Carbon-13 Magnetic Resonance. XXV.[1a-c] A Basic Set of Parameters for the Investigation of Tautomerism in Purines Established from Carbon-13 Magnetic Resonance Studies Using Certain Purines and Pyrrolo[2,3-*d*]pyrimidines

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Abstract: Carbon-13 chemical-shift data have been obtained for certain pyrrolo[2,3-*d*]pyrimidines and purines. From these data, sets of substituent parameters were determined which can be used to correct the bridgehead carbon chemical shifts for the effect of substitution on purines, when a proton at either the N7 or N9 position is replaced by a β-D-ribofuranosyl moiety or a methyl group. Other substituent effects on aromatic carbon chemical shifts caused by replacing a hydrogen atom at the C6 position of purine with an amino, oxo, or thione function are discussed. The carbon chemical-shift differences (Δδ) between purines and their corresponding pyrrolo[2,3-*d*]pyrimidines were observed to be highly reproducible.

I. Introduction

The study of biological compounds by means of carbon-13 magnetic resonance spectroscopy (^{13}C NMR) has been dramatically accelerated since the advent of Fourier transform (FT) techniques. The increased sensitivity of modern FT spectrometers has reduced the solubility limitations which plagued earlier work employing continuous wave methods and now permits one to investigate problems which were intractable a few years ago.

One such problem concerns tautomerism in purines. Tautomerism plays a significant role in many biochemical functions of nucleic acids and has been extensively studied by instrumental as well as theoretical methods.[2] Carbon-13 spectroscopy can provide important insights toward understanding tautomerism, because it is possible to determine quantitative populations of the different tautomeric forms from chemical-shift data. On the NMR time scale, tautomeric proton averaging is usually in the fast exchange region, and thus one observes only the weighted average of the contributing tautomeric forms. In order to estimate the tautomeric populations, it is necessary to determine the carbon chemical shifts for each tautomeric species. These chemical shifts can be obtained from certain model purines where the labile proton is replaced by either a methyl group or a β-D-ribofuranosyl moiety. Since such substituents affect the chemical shifts differently when compared with a hydrogen atom, it is essential to make the proper corrections on the chemical shifts of the model compounds. We now report certain parameters which can be used to correct chemical shifts in purine derivatives. These corrections were found to be more significant on proximate carbons; therefore, only the substituent effects on bridgehead carbons, which we define as α and β parameters, are considered in this study.

Two approximations were employed to establish these parameters (Figure 1). First, it was assumed that the sets of α and β parameters are similar for substituents on either the 7- or 9-position, even though purine derivatives are not symmetrical. Second, the sets of α and β parameters were assumed to be approximately the same whether the five-membered ring of the heterocyclic aglycon contains one nitrogen atom (pyrrolo[2,3-*d*]pyrimidines) or two nitrogen atoms (purines).[3]

To establish the validity of these two assumptions and to determine the sets of α and β parameters, we have studied various pyrrolo[2,3-*d*]pyrimidines[4] and purines (Table I).

II. Experimental Section

Carbon-13 spectra were obtained with a Varian XL-100-15 equipped for Fourier transform operation. Compounds were dissolved in dry spectroquality dimethyl sulfoxide (Me_2SO), and the concentrations for each compound discussed are given in the appropriate table. All carbon chemical shifts were calculated relative to the internal reference (Me_2SO) and converted to the Me_4Si scale using the formula $\delta_{Me_4Si} = (\delta_{Me_2SO} + 40.2_2 \text{ ppm}) + 7.4 \times 10^{-3}T$, where T is the temperature in °C.[5] Proton spectra were determined using either a Varian A-56/60 or XL-100-12 spectrometer. The methods used for the synthesis of compounds can be found in Table I. 8-Deuterio-6-methoxy-9-(β-D-ribofuranosyl)purine, 8-deuterio-6-methoxypurine, and 8-deuterio-7-methylhypoxanthine were prepared according to the general procedure in ref 6b.

