $2p_\pi$ populations on the N atom, and/or lower values of σ and *b*. The $2p_\pi$ electrons presumably come from the exocyclic NH_2 group, whose quadrupole coupling constant falls and asymmetry parameter rises owing to loss of $2p_\pi$ electrons, as we have previously pointed out, whereas the reverse occurs for N(1)H, implying a considerable contribution from structure (V): hence the shortening of both the C–O and C(4)–N(4) lengths in the cation.[20]

The $2p_\pi$ charge is predicted to be higher at N(3)H than N(1)H, and/or both σ and *b* lower, presumably because it is closer to the carbon atom carrying the NH_2 group. These are, however, rather tentative conclusions in view of the different hydrogen bonding to the two groups; the electronic effects are quite distinct, as can be seen from Figure 6 for N(3) in the supposedly isostructural series of the α-hydrochloride (1′), hydrobromide (2′), and hydroiodide (3′). Here $|q_{zz}|$ falls and $|q_{yy}|$ rises as the strength of hydrogen bonding increases, according to the value of the ^{2}H quadrupole coupling constant [28] in Table 6. In fact, the changes in q_{zz} (^{14}N) and q_{zz} (^{2}H) are almost linearly proportional to each other. The relative sign of the changes in the ^{14}N(3)H components, with $|q_{zz}|$ falling and $|q_{yy}|$ rising, suggests from equations (1—3) that variations in σ are responsible, the N–H populations increasing along the series $I^- < Br^- < Cl^-$. This behaviour is in marked contrast to the effects of hydrogen bonding on the N(1) quadrupole parameters of the imidazole ring,[1] which indicate that both σ- and π-charge density within the ring is affected.

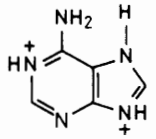
In conclusion, we analyse briefly the results for adenine dihydrobromide. Two Br quadrupole resonance frequencies are observed, overlapping to give one broad resonance at 77 K (Table 8); we therefore assume that the asymmetric unit is constituted of one molecule of the salt, so that only one set of ^{14}N frequencies for each N atom should be observed. This conclusion has been used in making the assignments



(V)



(VI)



(VII)

given in Table 1. One obvious feature of the results is the near equivalence of the N(7)H and N(9)H ^{14}N quadrupole parameters, implying nearly equal contributions from structures (VI) and (VII); Table 7 shows that two of the three NH ^{2}H quadrupole coupling constants have significantly lower values than the third and must be involved in stronger hydrogen bonding. One of the NH_2 protons is also involved in a significantly stronger hydrogen bond than the other. Now no structure for the dihydrobromide has yet been published, but that of the dihydrochloride is known [8] and shows features consistent with these observations. One of the NH_2 hydrogens [H_b in (II)] has a much shorter hydrogen-bond approach to Cl^- (2.18 Å) than the other (2.89, 2.34 Å), and N(1)H has a shorter approach to Cl^- (2.13 Å) than N(7) H (2.38 Å) which in turn has a shorter approach than N(9) (2.59, 2.70 Å).

As in cytosine and its salts, the NH_2 ^{14}N quadrupole coupling constant drops significantly on going from adenine to its dihydrobromide and η rises, changes which doubtless reflect the increase in C–NH_2 double bond character, and the short C–N bond length of 1.307 Å.[8] In the five-membered ring, protonation causes a drop in the ^{14}NH quadrupole coupling constant, just as in imidazole,[1] but both values in the adenine dication remain close to the other points in Figure 6 so presumably the orientation of the e.f.g. tensor is still that of Figure 7 and the changes in q_{zz} are due to a drop in $2p_\pi$ populations at the nitrogen atom. In contrast, the orientation of q_{zz} for N(1) in the six-membered ring is more uncertain, because of its high value of η (0.966); its position in Figure 6 close to the $\eta = 1$ line suggests that hydrogen bonding has increased σ, the N–H bond population, as occurs in the cytosine salts.

Acknowledgements

We thank the British Council for a research grant to one of us (M. L. S. G.), and Queen Elizabeth College, the S.E.R.C., and the University of London Central Research Fund for grants towards the purchase of equipment.

