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(19) Each of the four alkyl substituents could be expected to produce a bathochromic shift of 5 nm. See L. F. Fieser and M. Fieser, "Steroids", Reinhold, New York, N.Y., 1959.
(20) (a) H. Hart, P. M. Collins, and A. J. Waring, *J. Am. Chem. Soc.*, **88**, 1005 (1966), and references cited therein; (b) N. L. Allinger, J. C. Tai, and T. W. Stuart, *Theor. Chim. Acta*, **8**, 101 (1967).
(21) The splitting of the π levels in 1,4-pentadiene (ΔE = 0.34 eV) has been attributed to such a "through space" interaction: J. C. Bunzli, A. J. Burak, and D. C. Frost, *Tetrahedron*, **29**, 3735 (1973).
(22) H. W. Geluk, *Synthesis*, 652 (1970).
(23) This assumption, based on the approximation that the resonance interaction between degenerate orbitals is proportional to the overlap between them, has been successfully applied to other acyclic alkylated dienes, as discussed further in ref 2f, 6, and 12.

Carbon-13 Magnetic Resonance Spectra of C-Nucleosides. 3.[1a-c] Tautomerism in Formycin and Formycin B and Certain Pyrazolo[4,3-*d*]pyrimidines

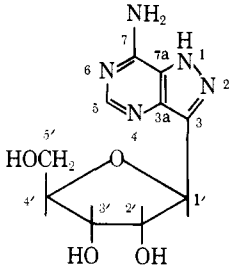
Marie-Thérèse Chenon,[1d] Raymond P. Panzica,[1e] James C. Smith,[1e] Ronald J. Pugmire,[1e] David M. Grant,*[1e] and Leroy B. Townsend*[1e]

Contribution from the CNRS, Service de Spectrochimie Infarouge et Raman, 94320 Thiais, France, and the Departments of Chemistry and Biopharmaceutical Sciences, University of Utah, Salt Lake City, Utah 84112. *Received September 18, 1975*

Abstract: Selected pyrazolo[4,3-*d*]pyrimidine heterocycles and nucleosides were examined by carbon-13 NMR spectroscopy. The carbon chemical shifts and line widths of certain aromatic and carbohydrate carbons were observed to be a function of temperature. Through an analysis of the carbon chemical shift data, the tautomeric populations of the C-nucleosides formycin (**4**) and formycin B (**10**) were determined. The prototropic N(1)H $\rightleftharpoons$ N(2)H process which occurs in the pyrazole portion of the heterocyclic aglycon was the only tautomeric process observed in these nucleosides and certain derivatives. The percentage of the N(2)H tautomer, a higher energy species, was shown to be dependent on the substituent at C7 in the pyrimidine portion of the pyrazolo[4,3-*d*]pyrimidine ring.

I. Introduction

Since the isolation[2,3] and characterization[4,5] of the C-nucleoside antibiotics formycin (4) and formycin B (**10**) from *Norcardia interforma*, numerous reports have appeared in the literature describing some of their biological and physicochemical properties.[1a,6] Formycin and formycin B, unlike their isomeric purine nucleoside counterparts adenosine and inosine, possess a C-glycosyl linkage. This unique linkage allows prototropic tautomerism to take place in the five-membered ring portion of the heterocyclic aglycon in contrast to adenosine or inosine. Two preliminary communications[1a,7] have recently described the effect such tautomeric processes have on the ^{13}C NMR spectra of formycin and formycin B. We have now extended our probe of all tautomeric processes in **4** and **10** by examining their ^{13}C NMR spectra as well as the ^{13}C spectra of certain model pyrazolo[4,3-*d*]pyrimidine heterocycles and nucleosides (Table I) in two solvents, i.e., dimethyl sulfoxide (Me_2SO) and hexamethylphosphoric triamide {$[(CH_3)_2N]_3PO$ or HMPT}, and as a function of temperature. Because of their physical properties, these solvents enabled us to evaluate tautomeric equilibria from an analysis of carbon chemical shift data in the fast and slow exchange rates.



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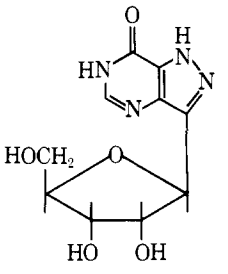

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II. Experimental Section

Carbon-13 NMR spectra were obtained with a Varian XL-100-15 spectrometer equipped with a Varian 620f computer for Fourier transform operation. Compounds were dissolved in dry, spectroquality Me_2SO or $[(CH_3)_2N]_3PO$ and the concentrations for each compound studied are provided in the appropriate tables. Cyclohexane was selected as an internal reference for this study since its chemical shift was observed to be nearly independent of temperature.[8] All carbon chemical shifts were observed with respect to the internal dioxane (ca. 2% v/v) line and then converted to the cyclohexane scale. The conversion formula (1) used for Me_2SO solutions is as follows:

$$\delta_{\text{cyclohexane}} = (\delta_{\text{dioxane}} + 40.0_0 \text{ ppm}) + 1.0 \times 10^{-3}T \quad (1)$$

where T is the temperature in degrees centigrade. A second formula (2) allows the conversion to the Me_4Si scale:

$$\delta_{Me_4Si} = (\delta_{\text{dioxane}} + 66.2_8 \text{ ppm}) + 2.0 \times 10^{-3}T \quad (2)$$

The conversion formulas (3 and 4) for $[(CH_3)_2N]_3PO$ solutions are as follows:

$$\delta_{\text{cyclohexane}} = \delta_{\text{dioxane}} + 40.2_3 \text{ ppm} \quad (3)$$

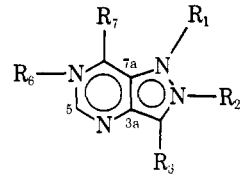
$$\delta_{Me_4Si} = (\delta_{\text{dioxane}} + 67.2_5 \text{ ppm}) + 1.2 \times 10^{-3}T \quad (4)$$

It should be pointed out that the chemical shift of dioxane in $[(CH_3)_2N]_3PO$ is independent of temperature and this accounts for the absence of the temperature term in eq 3. The ^1H NMR spectra were determined using either a Varian 56/60 or XL-100-12 spectrometer. The methods used for the synthesis of pyrazolo[4,3-*d*]-pyrimidine heterocycles and nucleosides can be found in Table I.

III. Results

Carbon-13 NMR spectra were determined using noise de-

Table I. Nomenclature of the Studied Pyrazolo[4,3-*d*]pyrimidine Derivatives



Compd	R_1	R_2	R_3	R_6	R_7		Synthetic method
1	H		β-D-ribofuranosyl		H	3-(β-D-Ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine	*a*
2	CH_3		CH_3		NH_2	7-Amino-1,3-dimethylpyrazolo[4,3-*d*]pyrimidine	*b*
3		CH_3	CH_3		NH_2	7-Amino-2,3-dimethylpyrazolo[4,3-*d*]pyrimidine	*b*
4	H		β-D-ribofuranosyl		NH_2	7-Amino-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (formycin)	*c*
5	CH_3		β-D-ribofuranosyl		NH_2	7-Amino-1-methyl-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine	*b*
6		CH_3	β-D-ribofuranosyl		NH_2	7-Amino-2-methyl-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine	*b*
7	H		H	H	O	Pyrazolo[4,3-*d*]pyrimidin-7-one	*d*
8	H		CH_3	H	O	3-Methylpyrazolo[4,3-*d*]pyrimidin-7-one	*e*
9	H		CH_3	CH_3	O	3,6-Dimethylpyrazolo[4,3-*d*]pyrimidin-7-one	*f*
10	H		β-D-ribofuranosyl	H	O	3-(β-D-Ribofuranosyl)pyrazolo[4,3-*d*]pyrimidin-7-one (formycin B)	*c*
11	H		2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl	H	O	3-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl) pyrazolo[4,3-*d*]pyrimidin-7-one	*g*