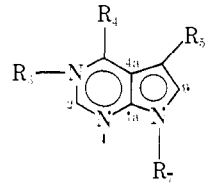
III. Results

Carbon-13 spectra were determined using noise decoupling and off-resonance conditions. This process permits a ready differentiation of quaternary carbons from the remaining carbons in the molecule. Selective proton decoupling experiments were conducted on certain compounds in order to facilitate the carbon assignments when any ambiguity existed between carbon lines.

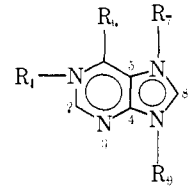
A. Proton Chemical Shifts. The ^1H NMR spectrum of pyrrolo[2,3-*d*]pyrimidine (I), excluding the NH signal, contains two singlets and two doublets. The singlets at 8.82 and 9.04 ppm were assigned to H2(H2)[4] and H4(H6), respectively, on the basis of previous ^1H NMR studies[6] conducted on purine (Table II). Likewise, previous ^1H NMR studies[7] on pyrrolo[2,3-*d*]pyrimidines made possible the assignment of the downfield doublet at 7.57 ppm to H6(H8) and the upfield doublet at 6.57 ppm to H5(N7).

The similarity with the proton chemical shifts (Table II) in purine (whose proton resonance sequence is H6, H2, and H8 for increasing field[7]) made the ^1H NMR assignments straightforward for 7-methylpurine (XII), 9-methylpurine (XIII), and nebularine (XIV). 6-Methoxy-9-(β-D-ribofuranosyl)purine (XXIII) with deuterium at C8 allowed us to distinguish between H2 (8.54 ppm) and H8 (8.62 ppm). Using 8-deuterio-6-methoxypurine, we have confirmed earlier findings[8] that the H8 signal (8.34 ppm) of 6-methoxypurine is upfield from the H2 resonance (8.49 ppm). Thus,

Table I. Nomenclature of the Studied Pyrrolo[2,3-*d*]pyrimidine and Purine Derivatives



	R_3	R_4	R_5	R_7		Synthetic method
I		H	H	H	Pyrrolo[2,3-*d*]pyrimidine	*a*
II		H	H	Ribose	7-(β-D-Ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine	*b*
III		NH_2	H	H	4-Aminopyrrolo[2,3-*d*]pyrimidine	*a*
IV		NH_2	H	CH_3	4-Amino-7-methylpyrrolo[2,3-*d*]pyrimidine	*c*
V		NH_2	H	Ribose	4-Amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (tubercidin)	*d*
VI		NH_2	CN	Ribose	4-Amino-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (toyocamycin)	*d*
VII		NH_2	$CONH_2$	Ribose	4-Amino-5-carboxamido-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (sangivamycin)	*e*
VIII	H	O	H	H	Pyrrolo[2,3-*d*]pyrimidin-4-one	*a*
IX	H	O	H	Ribose	7-(β-D-Ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one	*b*
X	H	S	H	H	Pyrrolo[2,3-*d*]pyrimidine-4-thione	*a*
XI	H	S	H	Ribose	7-(β-D-Ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-4-thione	*b*



	R_1	R_6	R_7	R_9		Synthetic method
XII		H	CH_3		7-Methylpurine	*f*
XIII		H		CH_3	9-Methylpurine	*f*
XIV		H		Ribose	9-(β-D-Ribofuranosyl)purine (nebularine)	*g*
XV		NH_2	CH_3		7-Methyladenine	*h*
XVI		NH_2	Ribose		7-(β-D-Ribofuranosyl)adenine	*i*
XVII		NH_2		CH_3	9-Methyladenine	*h, j*
XVIII		NH_2		Ribose	9-(β-D-Ribofuranosyl)adenine (adenosine)	*d*
XIX	H	O	CH_3		7-Methylhypoxanthine	*k*
XX	H	O	Ribose		7-(β-D-Ribofuranosyl)hypoxanthine	*i*
XXI	H	O		Ribose	9-(β-D-Ribofuranosyl)hypoxanthine (inosine)	*d*
XXII	CH_3	O		Ribose	1-Methyl-9-(β-D-ribofuranosyl)hypoxanthine	*l*
XXIII		OCH_3		Ribose	6-Methoxy-9-(β-D-ribofuranosyl)purine	*m*
XXIV	H	S	CH_3		7-Methylpurine-6-thione	*k*
XXV	H	S	Ribose		7-(β-D-Ribofuranosyl)purine-6-thione	*n*
XXVI	H	S		Ribose	9-(β-D-Ribofuranosyl)purine-6-thione (6-mercaptopurine riboside)	*m*
XXVII	CH_3	S		Ribose	1-Methyl-9-(β-D-ribofuranosyl)purine-6-thione	*o*
XXVIII		SCH_3		Ribose	6-Methylthio-9-(β-D-ribofuranosyl)purine	*p*

