8-azaadenosine[7-amino-3-(β-D-ribofuranosyl)-1,2,3-triazolo[4,5-d]pyrimidine] to be incorrect. An off-resonance experiment showed the correct sequence to be: C2 (157.1$_6$ ppm), C6 (156.5$_1$ ppm), C4 (149.1$_0$ ppm), C5 (124.4$_7$ ppm). The purine numbering system is used for the purpose of comparison with ref 19a.

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Carbon-13 Magnetic Resonance. XXVI.[1] A Quantitative Determination of the Tautomeric Populations of Certain Purines

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Abstract: The α and β carbon-13 chemical shift substituent parameters obtained for purines have been employed to investigate the tautomeric populations in this ring system. This procedure allows a quantitative determination of the predominant tautomeric forms of purine (I), adenine (II), hypoxanthine (III), 6-mercaptopurine (VI), and certain related purines. The study encompasses prototropic tautomerism in the imidazole moiety as well as lactam–lactim or thione–thiol tautomerism in the pyrimidine portion of the purine ring.

I. Introduction

The biological importance of purine tautomerism has stimulated a significant amount of research toward a better understanding of this phenomenon. A wide variety of experimental[3,5] and theoretical techniques[4,5] has been employed on purines in an effort to ascertain the relative populations of the various contributing structures. The majority of this work, however, has been qualitative, either eliminating "rare" tautomeric forms or determining the most stable structure of certain predominant pairs of tautomers. This prompted us to examine this problem by ^{13}C NMR spectroscopy and determine, quantitatively, the populations of the predominant tautomeric forms in solution.

Using this technique, it is possible to determine the population of various tautomeric species from an analysis of the chemical-shift data. At ambient temperature, the rate of tautomeric proton exchange usually exceeds the NMR time scale and, hence, one observes only a single resonance for each carbon with a chemical shift that is a weighted average of the contributing structures. In order to approximate the carbon chemical shifts of the various tautomeric forms, certain model compounds were examined[1] in which the labile hydrogen was replaced by a nonexchanging substituent such as a methyl group or a β-D-ribofuranosyl moiety at the N7 or N9 positions of purines. The effects on the purine carbon-13 chemical shifts, especially for the bridgehead carbons, associated with this type of substitution were clearly delineated, and sets of α and β parameters for these purines were established.[1]

In this study, these substituent parameters are now used to determine the tautomeric populations of purine (I), adenine (II), hypoxanthine (III), 6-mercaptopurine (VI), and certain analogs related to the latter two ring systems. This investigation includes a study of prototropic tautomerism of the imidazole moiety as well as lactam–lactim and thione–thiol tautomerism of the pyrimidine portion of the purine ring.

II. Experimental Section

A. Instrumentation. Carbon-13 spectra were obtained with a Varian XL-100-15 equipped with a Varian 620f computer for Fourier transform operation. Proton spectra were determined using a Varian A-56/60 or XL-100-12 spectrometer. Compounds were dissolved in dry, spectroquality dimethyl sulfoxide (Me_2SO) and concentrations for the purines studied are given in Tables I and II. All carbon-13 chemical shifts (in parts per million) were calculated relative to the internal reference (Me_2SO) and corrected to the Me_4Si scale using the temperature-dependent eq 1,[6]

$$\delta_{Me_4Si} = (\delta_{Me_2SO} + 40.2_2 \text{ ppm}) + 7.4 \times 10^{-3}T \qquad (1)$$

where T is the temperature in degrees centigrade.

B. Sample Preparation. Purine (I), adenine (II), hypoxanthine (III), 6-methoxypurine (V), and 6-mercaptopurine (VI) were obtained from commercial sources. 1-Methylhypoxanthine (IV),[7a] 1-methyl-6-mercaptopurine (VII),[7a] and 6-methylthiopurine (VIII)[7b] were synthesized by published procedures. 8-Deuterio-1-methyl-6-mercaptopurine and 8-deuterio-6-methoxypurine were prepared according to the general procedure described in ref 8c. All samples were checked for purity (TLC, uv, ^1H NMR) and dried prior to dissolving in Me_2SO.

III. Results

Carbon-13 spectra were obtained using noise decoupling and off-resonance conditions. Selective proton decoupling

Table I. Pertinent Proton Chemical Shifts[a] in Certain Purine Derivatives

Compd	Molarity	H2	H6	H8
I	2.1	8.85	9.05	8.54
V	0.32	8.49		8.35
VI	0.59	8.36		8.18
VII	0.90	8.66		8.33
VIII	0.36	8.68		8.40

[a] Chemical shifts are in parts per million with respect to Me_4Si. The temperature was ca. 37° and the solvent Me_2SO.

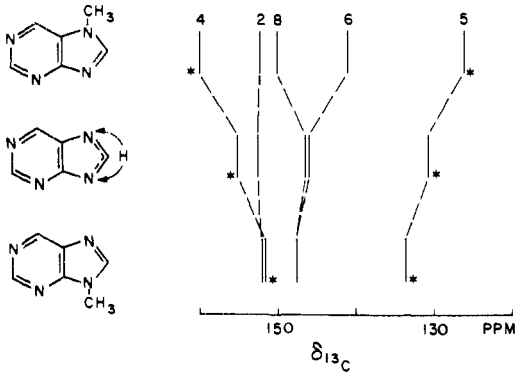

Figure 1. Correlation diagram for the ^{13}C chemical shifts (with respect to Me_4Si) of 7-methylpurine, purine, and 9-methylpurine. Asterisks refer to quaternary carbon lines.

experiments were conducted on certain compounds to facilitate the carbon assignments whenever ambiguity existed between two or more carbon lines.