[a] G. H. Milne and L. B. Townsend, *J. Chem. Soc., Perkin Trans. 1*, 2677 (1972). [b] L. B. Townsend, R. A. Long, J. P. McGraw, D. W. Miles, R. K. Robins, and H. Eyring, *J. Org. Chem.*, 39, 2023 (1974). [c] Commercial sources. [d] R. K. Robins, F. W. Furcht, A. D. Grauer, and J. W. Jones, *J. Am. Chem. Soc.*, **78**, 2418 (1956). [e] R. K. Robins, L. B. Holum, and F. W. Furcht, *J. Org. Chem.*, **21**, 833 (1956). [f] The authors wish to thank Mr. A. F. Lewis for the synthesis of this heterocycle. Experimental details will be published at a later date. [g] R. A. Long, A. F. Lewis, R. K. Robins, and L. B. Townsend, *J. Chem. Soc. C*, 2440 (1971).

Table II. Specific Proton Chemical Shifts[a] for Certain Pyrazolo[4,3-*d*]pyrimidine Derivatives in Me_2SO

Compd	Concn, M	H3	H5	H7	H1'
1	0.40		8.99	9.33	5.08
6	0.47		8.06		5.14
7	0.69	8.08	7.84		

[a] Chemical shifts are in parts per million with respect to Me_4Si. $T \simeq 37$ °C.

coupling and single-frequency off-resonance decoupling conditions. This process permits a ready differentiation of quaternary carbon signals from the remaining carbon resonances in the molecule. Selective proton decoupling experiments were conducted on certain pyrazolo[4,3-*d*]pyrimidines in order to facilitate the carbon assignments when any ambiguity existed between methine carbon lines. Coupled spectra were employed to assign quaternary carbon lines in some spectra by using the long-range (two and three bonds) coupling constants.

A. ^{1}H NMR Chemical Shifts. Selective proton decoupling experiments were required for the assignment of certain carbon chemical shifts of the pyrazolo[4,3-*d*]pyrimidine nucleosides **1** and **6**. This in turn necessitated the assignment of the proton spectra of these nucleosides (see Table II). In the ^{1}H NMR spectrum of 3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (**1**), the singlets at 8.99 and 9.33 ppm were assigned to the aromatic H5 and H7 protons, respectively, by analogy with data obtained for nebularine. The corresponding protons residing on the pyrimidine portion of nebularine were observed at 8.95 and 9.19 ppm.[9] It is noteworthy that the H1' resonance of **1** shifts upfield approximately −1 ppm with respect to its value in nebularine (6.06 ppm). This spectral feature is characteristic of C-nucleosides.[10] Since 7-amino-2-methyl-3-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**6**) has only one aromatic hydrogen, H5, the assignment of the downfield singlet at 8.06 ppm was unambiguous.

Conversely, the ^{1}H NMR spectrum of pyrazolo[4,3-*d*]-pyrimidin-7-one (**7**) was assigned from the ^{13}C spectrum of **7** by selective proton decoupling experiments. The singlets at 7.84 and 8.08 ppm were assigned to the H5 and H3 signals, respectively.

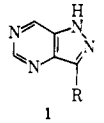
B. ^{13}C NMR Chemical Shifts. Carbon-13 chemical shifts of those compounds studied in Me_2SO solutions are summarized in Table III. This table provides a convenient comparison with other heterocyclic and nucleoside data in the literature; however, it must be emphasized that certain carbon chemical shifts found in this table are strongly influenced by temperature

Table III. Carbon-13 Chemical Shifts[a] of Certain Pyrazolo[4,3-*d*]pyrimidine Derivatives

	Concn,		Aglycon							Ribose				
Compd	M	*T*, °C	NCH_3	CCH_3	C3	C3a	C5	C7	C7a	C1'	C2'	C3'	C4'	C5'
1	0.40	40			143.4_4	140.8_2	150.3_6	143.0_0	131.8_2	77.6_3	74.2_8	71.9_0	85.4_8	62.3_5
2	0.92	38	38.5_3	10.2_6	139.4_9	141.5_1	150.9_7	150.9_1	121.4_8					
3	0.37	38	37.7_0	8.5_2	131.5_5	136.6_2	151.3_9	155.4_0	129.7_6					
4	2.0	37			143.1_7	138.3_1	151.4_1	151.5_7	123.3_6	78.1_8	75.3_2	72.5_6	86.0_8	62.5_6
5	0.12	40	39.0_0		142.1_2	140.2_4	151.0_5	151.2_5	122.1_1	78.1_8	74.8_4	72.3_6	85.9_8	62.6_2
6	0.47	37	38.9_7		133.3_9	136.0_6	152.0_5	155.7_8	129.8_5	75.8_7	75.2_5	72.1_2	86.2_3	62.1_3
7	0.39	40			133.5_6	138.9_9	142.8_6	153.2_0	126.8_1					
8	0.78	32		9.9_9	139.4_5	136.8_0	142.0_7	154.3_1	129.5_4					
10	1.6	40			144.3_9	136.4_7	143.1_5	153.2_9	128.2_3	77.5_3	74.9_2	72.0_5	85.6_0	62.5_0
11[b]	0.44	31			140.9_1	137.1_9	143.4_0	153.1_8	128.4_4	75.0_5[c]	72.9_0[c]	71.4_1[c]	79.2_1[c]	63.1_7[c]

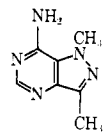
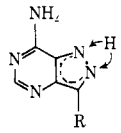
[a] Chemical shifts are in parts per million with respect to Me_4Si and were determined in Me_2SO. [b] δ (CH_3) = 20.3_0; 20.3_0; and 20.4_3 ppm. δ (C=O) = 169.3_9, 169.5_3, and 170.0_3 ppm. [c] Tentative assignments.

Table IV. Carbon-13 NMR Data[a] as a Function of Temperature for 3-(β-D-Ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine

Compound[b]	Solvent	Concn, M	Temp range, °C	Carbon	Linear variation: δ^c at 0 °C (ppm)	Linear variation: $10^3\,\Delta\delta/\Delta T$ (ppm/°C)
 1	Me_2SO	0.40	6–145	C3	117.0	4.0
				C3a	114.5	2.5
				C5	124.1	≃0
				C7	116.6	1.5
				C7a	105.5	2.5

[a] Chemical shifts are in parts per million with respect to cyclohexane. [b] R = β-D-ribofuranosyl. [c] Extrapolated values.