[a] J. Davoll, *J. Chem. Soc.*, 131 (1960). [b] J. F. Gerster, B. Carpenter, R. K. Robins, and L. B. Townsend, *J. Med. Chem.*, 10, 326 (1967). [c] R. H. Hammer, *J. Pharm. Sci.*, 55, 1096 (1966). [d] Commercial sources. [e] R. L. Tolman, R. K. Robins, and L. B. Townsend, *J. Am. Chem. Soc.*, 91, 2102 (1969). [f] R. J. Pugmire, D. M. Grant, L. B. Townsend, and R. K. Robins, *ibid.*, 95, 2791 (1973). [g] G. B. Brown and V. S. Weliky, *J. Biol. Chem.*, 204, 1019 (1953). [h] E. C. Taylor and P. K. Loeffler, *J. Am. Chem. Soc.*, 82, 3147 (1960). [i] R. J. Rousseau, R. K. Robins, and L. B. Townsend, *ibid.*, 90, 2661 (1968). [j] G. Shaw and D. N. Butler, *J. Chem. Soc.*, 4040 (1959). [k] R. N. Prasad and R. K. Robins, *J. Am. Chem. Soc.*, 79, 6401 (1957). [l] J. W. Jones and R. K. Robins, *ibid.*, 85, 193 (1963). [m] J. A. Johnson, Jr., H. J. Thomas, and H. J. Schaeffer, *ibid.*, 80, 669 (1958). [n] R. J. Rousseau, R. P. Panzica, S. M. Reddick, R. K. Robins, and L. B. Townsend, *J. Org. Chem.*, 35, 631 (1970). [o] The authors thank Dr. G. H. Milne and Professor A. D. Broom for an authentic sample of XXVII and the synthetic method for the preparation of this compound. [p] J. J. Fox, I. Wempen, A. Hampton, and I. L. Doerr, *J. Am. Chem. Soc.*, 80, 1669 (1958).

the downfield shift observed for the H8 resonance in XXIII results from the presence of a β-D-ribofuranosyl moiety at N9. This is consistent with an earlier report[9] that a ribosyl moiety can exert a deshielding effect on hydrogens at ring positions directly adjacent to the site of glycosylation. We have also observed this same effect for nebularine (XIV) with respect to purine (Table II). This deshielding effect also enabled us to assign the spectrum of 7-(β-D-ribofuranosyl)hypoxanthine (XX) by comparison with data obtained for 7-methylhypoxanthine (XIX) and 8-deuterio-7-methylhypoxanthine. The H2 signals of XIX and XX are very similar (8.02 and 7.95 ppm, respectively), but the H8 resonance in XX moves downfield 0.45 ppm with respect to the H8 signal observed in XIX. In the ^1H NMR spectrum of 1-methyl-9-(β-D-ribofuranosyl)hypoxanthine (XXII), the singlets at 8.34 and 8.40 ppm were assigned to H8 and H2, respectively, on the basis of the patterns observed in the ^{13}C NMR spectra from selective proton decoupling experiments.

B. Carbon-13 Chemical Shifts. Carbon-13 chemical shifts for the pyrrolo[2,3-*d*]pyrimidines and purines are summarized in Tables III and IV, respectively. As previously reported,[10] the resonance positions of the ribose carbon atoms do not exhibit large deviations because of variations of the aglycon in *N*-nucleosides. The resonance line sequence (from downfield to upfield) has been established[11] as: C1′,

Table II. Pertinent Proton Chemical Shifts[a] in Pyrrolo[2,3-*d*]pyrimidine and the Aglycon Moiety of Certain Purine Derivatives