A. Proton Chemical Shifts. Proton chemical shifts for certain purine derivatives are presented in Table I. Assignments for the H2, H6, and H8 signals of purine (I) were previously determined.[8] The downfield singlet observed in the spectra of 6-mercaptopurine (VI) and 6-methylthiopurine (VIII) was assigned to H2 on the basis of previous assignments.[9,10] The 8-deuterio derivatives of 6-methoxypurine (V) and 1-methyl-6-mercaptopurine (VII) were synthesized to provide an unequivocal means of distinguishing between the H2 and H8 signals in V and VII.

B. Carbon-13 Chemical Shifts. The ^{13}C chemical shifts for purine (I), adenine (II), hypoxanthine (III), 1-methylhypoxanthine (IV), 6-methoxypurine (V), 6-mercaptopurine (VI), 1-methyl-6-mercaptopurine (VII), and 6-methylthiopurine (VIII) are summarized in Table II. ^{13}C assignments of all other purines (heterocycles and nucleosides) used in the present investigation can be found in Table IV of the preceding paper in this issue.[1] The signals obtained for purine (I), dissolved in Me_2SO, were assigned by off-resonance conditions and selective proton decoupling (Figure 1).

Comparison of the off-resonance spectrum of adenine (II) with the spectra of 7-(β-D-ribofuranosyl)adenine[1] and adenosine[1] (Figure 2) provided an unequivocal assignment of the C2, C5, and C8 lines. The assignment of the C4 and C6 resonances was accomplished by comparing their relative peak intensities as the C4 signal is expected to be smaller than the C6 signal. The reduction of the C4 intensity is caused by a saturation effect due to the longer T_1 relaxation times associated with bridgehead carbons.[11] Therefore, the smaller of the two downfield singlets, which is at 151.3 ppm, was assigned to C4. Our data now confirm an earlier tentative assignment of adenine (II) reported from this laboratory.[12]

The carbon signals in the off-resonance spectrum of hypoxanthine (III) were assigned by a comparison with the

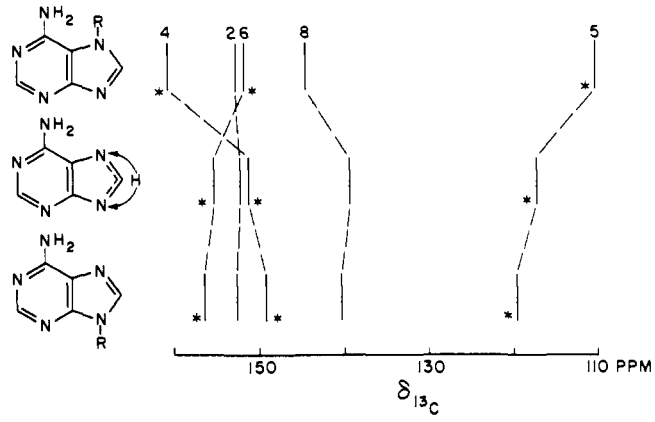

Figure 2. Correlation diagram for the ^{13}C chemical shifts (with respect to Me_4Si) of 7-(β-D-ribofuranosyl)adenine, adenine, and adenosine. Asterisks refer to quaternary carbon lines.

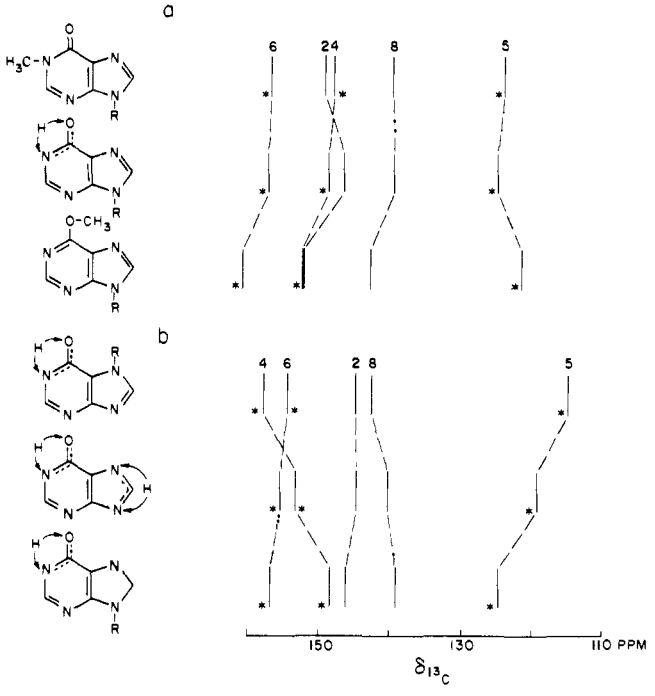

Figure 3. Correlation diagram for the ^{13}C chemical shifts (with respect to Me_4Si) of (a) 1-methyl-9-(β-D-ribofuranosyl)hypoxanthine, inosine, and 6-methoxy-9-(β-D-ribofuranosyl)purine and (b) 7-(β-D-ribofuranosyl)hypoxanthine, hypoxanthine, and inosine. Asterisks refer to quaternary carbon lines.