Table V. Carbon-13 NMR Data[a] as a Function of Temperature for 7-Aminopyrazolo[4,3-*d*]pyrimidine Derivatives

Compound[b]	Solvent	Concn, M	Temp range, °C	Carbon	Linear variation: δ^c at 0 °C (ppm)	Linear variation: $10^3\Delta\delta/\Delta T^d$ (ppm/°C)	$\delta\overline{N(1)H-N(2)H} - \delta\,N(1)H^e$ (ppm)
 2	Me_2SO	0.92	8–144	C3	112.9	7.0	0
				C3a	114.6	8.0	0
				C5	124.6	≃0	0
				C7	124.6	≃0	0
				C7a	94.9	5.8	0
				$N(1)CH_3$	12.3	−2.2	0
				CH_3	−16.0	−3.0	0
 4	Me_2SO	2.1	−14 to 145	C3	117.8	≃0	−1.4
		1.2	6–115	C3a	(112.3)[f]	7.8	(0.5)[f]
				C5	125.2	≃0	0
				C7	124.7	≃0	0.6
				C7a	96.0	2.0	1.3
	$[(CH_3)_2N]_3PO$	0.71	70–143	C3	118.0	≃0	−0.2
		0.49	−14 to 39	C3a	112.0	7.2	−0.2
				C5	124.3	2.0	0
				C7	124.8	≃0	0.3
				C7a	96.3	2.0	0.5

[a] Chemical shifts are in parts per million with respect to cyclohexane. [b] R = β-D-ribofuranosyl. [c] When the solution was not studied at 0 °C, these chemical shifts were calculated using their linear variations vs. temperature for the purpose of comparison with other data. For formycin (4) these values refer to the N(1)H tautomeric form. [d] These data refer to the N(1)H tautomeric form in the slow exchange region. [e] Difference between the chemical shifts of the compound undergoing a fast exchange tautomeric process and the chemical shifts of the N(1)H tautomeric species. [f] Estimated value.

because of the prototropic tautomeric process which takes place in the pyrazole moiety of the pyrazolo[4,3-*d*]pyrimidine ring. Therefore only the chemical shifts of each tautomeric species (Tables IV–VI) can be used for a precise evaluation of substituent effects. The notations N(*i*)H were used to represent the tautomeric forms of **4, 10,** and related derivatives, where *i* refers to the position of the labile hydrogen.

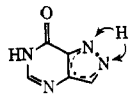
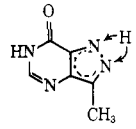
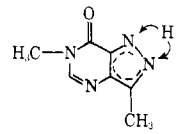
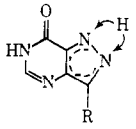
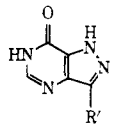
1. Assignment of the Ribose Carbon Atoms. The resonance line sequence of the β-D-ribofuranosyl carbon atoms for C-nucleosides is different from the established sequence[11] found in N-nucleosides. The C1′ line shifts upfield[12] with respect to its frequency range found in N-nucleoside ^{13}C NMR spectra, whereas the remaining carbon lines exhibit only minor changes, thus establishing the following sequence (from low field to high field) as: C4′, C1′, C2′, C3′, and C5′. In **6,** a selective proton decoupling experiment allowed one to ascertain the positions of the C1′ and C2′ signals. For 3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidin-7-one (**11**), the ribose carbons are only tentatively assigned. Acetylation of the 2′, 3′, and 5′ positions of the β-D-ribofuranosyl moiety induces large variations on the ribose carbon line frequencies. Since the major concern of this study was the determination of tautomeric populations, the β-D-ribofuranosyl carbon line sequence of **11** was not rigorously established.

2. Assignment of the Aglycon Resonances. a. 3-(β-D-Ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine. In the carbon-13 NMR spectrum of **1** (in Me_2SO), the C5 and C7 resonances were assigned to the lines at 150.4 and 143.0 ppm, respectively. These assignments were established by selective proton decoupling experiments. The upfield singlet (131.8 ppm) was assigned to C7a by analogy with purine data.[9] Comparison of data obtained from model systems enabled us to assign C3a to the singlet at 140.8 ppm (see section IV-A and Table VII). The line at 143.4 ppm was assigned to the C3 signal by default.

b. 7-Aminopyrazolo[4,3-*d*]pyrimidine Derivatives. The coupled spectrum of 7-amino-1,3-dimethylpyrazolo[4,3-*d*]-pyrimidine (**2,** in Me_2SO) provided a straightforward assignment of the carbon resonances of this heterocycle. The quartet (^2J = 6.8 Hz) at 139.5 ppm arises from the C3 carbon. The signal at 141.5 ppm was assigned to C3a; this carbon is coupled with the H5 proton (doublet, ^3J = 11.2 Hz) and with the methyl group (quartet, ^3J = 3.0 Hz) attached to the C3 position. The C5 resonance (151.0 ppm) appears as a large doublet (^1J = 198.2 Hz). The C7 line (150.9 ppm) is also a doublet because of the long-range coupling between C7 and H5. The C7a signal (121.5 ppm) is very broad (line width of ca. 11 Hz) and the fine structure was not resolved.

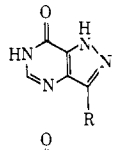
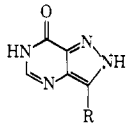
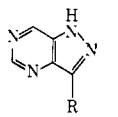
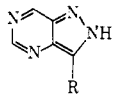
In the coupled spectrum of 1-methylformycin (**5,** in Me_2SO), the doublet (^1J = 199.3 Hz) at 151.1 ppm was assigned to the C5 resonance and the doublet (^3J = 11.0 Hz) at 151.3 ppm to the C7 signal. The C3 (142.1 ppm) and C3a (140.2 ppm) lines are reversed with respect to their positions in the spectrum of **2.** The C3 resonance shifts 2.6 ppm downfield, while the C3a signal moves −1.3 ppm upfield. This is consistent with data obtained from other pyrazolo[4,3-*d*]-pyrimidines when a β-D-ribofuranosyl moiety replaces a methyl group at the C3 position (see Table VIII). Additional evidence for these assignments is provided by the longer T_1

Table VI. Carbon-13 NMR Data[a] as a Function of Temperature for Some Pyrazolo[4,3-*d*]pyrimidin-7-one Derivatives

Compound[b]	Solvent	Concn, M	Temp range °C	Carbon	Linear Variation: δ^c at 0 °C N(1)H (ppm)	Linear Variation: δ^c at 0 °C N(2)H (ppm)	Linear Variation: $10^3 \Delta\delta/\Delta T^d$ (ppm/°C)	δN(1)H–N(2)H − δN(1)H[e] (ppm)
 7	Me_2SO	0.39	17–147	C3	107.6	97.1	≃0	−3.2
				C3a	113.2		≃0	−0.6
				C5	116.7		−2.7	0
				C7	126.7	130.3	≃0	1.1
				C7a	100.3		≃0	3.1
	$[(CH_3)_2N]_3PO$	0.42	1.5–99	C3	106.4	96.3	≃0	−3.1
				C3a	113.1	110.2	≃0	−0.5
				C5	115.9		≃0	0
				C7	126.3	130.2	≃0	1.1
				C7a	100.5	111.0	≃0	2.0
 8	Me_2SO	0.63	16–132	C3	*f*		−1.5	
				C3a	*f*		≃0	
				C5	*f*		−3.1	
				C7	*f*		≃0	
				C7a	*f*		3.5	
				CH_3	*f*		−4.5	
	$[(CH_3)_2N]_3PO$	0.68	−27 to 118	C3	114.2	105.1	≃0	−2.5
				C3a	111.1	108.4	≃0	−0.7
				C5	115.2		−1.5	0
				C7	126.4	130.6	≃0	1.2
				C7a	100.9	110.4	2.5	2.7
				CH_3	−16.1	−18.6	−5.0	−0.6
 9	$[(CH_3)_2N]_3PO$	0.68	−13 to 148	C3	114.2	105.3	≃0	−2.4
				C3a	110.8	108.0	≃0	−0.8
				C5	118.9		−5.0	0
				C7	126.5	130.4	≃0	1.3
				C7a	99.6	109.1	≃0	3.4
				$N(6)CH_3$	5.6		−2.0	0
				CH_3	*f*		−3.5	
 10	Me_2SO	1.6	−1 to 145	C3	118.3		3.0	−2.6
		0.75	11–120	C3a	110.4		≃0	0
		0.38	12–142	C5	117.0		−4.8	0
				C7	126.8		≃0	0.8
				C7a	101.6		2.0	2.0
				C1'	51.4		5.0	−0.5
	$[(CH_3)_2N]_3PO$	0.56	−18 to 165	C3	118.1	109.1	3.0	−2.7
				C3a	110.2	108.2	≃0	−0.4
				C5	115.5		≃0	0
				C7	126.2	130.2	≃0	1.2
				C7a	101.5	110.5	2.0	2.6
				C1'	52.2		5.0	−0.6
 11	Me_2SO	0.44	12–145	C3	114.7		≃0	0
				C3a	110.9		≃0	0
				C5	117.2		−3.8	0
				C7	126.9		≃0	0
				C7a	102.0		5.5	0