Compd	Concn, *M*	Aglycon Pp H2 / Pu H2	H4 / H6	H5	H6 / H8
I[b]	1.4	8.82	9.04	6.57	7.57
XII[c]	0.61	8.96	9.17		8.62
XIII[c]	1.3	8.97	9.17		8.60
XIV[c]	0.60	8.95	9.19		8.85
XIX[c]	0.30	7.95			8.14
XX[c]	0.16	8.02			8.59
XXII[c]	0.47	8.40			8.34
XXIII[c]	0.47	8.54			8.62
Purine[c]	2.1	8.85	9.05		8.54

[a] Chemical shifts are in parts per million with respect to Me_4Si. Temperature ~37°. Solvent Me_2SO. [b] Pyrrolo[2,3-*d*]pyrimidine (Pp) numbering. [c] Purine (Pu) numbering.

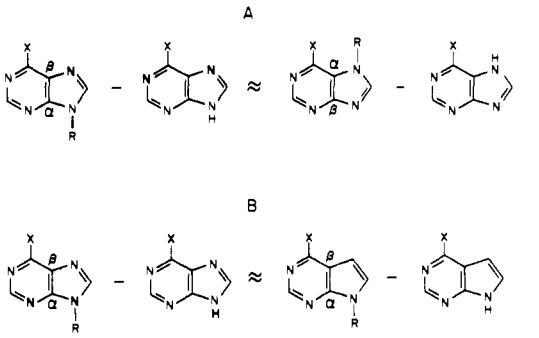


Figure 1. A. First Assumption. The sets of α and β parameters are similar for substituents (R) on either the 7- or 9-position of purines. B. Second Assumption. The sets of α and β parameters are approximately the same whether the five-membered ring of the heterocyclic aglycon contains one nitrogen atom (pyrrolo[2,3-*d*]pyrimidines) or two nitrogen atoms (purines).

Table III. Carbon-13 Chemical Shifts[a] in Pyrrolo[2,3-*d*]pyrimidine Derivatives

Compd	Concn, *M*	*T*, °C	Aglycon C2(C2)[f]	C4(C6)	C4a(C5)	C5(N7)	C6(C8)	C7a(C4)	Ribose C1'	C2'	C3'	C4'	C5'
I	1.4	40	150.8_8	148.8_8	118.2_2	99.4_5	127.2_4	151.2_5					
II	0.53	37	151.0_8	149.6_2	119.3_4	100.5_7	127.8_7	150.7_9	86.9_0	74.2_2	70.8_0	85.3_9	61.8_0
III[b]	0.50	32	151.1_9	157.2_3	102.3_0	99.2_0	121.5_4	150.0_0					
IV[b,c]	0.62	40	151.6_0	157.4_5	102.3_8	98.3_1	124.9_6	149.9_2					
V[b]	0.50	32	151.3_5	157.4_2	103.2_8	99.8_0	122.8_1	149.5_5	87.8_5	73.7_9	70.7_7	85.1_6	61.8_7
VI[d]	0.46	37	153.7_6	157.2_2	101.5_8	83.2_7	132.6_1	150.3_2	88.1_6	74.5_6	70.4_8	85.7_4	61.4_9
VII[e]	0.43	37	153.0_7	158.3_7	101.4_9	111.2_7	126.0_9	151.1_0	87.7_4	74.2_1	70.9_1	85.6_8	62.1_9
VIII	0.89	37	143.3_7	158.7_2	107.8_4	102.2_0	120.5_5	148.2_6					
IX	0.75	37	144.0_0	158.5_3	108.6_5	102.7_7	121.4_4	148.0_1	87.3_6	74.5_6	70.8_3	85.3_3	61.8_8
X	0.88	29	142.8_4	176.3_1	119.9_9	104.4_0	123.8_0	143.6_8					
XI	0.71	37	143.5_0	176.6_7	120.7_9	104.9_6	124.2_9	143.4_7	87.1_4	74.6_3	70.7_2	85.4_3	61.6_7

[a] Chemical shifts are in parts per million with respect to Me_4Si. [b] Chemical shifts observed with respect to internal dioxane and converted to the Me_4Si scale using the formula $\delta_{Me_4Si} = \delta_{dioxane} + (2.0 \times 10^{-3})T$ (°C) + 66.2_8 ppm.[5] [c] $\delta_{NCH_3} = 30.6_7$ ppm. [d] $\delta_{C\equiv N} = 115.5_5$ ppm. [e] $\delta_{C=O} = 166.7_2$ ppm. [f] Reference 4.