aromatic carbon lines found in the spectra of inosine[1] and 7-(β-D-ribofuranosyl)hypoxanthine[1] (Figure 3b). The C4 carbon singlet (153.2 ppm) was distinguished from the C6 resonance by its broad line pattern in the noise-decoupled spectrum. The large width observed for C4 and C5 lines is caused[13,14] by the tautomeric exchange of the labile proton [(N(7)H ⇄ N(9)H].[15] This large line width is absent in the spectra of inosine,[1] 7-(β-D-ribofuranosyl)hypoxanthine,[1] and 7-methylhypoxanthine,[1] where prototropic tautomerism in the imidazole moiety is eliminated. When the sample temperature of III was raised to 58°, both of the C4 and C5 lines became narrow because of the rapid exchange of the labile proton. The spectrum of 1-methylhypoxanthine (IV), which was recorded at 58°, was assigned by analogy with the data obtained for III. Like the hypoxanthine (III) spectrum, which was obtained at 58°, the spectrum of IV did not exhibit broad lines for C4 or C5, although their intensities were much smaller. This spectral feature of the bridgehead carbons (vide supra) permitted their assignment. This

Table II. Carbon-13 Chemical Shifts[a] in Certain Purine Derivatives

Compd	Concn, M^b	T, °C	CH_3	C2	C4	C5	C6	C8
Purine (I)	1.6	37		152.1_6	154.7_9	130.4_1	145.5_9	146.1_6
Adenine (II)	0.25	40		152.4_3	151.3_0	117.5_1	155.3_5	139.3_5
Hypoxanthine (III)	0.10	37		144.6_3	153.1_9	119.2^c	155.3_9	140.2_0
	0.32	58		144.6_1	153.3_1	119.3_3	155.4_2	140.0_7
1-Methylhypoxanthine (IV)	0.36	58	33.1_0	147.5_7	153.0_1	118.2_6	155.1_2	140.5_5
6-Methoxypurine (V)	0.89	58	53.6_6	151.2_8	155.1_6	118.1_2	159.2_9	142.5_1
6-Mercaptopurine (VI)	0.59	37		144.7_9	150.6_5	128.7_5	171.0_5	144.6_5
1-Methyl-6-mercaptopurine (VII)	0.90	40	39.8_1	147.6_4	149.2_3	128.2_5	171.6_9	145.0_9
6-Methylthiopurine (VIII)	0.36	37	11.2_7	151.5_4	150.1_0	129.3_9	158.5_0	143.2_1

[a] Chemical shifts are in parts per million with respect to Me_4Si. [b] All compounds were dissolved in Me_2SO. [c] The C5 signal of hypoxanthine exhibited a large line width at 37° which prevented the usual accuracy.

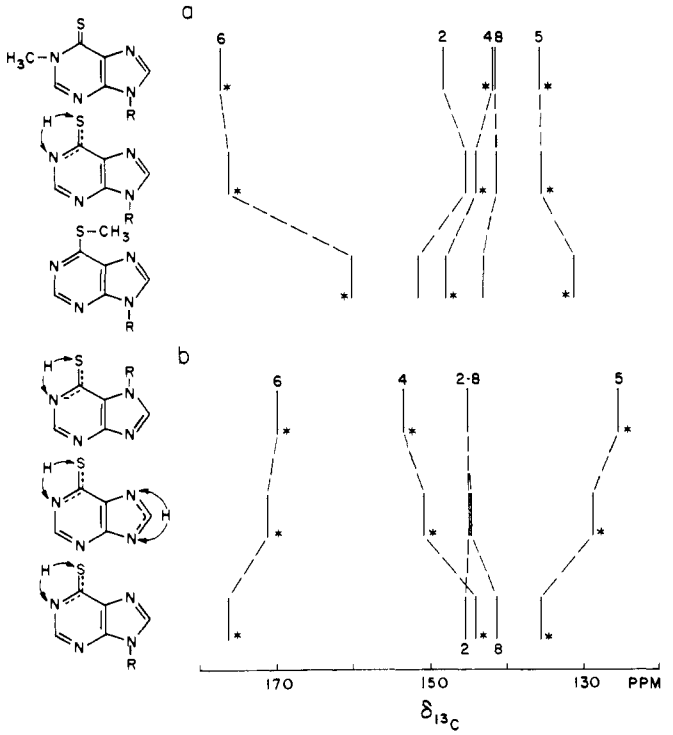

Figure 4. Correlation diagram for the ^{13}C chemical shifts (with respect to Me_4Si) of (a) 1-methyl-9-(β-D-ribofuranosyl)purine-6-thione, 9-(β-D-ribofuranosyl)purine-6-thione, and 6-methylthio-9-(β-D-ribofuranosyl)purine and (b) 7-(β-D-ribofuranosyl)purine-6-thione, 6-mercaptopurine (purine-6-thione), and 9-(β-D-ribofuranosyl)purine-6-thione. Asterisks refer to quaternary carbon lines.

same phenomenon allowed the differentiation of the C4 singlet from the C6 singlet in 6-methoxypurine (V). The C2, C5, and C8 resonances were assigned by a comparison with their respective signals found in the spectrum of 6-methoxy-9-(β-D-ribofuranosyl)purine.[1]