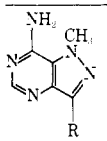
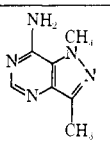
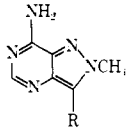
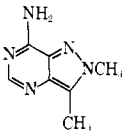
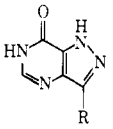
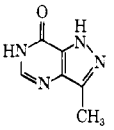
[a] Chemical shifts are in parts per million with respect to cyclohexane. [b] R and R' refer to the β-D-ribofuranosyl and 2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl moieties, respectively. [c] These chemical shifts refer to the two tautomeric forms N(1)H and N(2)H. When the solution was not studied at 0 °C, or when the tautomeric process was in a medium exchange rate at this temperature, these chemical shifts were calculated using the linear variations observed for the chemical shifts of the tautomeric forms in a slow exchange rate. [d] These data refer to the N(1)H and N(2)H tautomeric forms in the slow exchange region. [e] Difference between the chemical shifts of the compound undergoing a fast exchange tautomeric process and the chemical shifts of the N(1)H tautomeric species. [f] It was not possible to observe these chemical shifts in the slow exchange process because the sample froze prior to obtaining the data relative to the low temperature plateaus.

Table VII. Evaluation of the Carbon-13 Chemical Shifts[a] of the N(1)H Form of 3-(β-D-Ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine

Tautomeric form[b]		C3	C3a	C5	C7	C7a	C1'
	N(1)H obsd	118.3	110.4	117.0	126.8	101.6	51.4
	N(2)H evaluated	109.3[c]	108.4[c]	117.0[c]	130.8[c]	110.6[c]	49.1[d]
	Substituent effect (C=O) − (CH)		−2.8[e]	−6.9[e]	9.1[e]	−10.3[e]	0.3[e]
	N(1)H evaluated		113.2	123.9	117.7	111.9	51.1
	N(1)H obsd	117.0	114.5	124.1	116.6	105.5	51.4
	N(2)H evaluated		111.2	123.9	121.7	120.9	48.8

[a] Chemical shifts are in parts per million, with respect to cyclohexane, at 0 °C. Solvent: Me_2SO. [b] R = β-D-ribofuranosyl. [c] The differences in the chemical shifts of the the two tautomeric forms N(1)H and N(2)H are assumed to be the same in Me_2SO as in $[(CH_3)_2N]_3PO$. [d] Estimated value from C1' data of **5** and **6**. [e] Effects caused by the substitution of an oxo function for a hydrogen in purines and pyrrolo[2,3-*d*]pyrimidines (see Table VIII in ref 9).

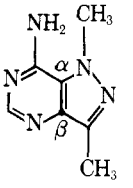
Table VIII. Substitution Effects[a] of a β-D-Ribofuranosyl Moiety vs. a Methyl Group at the C3 Position in Certain Pyrazolo[4,3-*d*]pyrimidines

Structure A	minus	Structure B[b]	Solvent	C3	C3a	C5	C7	C7a
	—		Me_2SO	2.6	−1.3	0.1	0.1	0.6
	—		Me_2SO	1.8	−0.6	0.7	0.4	0.1
	—		$[(CH_3)_2N]_3PO$	4.1[c]	−0.9[c]	0.3[c]	−0.2[c]	0.7[c]

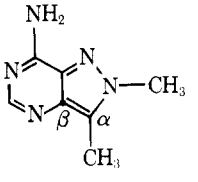
[a] Chemical shifts are in parts per million. Temperature 40 °C. [b] R = β-D-ribofuranosyl. [c] These data refer to the N(1)H tautomeric form.

relaxation time of the C3a resonance. This spectral feature is in agreement with the T_1 relaxation times measured for formycin (**4**).[14] These measurements revealed that the T_1's increased as follows: C5 < C7 < C3 < C7a < C3a. As usual, the upfield singlet at 122.1 ppm was assigned to the C7a carbon.

Likewise, in the ^{13}C NMR spectrum of 7-amino-2,3-dimethylpyrazolo[4,3-*d*]pyrimidine (**3**, in Me_2SO), the singlets at 131.6 and 129.8 ppm were assigned to the carbon atoms at positions C3 and C7a, respectively, on the basis of their intensities. These two carbon lines move −7.9 ppm (C3) and 8.3 ppm (C7a) compared to their positions in the spectrum of **2**. Again, this spectral feature is consistent with published data[9,13,16] concerning the effect of methyl substitution on carbon atoms adjacent to the site of substitution. If the reverse assignments for C3 and C7a were made, these lines must then move −9.7 and 10.1 ppm, respectively, as compared to their frequencies in the ^{13}C spectrum of **2**. Even though the magnitude of these chemical shift changes would be slightly larger than expected, they would still fall within acceptable limits, but the data would be inconsistent with other results arising from changing the C3 substituent, i.e., when a methyl group is replaced by a β-D-ribofuranosyl moiety (Table VIII). The C5 (151.4 ppm) and C7 (155.4 ppm) lines shifted downfield 0.4 and 4.5 ppm, respectively. This is in good agreement with the corresponding data (0.3 and 4.1 ppm) obtained for 7- and 9-methyladenines.[9] Although C3a occupies the β position in both **2** and **3**, its resonance frequency shifts −4.9 ppm in **3** as



2



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compared with its position in **2**. This result probably reflects the difference in electron density at this position in the two molecules.