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

Compd	Concn, *M*	*T*, °C	Aglycon CH_3	C2	C4	C5	C6	C8	Ribose C1'	C2'	C3'	C4'	C5'
XII	0.61	28	31.6_4	152.0_4	159.8_8	125.7_9	140.7_2	149.7_8					
XIII	1.3	40	29.3_5	151.8_6	151.3_8	133.4_6	147.4_4	147.4_4					
XIV	0.60	40		152.2_2	151.0_9	134.2_8	148.3_2	145.5_3	87.7_3	73.9_4	70.4_7	85.8_6	61.4_2
XV	0.07	32	33.7_6	152.3_1	159.8_2	111.7_7	151.9_1	145.9_4					
XVI	0.37	37		152.8_5	160.7_4	110.2_7	151.7_3	144.6_4	89.4_7	75.0_5	69.0_3	86.4_5	60.5_4
XVII	0.09	32	29.3_9	152.5_0	149.9_4	118.7_2	155.9_8	141.4_7					
XVIII	0.75	37		152.6_0	149.2_7	119.5_7	156.3_0	140.2_0	88.2_4	73.7_7	70.9_1	86.1_6	61.9_1
XIX	0.30	35	33.3_1	144.3_7	157.0_2	115.4_8	154.6_3	144.3_7					
XX	0.16	37		144.8_0	157.7_2	114.7_9	154.1_4	142.4_9	89.4_7	75.1_5	69.7_8	85.4_8	61.0_6
XXI	Satd soln	37		146.1_9	148.4_6	124.6_3	156.8_8	139.1_1	87.8_2	74.4_3	70.5_9	85.9_1	61.5_8
XXII	0.47	40	33.5_6	148.7_7	147.6_3	123.6_8	156.4_2	139.2_2	87.5_6	74.2_0	70.4_1	85.7_3	61.4_1
XXIII	0.47	40	54.0_3	151.6_7	151.8_3	121.2_4	160.4_6	142.3_3	87.8_0	73.8_8	70.5_9	85.8_2	61.4_0
XXIV	0.64	40	34.6_6	144.7_4	152.6_8	125.8_7	170.4_0	148.3_3					
XXV	0.40	39		144.9_9	153.3_4	125.3_2	169.8_3	144.9_9	89.1_4	75.7_5	68.9_8	84.6_8	60.3_6
XXVI	0.70	40		145.4_9	144.1_2	135.6_1	176.1_9	141.4_5	87.9_3	74.5_5	70.4_5	85.9_2	61.3_9
XXVII	0.45	39	40.4_2	148.4_9	142.0_1	135.7_8	177.4_3	141.6_0	87.6_7	74.3_2	70.2_8	85.7_8	61.2_5
XXVIII	0.50	38	11.2_6	151.5_3	148.0_1	131.3_4	160.4_6	143.0_6	88.0_2	73.9_6	70.3_8	85.8_3	61.3_7

[a] Chemical shifts are in parts per million with respect to Me_4Si.

C4′, C2′, C3′, and C5′. To simplify the presentation of the large quantity of data derived in the present study, we have divided the data related to the heterocyclic aglycons into two categories: (1) the pyrrolo[2,3-*d*]pyrimidines; and (2) the purines.

1. Assignments of the Pyrrolo[2,3-*d*]pyrimidine Aglycon Resonances.[4] Figure 2 shows the correlation diagram for the carbon-13 spectra of several pyrrolo[2,3-*d*]pyrimidines (I, III, VIII, and X). The upfield region of the off-resonance spectra exhibits two large doublets and a singlet for each compound. The methine carbons, with chemical shifts in the range 99.2–104.4 ppm, were assigned to C5(N7), while the remaining methine carbon lines (range from 120.6 to 127.2 ppm) were assigned to C6(C8) by analogy with the chemical shifts of the β and α carbons in pyrrole ($\delta_{C_\beta} = 108$ and $\delta_{C_\alpha} = 118$ ppm).[12] By analogy with certain purines,[13,14] the upfield quaternary resonance lines were assigned to C4a(C5). These signals exhibit a strong dependence on the