The C2 and C8 carbon resonance positions of 6-mercaptopurine (VI) were identified by selective proton decoupling in spite of near degeneracy of these two lines. A comparison with the spectra of 7-(β-D-ribofuranosyl)purine-6-thione[1] and 9-(β-D-ribofuranosyl)purine-6-thione[1] made the assignments for all the other aromatic carbon lines straightforward (Figure 4b). For 1-methyl-6-mercaptopurine (VII), the C2 and C8 signals were distinguished by selective proton decoupling, while the similarity of the C4, C5, and C6 chemical shifts to those found in 6-mercaptopurine led to their assignment. Similar procedures, as performed on VII, identified all carbon lines in the spectrum of 6-methylthiopurine (VIII). Methylation of the sulfur atom produces a significant upfield shift at C6 (−12.6 ppm with respect to VI), while the C2 resonance experiences a downfield (6.8 ppm) shift. The resonance positions for C4 and C5 are changed slightly from their respective position in VI.

IV. Discussion

A. General Considerations. As pointed out in the preceding paper in this issue,[1] substituent parameters have been determined for only the C4 and C5 carbons of the purine ring. It has been demonstrated,[1] that the α- and β-substituent parameters for these positions can be used in a reverse process, i.e., whether the substituent resides on the N7 or the N9 position of the purine ring. Thus, the prototropic tautomerism which occurs in the imidazole portion of the purine ring was investigated from the chemical shifts of the C4 and C5 carbons. Using either the chemical shifts for the C4 or C5 carbons, the percentage of the $N(7)H^{15}$ tautomer was calculated using eq 2 and 3,

$$[\%\ N(7)H]_{C4} = \frac{\delta[Pu]_{C4} - \{\delta[N(9)R\cdot Pu]_{C4} - \alpha\}}{\{\delta[N(7)R\cdot Pu]_{C4} - \beta\} - \{\delta[N(9)R\cdot Pu]_{C4} - \alpha\}} \quad (2)$$

$$[\%\ N(7)H]_{C5} = \frac{\delta[Pu]_{C5} - \{\delta[N(9)R\cdot Pu]_{C5} - \beta\}}{\{\delta[N(7)R\cdot Pu]_{C5} - \alpha\} - \{\delta[N(9)R\cdot Pu]_{C5} - \beta\}} \quad (3)$$

where $\delta[Pu]_i$ is the chemical shift of either C4 or C5 in the unsubstituted purine, and $\delta[N(9)R\cdot Pu]_i$ are the C4 or C5 chemical-shift values of the N7- or N9-substituted purine, where α and β are the substituent parameters[1] which must be used to correct the chemical shifts of the model compounds for the effect of the substituent. These parameters are dependent on the nature of the R substituent, i.e., whether it is a methyl group or β-D-ribofuranosyl moiety (see Table X in the preceding paper in this issue[1]).

Lactam–lactim tautomerism or thione–thiol tautomerism which could occur in the pyrimidine portion of hypoxanthine and 6-mercaptopurine, respectively, was investigated by using the chemical shift of the C6 carbon. The relative position of the C6 carbon resonance, e.g., in 1-methyl-9-(β-D-ribofuranosyl)hypoxanthine, inosine, and 6-methoxy-9-(β-D-ribofuranosyl)purine, permitted the calculation of the amount of N(1)H tautomer from eq 4,

$$[\%N(1)H]_{C6} = \frac{\delta[Pu]_{C6} - \{\delta[C(6)R\cdot Pu]_{C6} - y\}}{\{\delta[N(1)R\cdot Pu]_{C6} - z\} - \{\delta[C(6)R\cdot Pu]_{C6} - y\}} \quad (4)$$

where $\delta[Pu]_{C6}$ is the C6 chemical shift of the purine possessing lactam–lactim or thione–thiol tautomerism, i.e.,

Table III. Calculation of the Percentage of the N(7)H Tautomeric Form of Purine from C4 and C5 Chemical-Shift Data

Compd	C4	C5
Purine (I)	154.8	130.4
7-Methylpurine[a]	159.9	125.8
(CH_3 – H) parameters[b]	0.3 (β)	−0.2 (α)
9-Methylpurine[a]	151.4	133.5
(CH_3 – H) parameters[b]	−0.2 (α)	0.3 (β)
% N(7)H	40	39

[a] Chemical shifts from Table IV, ref 1. [b] Parameters from Table X, ref 1.

Table IV. Calculation of the Percentage of the N(7)H Tautomeric Form in Adenine from the C4 and C5 Chemical-Shift Data

Compd	C4	C5
Adenine (II)	151.3	117.5
7-(β-D-Ribofuranosyl)adenine[a]	160.7	110.3
(β-D-Ribofuranosyl – H) parameters[b]	1.0 (β)	−0.5 (α)
Adenosine[a]	149.3	119.6
(β-D-Ribofuranosyl – H) parameters[b]	−0.5 (α)	1.0 (β)
% N(7)H	15	14

[a] Chemical-shift data from Table IV, ref 1. [b] Parameters from Table X, ref 1.

C(6)=X ⇄ C(6)—XH, and δ[C(6)R·Pu]$_{C6}$ is the C6 chemical shift for purines with a XCH_3 group on position 6, where δ[N(1)R·Pu]$_{C6}$ is the C6 chemical shift when a methyl group is attached to the N1 position of the purine. The correction factors (y,z) which were used for the XCH_3 and N(1)CH_3 substituents will be discussed in Section IV-D.