The coupled spectrum of 2-methylformycin (**6**, in Me_2SO) provides an easy differentiation of the carbon resonances for this compound. The C3a signal corresponds to the pattern observed at 136.1 ppm. This resonance appears as a set of doublets arising from the coupling with H5 (^3J = 12.2 Hz) and

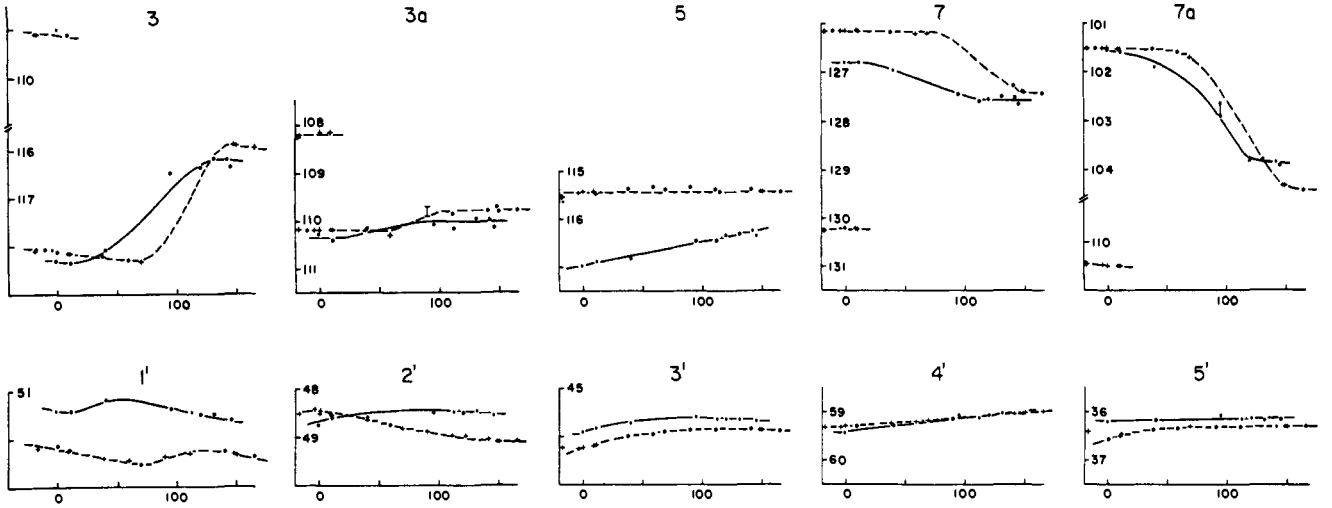


Figure 1. Carbon chemical shifts of formycin B (**10**) as a function of temperature. The chemical shifts are in parts per million with respect to cyclohexane. Portions of the curve were estimated when data were not available in a particular temperature region. Solvent: Me_2SO (—); $[(CH_3)_2N]_3PO$ (- - -).

with H1′ (^3J = 4.1 Hz). The C5 line (152.1 ppm) appears as a large doublet (^1J = 198.5 Hz) and the C7 resonance (155.8 ppm) is also split into a doublet (^3J = 9.8 Hz) due to the long-range coupling between the C7 carbon and the H5 proton. The C3 (133.4 ppm) and C7a (129.9 ppm) signals were assigned by analogy with the data from **3.** In both compounds, **6** and **3,** the intensity of the signal for C3 is larger than for C7a which agrees with the T_1 measurements performed on formycin (**4,** vide supra).

The chemical shifts[1a] and line widths[1a,7] of formycin (**4**) are strongly influenced by changes in temperature (see Table V and section III-B-3). At 40 °C, some lines are very broad; however, a coupled spectrum (in Me_2SO) obtained at this temperature provides sufficient information to assign all carbon resonances. The C3a signal appears as a doublet (^3J = 14.3 Hz) at 138.3 ppm, but the fine structure was not resolved. The large doublet (^1J = 198.7 Hz) observed at 151.4 ppm was assigned to the C5 signal while the doublet (^3J = 9.1 Hz) at 151.6 ppm, which arises from coupling with the H5 proton, was assigned to C7. The C3 and C7a signals which are very broad at this temperature were assigned by comparison of the data from 1-methylformycin (**5**) and 2-methylformycin (**6**). The chemical shifts of formycin in $[(CH_3)_2N]_3PO$ (HMPT) are very similar to those found in Me_2SO (Table V).

c. Pyrazolo[4,3-*d*]pyrimidin-7-one Derivatives. The chemical shifts for the category of compounds where the amino group at C7 is replaced by an oxo function are also strongly dependent on temperature (Table VI). At ambient temperature, the noise-decoupled spectrum of pyrazolo[4,3-*d*]pyrimidin-7-one (**7,** in Me_2SO) exhibits four broad lines and one narrow line. By subjecting this compound to a single-frequency off-resonance decoupling experiment, the narrow line at 142.9 ppm was transformed into a large doublet and was assigned to the C5 carbon. The broad line which was present in the noise-decoupled spectrum at 133.6 disappeared into the baseline. Therefore, this line was assigned to the C3 signal. The C7a and C7 resonances were assigned to the upfield (126.8 ppm) and downfield (153.2 ppm) singlets, respectively, by analogy with certain purine data.[9,17] The singlet at 139.0 ppm was assigned to C3a by default.

The ^{13}C spectrum of 3-methylpyrazolo[4,3-*d*]pyrimidin-7-one (**8,** in Me_2SO) was easily assigned by comparing the carbon chemical shifts with those of **7.** The addition of the methyl group at C3 shifts this carbon signal 5.9 ppm downfield and the frequency of the adjacent C3a carbon −2.2 ppm upfield. This assignment is consistent with the behavior of these lines vs. temperature (see section III-B-3). The limited solubility of 3,6-dimethylpyrazolo[4,3-*d*]pyrimidin-7-one (**9**) in Me_2SO prevented a thorough temperature study of this heterocycle. The data obtained for **9** in $[(CH_3)_2N]_3PO$ are summarized in Table VI. The methyl group at N6 affects mainly the C5 resonance causing a 3.7 ppm downfield substituent shift with respect to its position in **8.** This same trend is observed in 1-methylpurine nucleosides.[9]

The C3 (144.4 ppm) and C5 (143.2 ppm) signals in the spectrum of formycin B (**10,** in Me_2SO) were distinguished by a single-frequency off-resonance decoupling experiment. The remaining carbons of the heterocyclic aglycon were assigned by a comparison of the chemical shift data with those of **8.** As expected, the substitution of a 2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl moiety (**11**) or a β-D-ribofuranosyl moiety (**10**) for a methyl group influences mainly the C3 resonance position.

3. Temperature Dependence of ^{13}C NMR Data. The variation in carbon chemical shifts and line widths of formycin as a function of temperature were depicted earlier.[1a] We have now obtained additional data using cyclohexane as our internal standard and these new data indicate that for certain carbons the chemical shifts can fall into three distinct plateaus. This trend holds true in both solvents (Me_2SO and $[(CH_3)_2N]_3PO$) for those heterocycles and nucleosides which can undergo tautomerism. Figure 1 illustrates the effect of temperature on the carbon chemical shifts of formycin B (**10**) and is a typical example of this pattern. The aromatic carbon chemical shifts (except for the C5 resonance) vary linearly with temperature except for a positive (downfield) or negative (upfield) displacement which takes place over a short temperature range. As mentioned above, the C5 chemical shifts for all compounds examined do not exhibit any nonlinear variation over the temperature range employed in this study. This was also the case for all the aromatic carbon chemical shifts of **1, 2,** and **11.** The β-D-ribofuranosyl carbon chemical shifts exhibit little temperature dependence with the exception of the C1′ carbon chemical shift in formycin B. A similar occurrence was observed for the C3 methyl resonances in **8** and **9.** In these compounds, the C1′ chemical shift of formycin B (**10**) or the C3 methyl chemical shifts of **8** and **9** are strongly influenced by temperature and exhibit a nonlinear displacement similar to that described above. It is important to note that data obtained from solutions of different concentrations fit the same curves.