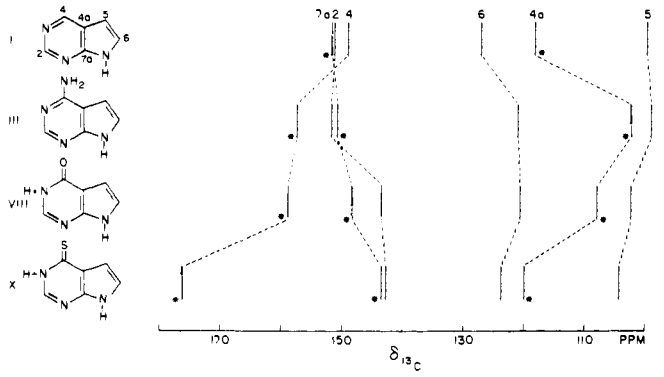


Figure 2. Correlation diagram of certain pyrrolo[2,3-*d*]pyrimidines showing the effect of the C4 substituent on the ^{13}C chemical shifts (with respect to Me_4Si). The asterisks refer to quaternary carbon lines.

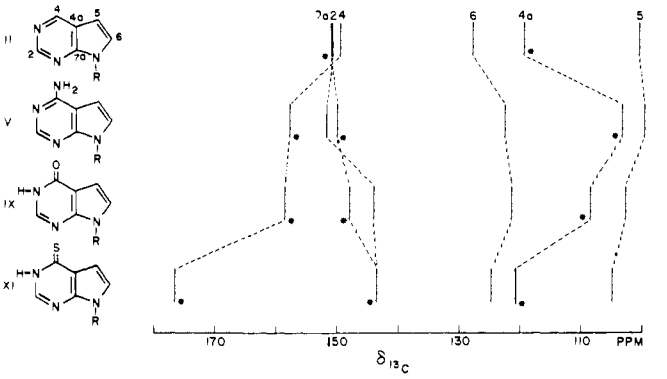


Figure 3. Correlation diagram of certain pyrrolo[2,3-*d*]pyrimidine nucleosides (R = β-D-ribofuranosyl) showing the effect of the C4 substituent on the ^{13}C chemical shifts (with respect to Me_4Si). The asterisks refer to quaternary carbon lines.

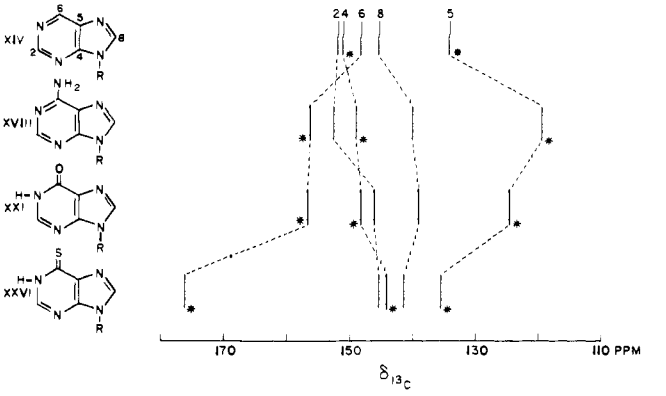


Figure 4. Correlation diagram of certain purine nucleosides (R = β-D-ribofuranosyl) showing the effect of the C6 substituent on the ^{13}C chemical shifts (with respect to Me_4Si). The asterisks refer to quaternary carbon lines.

nature of the substituent at C4(C6), in contrast to the C5(N7) and C6(C8) resonance positions which exhibit narrower ranges of chemical-shift variations. It is noteworthy that such variations on the resonance positions of C5(N7) and C6(C8) reflect a long-range inter-ring effect caused by substitution at C4(C6) (see Table VIII and section IV-B). The downfield portion of the off-resonance spectrum of I consists of two doublets and a singlet. This singlet obviously corresponds to C7a(C4), and we used a proton selective decoupling experiment to assign the doublets at 150.9 and 148.9 ppm to C2(C2) and C4(C6), respectively. For compounds III, VIII, and X, the downfield doublet was assigned to C2(C2). The resonance positions of C4(C6) and C7a(C4) were assigned on the assumption that the former should be much more sensitive to substituent effects than the latter. This assumption is substantiated by the study of substituent effects in benzene, pyridine,[15a] and pyrimidine.[15b]