B. Tautomerism in Purine. 7-Methylpurine and 9-methylpurine were selected as models to represent the two predominant tautomeric forms [N(7)H and N(9)H][5] of purine (I). The specific α and β parameters[1] were applied to the methylpurine chemical shifts in order to correct for the difference between a methyl group and a hydrogen. The corrections as well as the percentage of the N(7)H tautomer are given in Table III. The chemical-shift values (in Me_2SO)[16] for the C4 and C5 carbons (eq 2 and 3) of purine, 7-methylpurine, and 9-methylpurine give essentially identical values for the percentage of the N(7)H tautomer of purine, i.e., 40 and 39%, respectively. These data corroborate previous findings[17] from this laboratory concerning the tautomeric populations of purine and are in good agreement with theoretical calculations[5] which predict that the N(7)H and N(9)H tautomers of purine are of comparable energy.

C. Tautomerism in Adenine. Adenosine [9-(β-D-ribofuranosyl)adenine] and 7-(β-D-ribofuranosyl)adenine were selected as models for the investigation of the N(7)H ⇄ N(9)H tautomeric population of adenine (II). Figure 2 demonstrates the relationship between the chemical shifts in adenine, per se, and the model 7- and 9-ribosyladenines. Using the appropriate α and β parameters[1] for this pair of nucleosides, the percentage of the N(7)H tautomer was calculated using eq 2 and 3 (see Table IV). A significant decrease in the population of the N(7)H tautomer of adenine (15%) was found as compared with purine (40%). This result is in excellent agreement with experimental data derived from other methods. Eastman[3] estimated the amount of the N(7)H tautomer of adenine to be 6% in isobutyl alcohol at −103°C using fluorescence experiments. A temperature jump relaxation experiment allowed Dreyfus et al.[3a] to determine the amount of the N(7)H tautomer, in aqueous solution (20°), to be about 22%. Therefore, all experimental data including our results confirm the theoretical prediction[4b,4c,5] that the N(9)H tautomer of adenine is the most stable form.

A spectral (uv and ir) investigation and pK_a determination[18] of adenosine, 1-methyladenosine, and 6-dimethylamino-9-(β-D-ribofuranosyl)purine led to the conclusion that adenosine exists primarily in the *amine* form in aqueous solution. Likewise, CNDO/2 calculations[4c,5] predicted the amine form of adenine to be approximately 27 kcal/mol more stable than the imine tautomer. For these reasons, further study of amine–imine tautomerism in adenine (II) was not pursued by ^{13}C NMR spectroscopy.

Table V. Investigation of the Lactam–Lactim Tautomerism in the Pyrimidine Ring of Inosine from C6 Chemical-Shift Data

Compd	C6
Inosine[a]	156.9
1-Methyl-9-(β-D-ribofuranosyl)hypoxanthine[a]	156.4
(NCH_3 – H) correction[b]	−0.5 (z)
6-Methoxy-9-(β-D-ribofuranosyl)purine[a]	160.5
% N(1)H	88[c]
	100[d]

[a] Chemical-shift data from Table IV, ref 1. [b] Correction factor, section IV-D-1. [c] Calculated without correction factor. [d] Calculated with correction factor.

D. Tautomerism in Hypoxanthine and Certain Analogs. In contrast to adenine, hypoxanthine (III) exhibits two types of tautomerism, prototropic (imidazole moiety), and lactam–lactim (pyrimidine portion). Key derivatives of hypoxanthine (III) were synthesized in order to make an individual investigation of both processes.

1. Lactam–Lactim Tautomerism. The lactam–lactim tautomerism in hypoxanthine has been examined by several investigators. Wolfenden[18] concluded from a spectral (ir and uv) investigation and pK_a determination on inosine [9-(β-D-ribofuranosyl)hypoxanthine], 1-methylinosine, and 6-methoxy-9-(β-D-ribofuranosyl)purine that the keto [C(6)=O] and enol [C(6)—OH] forms of inosine, in aqueous solution, were equally favored. On the contrary, Psoda and Shugar[19] who reexamined the same three nucleosides by ir and uv (in aqueous and Me_2SO solutions) found the neutral form of inosine to exist predominantly in the keto (lactam) form. These same conclusions were also reached by Medeiros and Thomas[20] who examined the Raman spectra of the three nucleosides.

In order to study the lactam–lactim tautomerism by ^{13}C NMR spectroscopy, the C6 carbon chemical shifts[1] of the above mentioned nucleosides were examined, and a comparison of their aromatic carbon chemical shifts is shown in Figure 3a. A precise examination of this type of tautomerism is difficult because of the substituent parameters needed to correct the chemical shifts of the required model compounds, i.e., 1-methyl-9-(β-D-ribofuranosyl)hypoxanthine and 6-methoxy-9-(β-D-ribofuranosyl)purine. It has been shown[21] that the perturbations, on the carbon chemical shifts produced by methylation at position N1 of purine (I), are comparable to those produced when a proton resides at this position. A recent ^{13}C NMR investigation[22] on 2-pyridone[23] has corroborated this finding and revealed that methylation of the pyridine nitrogen produces only a small upfield shift (−0.5 ppm) on the C2 resonance position of *N*-methyl-2-pyridone. Thus, this value (−0.5 ppm) was used as the N1-methylation parameter (z) to correct the C6

Table VI. The Calculation of the Tautomeric Populations in the Imidazole Ring of Certain Hypoxanthines from C4 and C5 Chemical-Shift Data