The temperature dependence of certain chemical shift data are summarized in Tables IV–VI. The use of concentrated solutions allowed us to collect data on nonviscous solutions below the freezing point (18 °C) of pure, dry Me_2SO. Using

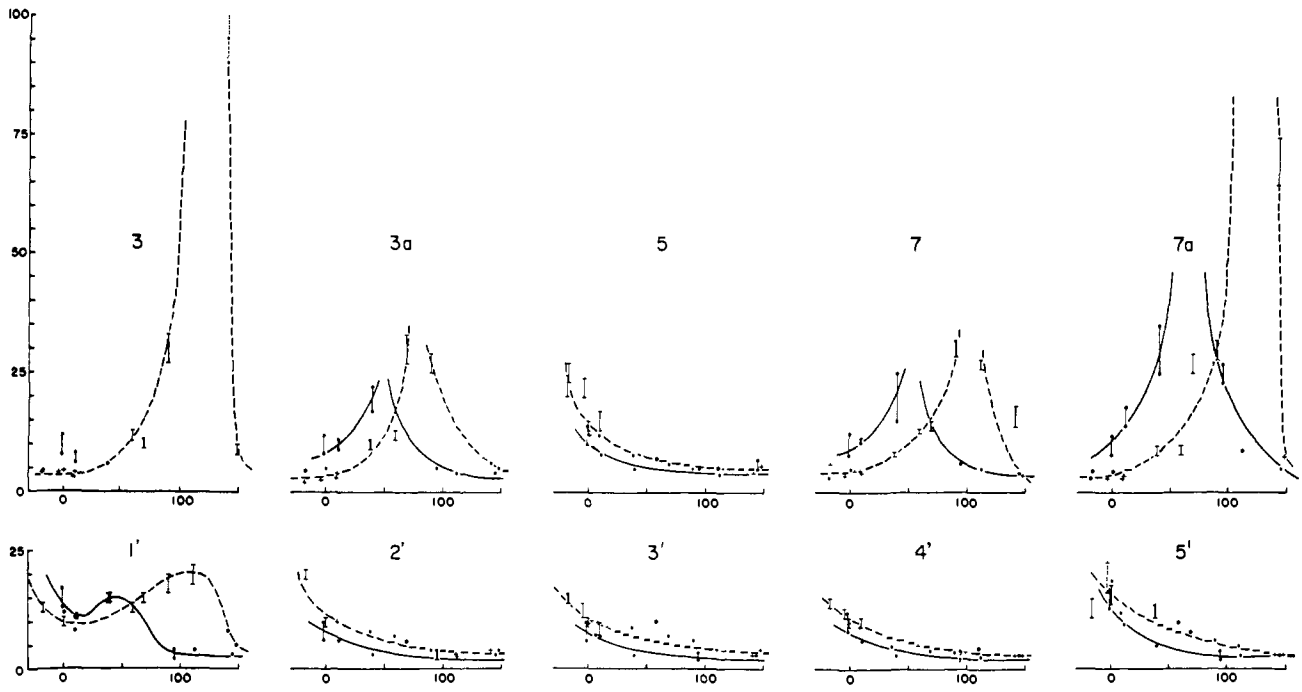


Figure 2. Carbon line widths of formycin B (**10**) as a function of temperature. Portions of the curve were estimated when data were not available in a particular temperature region. Solvent: Me_2SO (—); $[(CH_3)_2N]_3PO$ (- - -).

$[(CH_3)_2N]_3PO$, it was possible to freeze out both tautomeric species [N(1)H and N(2)H] of **7, 8, 9,** and **10.** The lines of the N(2)H tautomer were assigned by assuming that the variations of the chemical shifts of the C3 and C7a carbons are nearly equal in magnitude, but opposite in sign $\{[\delta_{C3}N(1)H - \delta_{C3}N(2)H] \simeq -[\delta_{C7a}N(1)H - \delta_{C7a}N(2)H]\}$. The linear variation data were determined in the slow exchange region and reflect the intrinsic temperature dependence of the chemical shifts of the tautomeric forms, apart from the equilibrium.

Substantial variations in line widths (Figure 2) are also observed for certain carbons. Quaternary carbon line widths remain narrow[18] as long as their corresponding chemical shifts fall on one of the three temperature plateaus. When the chemical shifts lie in the area of inflection, the lines become very broad and in some cases the signals disappear into the noise. On the other hand, the protonated carbons exhibit narrow lines at high temperatures and progressively broaden as the temperature is decreased. The C3 resonance in **7,** the C3 methyl resonance in **8** and **9,** and the C1′ carbon line in **10** display additional line broadening (Figure 2) in the range where their chemical shifts rise to a different plateau.

IV. Discussion

The observed variations in chemical shifts and line widths reflect the changes in the rate of prototropic tautomeric exchange.[1a] The C3 and C7a carbons which are adjacent to the N1 and N2 nitrogens of the pyrazole portion of the heterocyclic moiety are extremely sensitive to tautomeric averaging. Even the remote carbons C3a and C7 of aglycon, C1′ of the β-D-ribofuranosyl moiety, and the C3 methyl resonance are affected by this process.

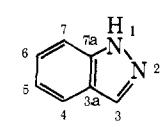
A. Evaluation of the Tautomeric Populations. It is possible to evaluate the populations of the two predominant tautomeric forms [N(1)H and N(2)H] of formycin B (**10**) and 3-methylpyrazolo[4,3-*d*]pyrimidin-7-one (**8**) in the slow exchange region. This can be accomplished by integrating the area under their resonance lines, assuming that they were recorded under identical saturation conditions. Taking into account the intrinsic temperature dependence of the chemical shifts of the tautomers apart from the equilibrium (see the linear variation data in Tables V and VI), the populations of the N(1)H and N(2)H tautomeric forms can also be determined in the fast exchange region. For the *i*th carbon which is affected by tautomerism, one has the relationship:

$$\% \text{ N(2)H} = \frac{\delta_i[\text{compound}] - \delta_i[\text{N(1)H}]}{\delta_i[\text{N(2)H}] - \delta_i[\text{N(1)H}]}$$

It was assumed that the chemical-shift differences between the two tautomers are similar in Me_2SO and $[(CH_3)_2N]_3PO$.

1. 3-(β-D-Ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (1). The chemical shifts of **1** varied linearly throughout the temperature range (6–145 °C) employed and the line widths for the quaternary carbon resonances remained narrow. Thus, this nucleoside exists in one predominant tautomeric form or is undergoing tautomerism at a very rapid rate of exchange on the NMR time scale. It is possible, however, to evaluate the chemical shifts of the N(1)H and N(2)H tautomers of **1** by a comparison of the chemical shift data determined for these tautomers in formycin B (**10**) and considering the substituent effect of the 7 oxo group[9] (Table VII). A comparison of the observed chemical shifts with the set of estimated values provides strong evidence that **1** exists predominantly in the N(1)H form over the temperature range used in this study. The only significant discrepancy in this analysis is for C7a where the experimental value does not fall near the range of the estimated value for the two forms [N(1)H and N(2)H]. Even in this case, however, the data preferentially support the N(1)H form.

In a similar study conducted on indazole (**12**) in methyl



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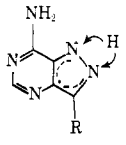
sulfoxide (30–145 °C), a continuous, linear variation of the carbon chemical shifts was also observed and the line widths for the carbon resonances remained narrow. This suggests that this heterocycle exists only in one tautomeric form and corroborates earlier reports[19] that indazole exists exclusively in one tautomeric form, the N(1)H species.