References

1 M. L. S. Garcia, J. A. S. Smith, P. M. G. Bavin, and C. R. Ganellin, preceding paper.
2 H. Budak, M. L. S. Garcia, I. C. Ewart, I. J. F. Poplett, and J. A. S. Smith, *J. Magn. Reson.*, 1979, **35**, 309.
3 D. T. Edmonds and P. A. Speight, *J. Magn. Reson.*, 1972, **6**, 265.
4 R. Blinc, M. Mali, R. Osredkar, A. Prelesnik, J. Seliger, and I. Zupančic, *J. Chem. Phys.*, 1972, **57**, 5087.
5 D. T. Edmonds, *Phys. Rep., Sect. C*, 1977, **29**, 233.

6 M. J. R. Hoch and E. R. Andrew, *Chem. Phys. Lett.*, 1977, **48**, 377.
7 Y. Hiyama, L. G. Butler, W. A. Olsen, and T. L. Brown, *J. Magn. Reson.*, 1981, **44**, 483.
8 T. J. Kistenmacher and T. Shigematsu, *Acta Crystallogr., Sect. B*, 1974, **30**, 66.
9 E. Schempp and M. Chao, *J. Magn. Reson.*, 1977, **27**, 303, and references therein.
10 E. Schempp and P. J. Bray, *J. Chem. Phys.*, 1967, **46**, 1186.
11 G. O. Sørenson, *J. Mol. Spectrosc.*, 1967, **22**, 325.
12 G. L. Blackman, R. D. Brown, F. R. Burden, and I. R. Elsum, *J. Mol. Spectrosc.*, 1976, **60**, 63; D. Christen, J. H. Griffiths, and J. Sheridan, *Z. Naturforsch., Teil A*, 1982, **37**, 1378.
13 C. I. H. Ashby, C. P. Cheng, and T. L. Brown, *J. Am. Chem. Soc.*, 1978, **100**, 6057.
14 M. H. Palmer, I. Simpson, and R. H. Findlay, *Z. Naturforsch., Teil A*, 1981, **36**, 34.
15 M. H. Palmer, F. Scott, and J. A. S. Smith, *Chem. Phys.*, 1983, **74**, 9.
16 L. Nygaard, J. T. Nielsen, J. Kirchheimer, G. Maltesen, J. Rastrup-Andersen, and G. O. Sørensen, *J. Mol. Struct.*, 1969, **3**, 491.
17 D. Voet and A. Rich, *Prog. Nucl. Acid Res. Mol. Biol.*, 1970, **10**, 183.
18 D. L. Barker and R. E. Marsh, *Acta Crystallogr.*, 1964, **17**, 158; R. J. McLure and B. M. Craven, *Acta Crystallogr., Sect. B*, 1973, **29**, 1234.
19 C. E. Bugg, U. T. Thewalt, and R. E. Marsh, *Biochem. Biophys. Res. Commun.*, 1968, **33**, 436.
20 N. S. Mandel, *Acta Crystallogr., Sect. B*, 1977, **33**, 1079.
21 J. S. Kwiatkowski and B. Pullman, *Adv. Heterocycl. Chem.*, 1975, **18**, 199.
22 M. Rossi, L. G. Marzilli, and T. J. Kistenmacher, *Acta Crystallogr., Sect. B*, 1978, **34**, 2030.
23 A. R. Katritzky and A. J. Waring, *J. Chem. Soc.*, 1963, 3046.
24 K. G. Rao, *Curr. Sci.*, 1975, **44**, 224.
25 D. T. Edmonds, M. J. Hunt, and A. L. Mackay, *J. Magn. Reson.*, 1975, **20**, 505.
26 M. J. Hunt and A. L. Mackay, *J. Magn. Reson.*, 1976, **22**, 295.
27 M. J. Hunt and A. L. Mackay, *J. Magn. Reson.*, 1974, **15**, 402.
28 N. S. Goldstein and J. L. Ragle, *J. Chem. Phys.*, 1979, **70**, 5072.

Received 14*th July* 1982; *Paper* 2/1197