A. Calculated Denominators for Equations 2 and 3	C4	C5
7-(β-D-Ribofuranosyl)hypoxanthine[a]	157.7	114.8
(β-D-Ribofuranosyl − H) parameters	0.8 (β)	−0.3 (α)
Inosine[a]	148.5	124.6
(β-D-Ribofuranosyl − H) parameters	−0.3 (α)	0.8 (β)
Denominators	8.1 (eq 2)	−8.7 (eq 3)

B. N(7)H Percentage for 1-Methylhypoxanthine (IV)	C4	C5
1-Methylhypoxanthine (IV)	153.0	118.3
1-Methyl-9-(β-D-ribofuranosyl)hypoxanthine[a]	147.6	123.7
(β-D-Ribofuranosyl − H) parameters[b]	−0.3 (α)	0.8 (β)
% N(7)H	63	53
Average %	58	

C. N(7)H Percentage for 6-Methoxypurine (V)	C4	C5
6-Methoxypurine (V)	155.2	118.1
6-Methoxy-9-(β-D-ribofuranosyl)[a]	151.8	121.2
(β-D-Ribofuranosyl − H) parameters[b]	−0.3 (α)	0.8 (β)
% N(7)H	38	26
Average %	32	

[a] Chemical shifts from Table IV, ref 1. [b] Parameters from Table X, ref 1.

chemical shift of 1-methyl-9-(β-D-ribofuranosyl)hypoxanthine. The absence of any suitable methoxyl vs. hydroxyl (C·OCH$_3$ − C·OH) substituent parameters (y) prevented a refinement of the chemical shifts of 6-methoxy-9-(β-D-ribofuranosyl)purine.

The percentage of the N(1) tautomer of inosine was calculated with and without the substituent correction parameter. Without the correction parameter the population of the N(1)H (lactam) form is evaluated at ≥88% while, with this parameter (z), the percentage may be as high as 100% (see Table V). Both of these values are consistent with prior findings[19,20] which concluded that inosine exists predominantly (>99%)[20] in the lactam form.

2. Prototropic Tautomerism (Imidazole Moiety). In order to measure the prototropic tautomerism in the imidazole moiety of hypoxanthine (III), the lactam–lactim tautomerism in the pyrimidine portion was prevented by using certain methylated purines, e.g., 1-methylhypoxanthine (IV, methyl group at N1) and 6-methoxypurine (V, methyl group on the C6 oxygen). 1-Methyl-9-(β-D-ribofuranosyl)hypoxanthine[1] and 6-methoxy-9-(β-D-ribofuranosyl)purine,[1] whose heterocyclic aglycons possess these methylation features, were used as models for the N(9)H tautomeric form. It was assumed[24] that the difference ($\Delta\delta$) between the chemical shifts of the C4 (or C5) carbons in 6-methoxy-9-(β-D-ribofuranosyl)purine and 6-methoxy-7-(β-D-ribofuranosyl)purine is approximately the same as the difference observed for 9-(β-D-ribofuranosyl) hypoxanthine (inosine) and 7-(β-D-ribofuranosyl)hypoxanthine. Therefore, the data obtained for inosine[1] and 7-(β-D-ribofuranosyl)hypoxanthine[1] were used in the denominators of eq 2 and 3. Furthermore, the α and β parameters[24] derived when C6 bears an oxo group were employed to correct the chemical shifts of 1-methyl-9-(β-D-ribofuranosyl)hypoxanthine and 6-methoxy-9-(β-D-ribofuranosyl)purine. The data used for the calculation of the N(7)H populations are summarized in Table VI. The discrepancy between the percentage calculated from the C4 and C5 chemical-shift data probably reflects the error arising from the inadequacies of the above approximations.[24] However, this variation does not alter the conclusions arrived at for the position of equilibrium between the N(7)H,N(9)H tautomeric pair. It is noteworthy that the percentage of the N(9)H form (68%) is greater when a methoxy group resides at position C6, while the N(7)H tautomer predominates (58%) when the hypoxanthine ring is locked in the lactam form. These same trends are observed for the thiopurine derivatives (see section IV-E-2).

Table VII. Investigation of the Thione–Thiol Tautomerism in the Pyrimidine Ring of 9-(β-D-Ribofuranosyl)purine-6-thione from C6 Chemical-Shift Data[a]

Compd	C6
9-(β-D-Ribofuranosyl)purine-6-thione	176.2
1-Methyl-9-(β-D-ribofuranosyl)purine-6-thione	177.4
6-Methylthio-9-(β-D-ribofuranosyl)purine	160.5
% N(1)H	93

[a] Chemical shift from Table IV, ref 1.