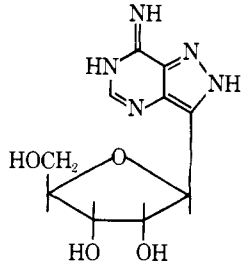
Table IX. Evaluation of the Tautomeric Populations of 7-Amino-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine

Compound[a]	Carbon	δ(6) − δ(5) (ppm)	Me_2SO δN(1)H–N(2)H − δN(1)H (ppm)	Me_2SO % N(2)H	$[(CH_3)_2N]_3PO$ δN(1)H–N(2)H − δN(1)H (ppm)	$[(CH_3)_2N]_3PO$ % N(2)H fast exchange
	C3	−8.7	−1.4	16	−0.2	2
	C3a	−4.2	(0.5)[b]	(12)[b]	−0.2	5
	C7	4.5	0.6	13	0.3	7
	C7a	7.7	1.3	17	0.5	6
				15 ± 2[c]		5 ± 2[c]

4

[a] R = β-D-ribofuranosyl. [b] Estimated value. [c] Standard deviation.

2. Formycin (4). It was not possible to observe the N(2)H tautomeric species of formycin at low temperatures. Presumably, this was due to the limited amount of this tautomer at these temperatures. However, a concentrated sample (in Me_2SO) did allow an accurate measurement of the N(1)H carbon chemical shifts in the slow-exchange rate region at low temperatures. The difference of the chemical shifts for 1-methylformycin (**5**) and 2-methylformycin (**6**) was used as an approximation for the difference of the chemical shifts of the two tautomeric species; δ[N(1)H] − δ[N(2)H]. It is difficult to correct these chemical shifts for substituent effects (methyl group vs. a hydrogen)[9] as only one of the carbons adjacent to the site of substitution is a bridgehead carbon. Thus, the α and β parameters[9] established for the purine system cannot be used. Since the displacement of the chemical shifts from one temperature plateau to another are small, this approximation should not induce a large error in evaluating the percentages of the tautomeric populations. The calculated percentages of the N(2)H tautomer are summarized in Table IX. They are consistent with recent experimental data[6b] obtained from magnetic circular dichroism spectroscopy which suggest that formycin in solution exists predominantly in the N(1)H form. The possibility of another tautomeric form (**13**) of formycin



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has recently been suggested.[20] This *imine* tautomer was estimated to account for 1% of the total equilibria. In formycin, amine–imine tautomerism would affect principally the C5 and C7 chemical shifts. Only a continuous linear variation was observed for the chemical shift of C5 in the range −14 to 145 °C, and on this basis it is concluded that the population of the *imine* tautomer is less than the calculations suggest.

3. Pyrazolo[4,3-*d*]pyrimidin-7-one Derivatives. The signals for both tautomeric forms [N(1)H and N(2)H] were observed in compounds **7, 8, 9,** and **10** using $[(CH_3)_2N]_3PO$ as solvent. Integration of the specific signals provided the percentage of the N(2)H tautomer, in the slow exchange region, for compounds **8** and **10.** For pyrazolo[4,3-*d*]pyrimidin-7-one (**7**) some lines were also observed corresponding to the N(2)H tautomer in Me_2SO (Table VII). The average of these data with those obtained from $[(CH_3)_2N]_3PO$ was used as the difference of the chemical shifts for the two tautomeric species.

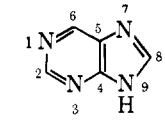
Because of the low solubility of 3-methylpyrazolo[4,3-*d*]-pyrimidine (**8**) in Me_2SO, it was not possible to obtain data below the coalescence temperature without freezing the solution. Therefore, to evaluate the chemical shifts of the N(1)H tautomer, the differences between the solvent effects of Me_2SO and $[(CH_3)_2N]_3PO$ were assumed to be the same in **8** as they were in **7.** This approximation permits the estimation of the tautomeric populations of **8** in Me_2SO solution.

It is obvious from the comparison of the chemical shift data of **10** and **11** (Table VI) that, in Me_2SO, **11** exists predominantly as the N(1)H tautomer in the temperature range in which it was studied.

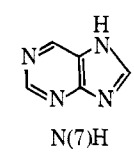
All N(2)H populations are summarized in Table X.

The excellent agreement between the data obtained for **8** and **9** (Tables VI and X) rules out any significant tautomeric process, i.e., lactam–lactim tautomerism, in the pyrimidine portion of these heterocycles. It also indicates that the labile hydrogen resides almost exclusively on the N6 position. Such conclusions can be extended to **7, 10,** and **11.** These findings are consistent with previous results[17] reported from this laboratory which have shown that hypoxanthine, inosine, and related derivatives exist predominantly in the lactam [N(1)-H][22] form.

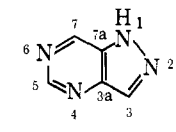
B. Factors Influencing the Tautomeric Populations. Prototropic tautomerism which occurs in the imidazole moiety of purine heterocycles does not affect the bond structure of the entire molecule. The nature of the substituent at C6,[22] however, does influence to a certain degree the relative populations of the N(7)H and N(9)H tautomers. In contrast, prototropic tautomerism in the pyrazole portion of the pyrazolo[4,3-*d*]-pyrimidine ring does influence the bond structure of this heterocycle. When the labile hydrogen resides on the N2 position, this heterocycle assumes a high degree of fixed bond character. Such structures can be expected to exhibit higher energy than the tautomeric N(1)H species. A case in point is 3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (**1**) where the population of the more favorable (lower energy) tautomer, N(1)H, has been determined to be 100% (see Table VII). On the other hand, it was shown (Tables IX and X) that the N(2)H tautomer which possesses more fixed bond character increases in population when an electron releasing substituent is attached to the C7 position. Thus, the observed differences in tautomeric populations of formycin (**4;** 85% N(1)H) and formycin B (**10;**



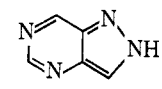
N(9)H



N(7)H

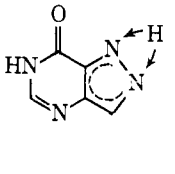


N(1)H

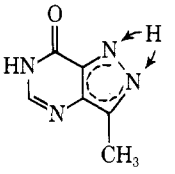


N(2)H

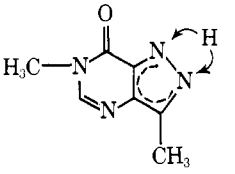
Table X. Evaluation of the Tautomeric Populations of Some Pyrazolo[4,3-*d*]pyrimidin-7-one Derivatives[a]



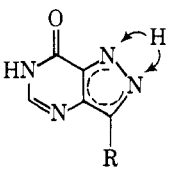
7



8



9



10

Carbon	δN(2)H − δN(1)H (ppm)	Me$_2$SO $\overline{\delta N(2)H-N(1)H}$ − δN(1)H (ppm)	Me$_2$SO % N(2)H fast exchange	[(CH$_3$)$_2$N]$_3$PO $\overline{\delta N(2)H-N(1)H}$ − δN(1)H (ppm)	[(CH$_3$)$_2$N]$_3$PO % N(2)H fast exchange	[(CH$_3$)$_2$N]$_3$PO % N(2)H slow exchange
			7			
C3	−10.3	−3.2	31	−3.1	30	
C3a	−2.9	−0.6	21	−0.5	17	
C7	3.8	1.1	29	1.1	29	
C7a	10.5	3.1	30	2.9	28	
			28 ± 5[b]		26 ± 6[b]	
			8			
C3	−9.1	−2.2[c]	24	−2.5	27	20
C3a	−2.7	−0.7[c]	26	−0.7	26	20
C7	4.2	1.2[c]	29	1.2	29	26
C7a	9.5	2.5[c]	26	2.7	28	28
CH$_3$	−2.5			−0.6	24	30
			26 ± 2[b]		27 ± 2[b]	25 ± 5[b]
			9			
C3	−8.9			−2.4	27	
C3a	−2.8			−0.8	29	
C7	3.9			1.3	33	
C7a	9.5			3.4	36	
					31 ± 4[b]	
			10			
C3	−9.0	−2.6	29	−2.7	30	20
C3a	−2.0			−0.4	20	30
C7	4.0	0.8	20	1.2	30	28
C7a	9.0	2.0	22	2.6	29	32
C1′	−2.3[d]	−0.5	22	−0.6	26	
			23 ± 4[b]		27 ± 4[b]	28 ± 5[b]

[a] R = β-D-ribofuranosyl. [b] Standard deviation. [c] See section IV-A-3. [d] Estimated value from C1′ data of **5** and **6**.

77% N(1)H) as compared to **1** are undoubtedly the result of the electronic contribution of the amino or oxo substituent to the heterocyclic aglycon.

The substituent which resides at position 3 of the aromatic aglycon, i.e., a hydrogen atom, methyl group, or β-D-ribofuranosyl moiety, does not influence the tautomeric populations in a given series (Table X). On the other hand, when the substituent at C3 is a 2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl group, as in the case of **11**, the N(1)H tautomer exists exclusively. Steric crowding of the N2 position may be an explanation for the predominance of this species.

In [(CH$_3$)$_2$N]$_3$PO solutions, the similarity of the linear variations of the chemical shifts for those compounds studied in the slow and fast exchange regions indicate that the tautomeric populations do not vary appreciably with temperature. This is corroborated by the evaluation of the tautomeric populations in both regions which are in good agreement (Table X). Therefore the enthalpy (ΔH) of the equilibrium between N(1)H $\rightleftharpoons$ N(2)H is probably very small. A similar conclusion was arrived at for the tautomeric equilibrium N(7)H $\rightleftharpoons$ N(9)H[22] in adenine.[23] In Me$_2$SO solutions, the lines corresponding to the N(2)H tautomeric form were generally not observed. Only in the case of pyrazolo[4,3-*d*]pyrimidin-7-one (**7**) were two of the carbon signals (C3 and C7) of this tautomer visible. This prevented any evaluation of the tautomeric populations in the slow exchange region. Furthermore, the somewhat high freezing points of the Me$_2$SO solutions also prohibited a thorough study of tautomerism in this same region. However, the limited amount of available data suggests that the linear variations of the chemical shifts, as a function of temperature, have similar slopes at low and high temperatures.[24] Therefore, it seems reasonable that the conclusion reached for the [(CH$_3$)$_2$N]$_3$PO solutions can be extended to the Me$_2$SO solutions.

Although our line-shape data were not precise enough to allow a detailed analysis, it was possible to evaluate the coalescence temperatures of the signals affected by the tautomeric process. Considering the equilibrium constants,[25] the line widths minus the contributions of tautomeric broadening, and the chemical shifts of the two tautomeric forms, N(1)H and N(2)H, a reasonable estimate of the enthalpy of activation, $\Delta H^{\ddagger}$, for the process N(1)H $\rightarrow$ N(2)H was made using the Eyring equation. In [(CH$_3$)$_2$N]$_3$PO solution, $\Delta H^{\ddagger}$ is $\simeq 8 \pm 2$ kcal/mol for compounds **7**, **8**, and **9**. This value increases slightly for formycin B (**10**). The enthalpies of activation decrease nearly 4 kcal in corresponding Me$_2$SO solutions. The larger enthalpies of activation in [(CH$_3$)$_2$N]$_3$PO most probably reflect some specific interaction between the labile hydrogen and the very polar hexamethylphosphoric triamide (the dipole moment for [(CH$_3$)$_2$N]$_3$PO is approximately 4.8 D and for Me$_2$SO it is 4.0 D).[26]

V. Conclusion

A quantitative study of tautomeric populations for certain pyrazolo[4,3-*d*]pyrimidines has been conducted using ^{13}C NMR spectroscopy. It was shown that prototropic tautomer-

ism causes variations on certain carbon chemical shifts and line widths, especially at those carbons which are directly adjacent to the nitrogens which participate in this phenomenon. In the case of formycin (**4**) no carbon chemical-shift variations were observed which would account for any appreciable amine–imine tautomerism. Similarly, lactam–lactim tautomerism for those derivatives which have a 7-oxo function was not observed. Our data establish that the labile hydrogen in this portion of the pyrazolo[4,3-*d*]pyrimidine ring resides exclusively on the nitrogen (N6) adjacent to the oxo function. Therefore, the major tautomeric process observed in those derivatives studied is N(1)H $\rightleftharpoons$ N(2)H. This prototropic process appears to be dependent on the substituent residing at C7, e.g., a hydrogen, amino, or oxo group. When a hydrogen atom is bonded to the C7 position of the pyrazolo[4,3-*d*]pyrimidine aglycon, the N(2)H tautomeric form was not detected. On the other hand, when an amino or oxo substituent is attached to this position, prototropic exchange between the N(1)H and N(2)H tautomers occurs. The data indicate that the less favorable (higher energy) N(2)H form is stabilized to a greater degree by the presence of an oxo group at this position as compared to an amino group. A possible explanation for the varying extent of tautomerism when the substituent at C7 is either a hydrogen, amino, or oxo group is their different electronic (resonance and/or inductive) influence on the pyrazolo[4,3-*d*]pyrimidine ring system.

Acknowledgments. Support for this work was provided by a grant from the Research Resources Branch, National Institutes of Health (RR-0574), and a research contract from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education and Welfare (NO1-CM-23710 and NO1-CM-43806).

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pH Dependence of Carbon-13 Nuclear Magnetic Resonance Shifts of Tetracycline. Microscopic Dissociation Constants

G. L. Asleson and C. W. Frank*

Contribution from the Department of Chemistry, University of Iowa, Iowa City, Iowa 52242. *Received November 14, 1975*

Abstract: Carbon signals of tetracycline hydrochloride were found to be very sensitive to pH in a 50/50 DMSO:water solvent system. The curves obtained by plotting the chemical shift of a signal vs. pH were related to the pK_a values which were determined to be 4.4, 8.1, and 9.8 in the mixed solvent system. These are compared to 3.3, 7.7, and 9.7 for tetracycline in aqueous solution. Microdissociation constants were determined and compared with values obtained by previous workers.

Proton NMR has been applied in the study of tetracyclines to elucidate stereochemical and structural features,[1-11] to determine microscopic dissociation constants,[12,13] to monitor the kinetics of epimerization at the 4 position,[14] and to study the binding sites of a number of metal ions.[15] Prior applications of ^{13}C NMR to tetracyclines have been the partial[16] and complete[17] assignment of ^{13}C NMR spectra, and studies concerning metal ion binding[18] and the effect of electrolytes on the metal binding sites of tetracycline.[19]

Previous discussions by Dias et al.[20] and Rigler et al.[12] indicated that it is possible that the microscopic dissociation constants of the tetracyclines are more reflective of the pro-