3. Tautomerism in Hypoxanthine. As mentioned above, both forms (prototropic and lactam–lactim) of tautomerism can occur simultaneously in hypoxanthine (III, Figure 3b) and to a certain degree, each process affects every carbon chemical shift (see Tables IV and V of the preceding paper in this issue[1]). Since these two types of tautomerism were studied individually, i.e., lactam–lactim through an analysis of the C6 chemical-shift data and prototropic tautomerism through the C4 and C5 chemical-shift data, it is difficult to evaluate the cross-effect of the combined processes. However, the nearly identical chemical shifts for the C4, C5, and C6 carbons (see Table II) in III and IV suggest that the populations of the tautomeric forms are approximately the same for these two compounds. Therefore, it is possible to evaluate the tautomeric N(1)H–N(7)H and N(1)H–N(9)H populations of hypoxanthine (III). The data suggest that the N(1)H–N(7)H tautomer is nearly equal to 58%, while the N(1)H–N(9)H tautomer is nearly equal to 42%.[25] Support for the N(1)H form rather than the N(3)H form is provided by the similarity of the C8 chemical shifts in III and IV. It has been demonstrated[21] that, when a methyl group is attached to the N3 position of purine, the C8 chemical shift moves upfield ca. −4 ppm when compared

Table VIII. The Calculation of the Tautomeric Populations in the Imidazole Ring of Certain 6-Mercaptopurines from C4 and C5 Chemical-Shift Data

A. Calculated Denominators for Equations 2 and 3	C4	C5
7-(β-D-Ribofuranosyl)purine-6-thione[a]	153.3	125.3
(β-D-Ribofuranosyl − H) parameters[b]	0.8 (β)	−0.2 (α)
9-(β-D-Ribofuranosyl) purine-6-thione[a]	144.1	135.6
(β-D-Ribofuranosyl − H) parameters[b]	−0.2 (α)	0.8 (β)
Denominators	8.2 (eq 2)	−9.3 (eq 3)
B. N(7)H Percentage for 1-Methyl-6-mercaptopurine (VII)	**C4**	**C5**
1-Methyl-6-mercaptopurine (VII)	149.2	128.3
1-Methyl-9-(β-D-ribofuranosyl)purine-6-thione[a]	142.0	135.8
(β-D-Ribofuranosyl − H) parameters[b]	−0.2 (α)	0.8 (β)
% N(7)H	85	72
Average %	79	
C. N(7)H Percentage for 6-Methylthiopurine (VIII)	**C4**	**C5**
6-Methylthiopurine (VIII)	150.1	129.4
6-Methylthio-9-(β-D-ribofuranosyl)purine[a]	148.0	131.3
(β-D-Ribofuranosyl − H) parameters[b]	−0.2 (α)	0.8 (β)
% N(7)H	23	12
Average %	18	

[a] Chemical shifts from Table IV, ref 1. [b] Parameters from Table X, ref 1.

with 1-methylpurine. Such an effect was not observed in the C8 chemical-shift data of hypoxanthine (III) and 1-methylhypoxanthine (IV), which would suggest that the contribution of the N(3)H tautomer is negligible.

These results are consistent with the CNDO/2 calculation performed on hypoxanthine,[5] which predict that the N(1)H–N(7)H tautomer is slightly more stable than the N(1)H–N(9)H tautomer and considerably more stable than any of the N(3)H forms. A recent ^{1}H NMR and uv study[3b] suggested that the predominant tautomeric form of hypoxanthine in dilute *aqueous* solution was N(1)H–N(9)H. This discrepancy with our results may arise from the different solvents employed or on the other hand may only reflect experimental errors in both methods.

E. Tautomerism in 6-Mercaptopurine and Certain Derivatives. Like hypoxanthine, tautomerism can occur in both rings of 6-mercaptopurine (VI). Accordingly, the same approach discussed in section IV-D was used to investigate the various tautomeric processes.

1. Thione–Thiol Tautomerism. 1-Methyl-9-(β-D-ribofuranosyl)purine-6-thione[1] and 6-methylthio-9-(β-D-ribofuranosyl)purine[1] were selected as models for the study of tautomerism in 9-(β-D-ribofuranosyl)purine-6-thione.[1] The relationships of the aromatic carbon chemical shifts[1] of these nucleosides are shown in Figure 4a.

Because of the lack of a suitable set of models (vide supra, section IV-D-1) from which the appropriate substituent parameters could be obtained, the C6 chemical shifts were used without any correction in eq 4 to determine the percentage of the N(1)H (thione) tautomer. This percentage was found to be ca. 93% (Table VII). Again, this result is in good agreement with data obtained by other techniques which suggest that 6-mercaptopurine and related systems preferentially exist in the thione form in solution.[5,26] The similarity of the chemical-shift differences, $\Delta\delta$ [C(6)=S − C(6)H, Table VIII in the preceding paper of this issue[1]], observed for the other thione derivatives we have studied, i.e., pyrrolo[2,3-*d*]pyrimidine-4-thione[1] and 7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-4-thione,[1] suggest that the thione form also predominates in these compounds.

2. Prototropic Tautomerism (Imidazole Moiety). The prototropic tautomerism in 6-mercaptopurine was investigated using similar methods and approximations described for hypoxanthine (section IV-D-2). Employing 1-methyl-9-(β-D-ribofuranosyl)purine-6-thione[1] and 6-methylthio-9-(β-D-ribofuranosyl)purine[1] as models for the N(9)H form, the percentage of the N(7)H form was found to be approximately 79% for VII and 18% for VIII (Table VIII). Once again, discrepancies of about 10% are noted in the results obtained from the C4 (eq 2) and C5 (eq 3) data, and this range is probably a reflection of the accuracy of the approximations used in these calculations.

It is noteworthy that the thione form of 1-methyl-6-mercaptopurine (VII) seems to stabilize the N(7)H tautomer. On the other hand, when the sulfur atom is methylated, VIII [C(6)–SCH_3], the apparent combination of electronic and steric effects reverses the populations of the N(7)H and N(9)H tautomers. That 6-methylthiopurine (VIII) exists predominantly as the N(9)H tautomer is supported by dipole moment measurements.[3d] A recent spectroscopic study (^{1}H NMR and uv) by Reichman et al.[3d] indicated the presence of both N(7)H and N(9)H tautomers in VIII, but their data are not sufficient to distinguish, quantitatively, the predominant tautomeric form. The data in Table VIII clearly demonstrate the utility of ^{13}C NMR spectroscopy in providing reasonably good quantitative estimates of tautomeric populations.

3. Tautomerism in 6-Mercaptopurine. Like hypoxanthine, both types of tautomerism (prototropic and thione–thiol) can occur simultaneously in 6-mercaptopurine (VI). Using the same approach as before (section IV-C-3), the tautomeric populations can be estimated from a comparison of the chemical shifts of 1-methyl-6-mercaptopurine (VII) and 6-mercaptopurine (VI). The similarity of the C4, C5, and C6 chemical-shift data suggests that the percentages of the tautomeric forms are approximately the same in these two compounds, viz., 79% N(1)H–N(7)H and 21% N(1)H–N(9)H.[27] Again no large amount of the N(3)H forms is expected on the basis of the C8 data. These results support the theoretical CNDO/2 calculations on VI, which predict that the N(7)H tautomer is more stable than the N(9)H form.[5]

Lichtenberg et al.[3b] suggested from spectroscopic experiments (^{1}H NMR and uv) that the predominant form of 6-mercaptopurine in *aqueous* solution is N(1)H-N(9)H. Unlike the disagreement with hypoxanthine where our data indicated a nearly 1:1 tautomeric mixture, the 4:1 results determined for the N(1)H-N(7)H/N(1)H-N(9)H tautomers of 6-mercaptopurine (VI) represent a significant departure from the work of Lichtenberg et al.[3b] and cannot be justified on the basis of a change in solvent. A possible explanation for this variance is that the ultraviolet experimental data[3b] were not corrected for the substituent effect of a methyl group vs. a hydrogen atom.

V. Conclusion

Using model compounds where the labile hydrogen is replaced by a nonexchanging group, usually a methyl group, several spectroscopic techniques[5] (mainly uv and ^{1}H NMR) and dipole moment measurements have been employed to qualitatively investigate tautomeric processes. This present study now indicates that ^{13}C NMR spectroscopy is a very sensitive technique for the investigation of tautomerism as it is possible to evaluate, quantitatively, the tautomeric populations from carbon chemical-shift data.[28] The corrections for the substitution of a methyl or β-D-ribofuranosyl moiety vs. a hydrogen atom, determined in the previous paper in this issue,[1] have been employed to evaluate the percentages of the various tautomeric species in a variety of purines. In general, our data are in good qualitative agreement with those results obtained by other techniques and provide direct evidence to substantiate previous[5] theoretical conclusions regarding populations of various tautomeric forms.

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(1) (a) Paper XXV: M.-Th. Chenon, R. J. Pugmire, D. M. Grant, R. P. Panzica, and L. B. Townsend, *J. Am. Chem. Soc.*, the previous paper in this issue. (b) All experimental work described in this paper was conducted at the University of Utah. (c) Presented in part at the Fourth International Congress of Heterocyclic Chemistry, Salt Lake City, Utah, July 1973, A-24. As this manuscript was being put in final form, the work of M. C. Thorpe, W. C. Coburn, Jr., and J. A. Montgomery [*J. Magn. Reson.*, **15**, 1981 (1974)] appeared reconfirming the assignments in four of the compounds discussed herein. The quantitative discussion of tautomerism, which constitutes the principal contribution of this work, has not been reported by other workers.

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(15) The N(7)H notation refers to that purine tautomer which possesses a hydrogen at position N7 of the purine ring. Similarly the N(9)H notation stands for the N9 tautomer.

(16) We have calculated the percentage of the purine N(7)H tautomer in water (H_2O) using the C4 and C5 chemical-shift data obtained for aqueous solutions of purine, 7-methylpurine, and 9-methylpurine [50%, eq 2; 66%, eq 3 (average 58%)]. The α and β parameters used to correct for methyl substitution on the imidazole portion of the purine ring were those derived from our Me_2SO study. Thus, the discrepancy between the percentages calculated from the C4 and C5 chemical-shift data is probably the result of the solvent dependence of the α and β parameters. We are now establishing a set of α and β parameters for water in our laboratory, and these data will be forthcoming.

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(25) If 100% of the N(1)H form is assumed, percentages for the N(7)H form can be evaluated from the C4 and C5 chemical-shift data of hypoxanthine (III), 7-(β-D-ribofuranosyl)hypoxanthine,[1] and inosine,[1] ca., 54 and 53%, respectively (see Figure 3b and Tables II and VI-A).

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(27) If 100% of the N(1)H form is assumed, percentages for the N(7)H form can be evaluated from C4 and C5 chemical-shift data of 6-mercaptopurine (VI), 7-(β-D-ribofuranosyl)purin-7-thione, and 9-(β-D-ribofuranosyl)purin-6-thione, ca. 78 and 65%, respectively (see Figure 4b and Tables II and VIII-A).

(28) Corrections for substituent effects on ^{13}C data of azoles seem to be very difficult to evaluate,[29] but an approach using *N*-methyl derivatives in combination with ^{13}C chemical-shift titration curves succeeds to determine the tautomeric equilibrium of the imidazole ring in several derivatives of histidine.[30]

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