Since the effect of substitution of a β-D-ribofuranosyl moiety for a proton at N7(N9) is expected to be small, there is no ambiguity in assigning the spectra of the nucleosides II, V, IX, and XI (see Figure 3) by analogy with those of their parent heterocycles (I, III, VIII, and X, see Figure 2). Also, the chemical shifts of III and IV are similar (see Table III).

Because of their biological importance,[16] the nucleoside antibiotics toyocamycin (VI) and sangivamycin (VII) were included in this series of pyrrolo[2,3-*d*]pyrimidine derivatives. As expected, substitution at C5(N7) affects mainly the C5(N7) and C6(C8) resonance positions. For toyocamycin (VI), the C5(N7) resonance was assigned to the singlet at 83.3 ppm. This −16.5 ppm upfield shift from its resonance position in tubercidin (V) is in good agreement with the −15.5 ppm shift due to substitution of a cyano group in benzene and pyridine.[15a] The resonance of C6(C8) exhibits a downfield shift of 9.8 ppm, while only small perturbations were observed on the chemical shift of the C4(C6), C4a(C5), and C7a(C4). A long-range inter-ring effect influences the resonance position of C2(C2) by 2.4 ppm. The cyano resonance was found at 115.6 ppm, similar to the corresponding value (118.7 ppm) in benzonitrile.[17]

The resonance position of C4a(C5) is not very sensitive to the nature of the substituent at C5(N7) (103.3 ppm in V and 101.6 ppm in VI), allowing us to assign the singlet observed at 101.5 ppm in the sangivamycin (VII) spectrum as the C4a(C5) resonance. The C6(C8) signal, which is readily identified by its doublet pattern, exhibits a downfield shift of 3.3 ppm with respect to its frequency in V, while the C2(C2) and C7a(C4) resonances were readily assigned by analogy with the chemical shifts in V and VI. Again a long-range inter-ring effect of 1.7 ppm on the C2(C2) resonance position is observed. The singlet at 111.3 ppm was assigned to C5(N7) by default. This line exhibits a downfield shift of 11.5 ppm (compared with V) because of substitution of a $CONH_2$ group for a hydrogen.

2. Assignments of the Purine Aglycon Resonances. Purine Derivatives. ^{13}C NMR spectra of aqueous solutions of methyl derivatives of purine have already been reported.[14] The C4, C5, and C6 resonances for 7-methylpurine (XII) in Me_2SO were assigned by analogy with the data obtained for their resonance positions in water. Assignments of the C2 and C8 signals were made by a selective proton decoupling experiment. For 9-methylpurine (XIII) in Me_2SO, the change of solvent affects mainly the C8 frequency. A selective proton decoupling experiment demonstrated that the C8 resonance moves upfield directly beneath the C6 line (147.4 ppm). The assignment of the other lines was straightforward.

In nebularine (XIV), the upfield singlet (134.3 ppm) was assigned to C5 by comparison with other purine data.[10,13,14] Selective proton decoupling experiments were used to distinguish between the three doublets: C2 at 152.2 ppm; C6 at 148.3 ppm; and C8 at 145.5 ppm. Assignments of the C2 and C4 resonance positions were reversed with respect to previous work in this laboratory using a field sweep instrument.[10] The high sensitivity of the FT technique permitted off-resonance analysis of the spectrum which proved that the correct sequence for carbon-13 resonances in nebularine is C2, C4, C6, C8, and C5 in the direction of increasing field.

Adenine Derivatives.[18] The data and assignments for adenosine (XVIII, Table IV) are consistent with previous findings in this laboratory.[10] When the site of attachment of the β-D-ribofuranosyl moiety is changed from N9 (XVIII) to N7 (XVI), one expects a downfield shift of the C4 signal approximately equivalent to an upfield shift on the C5 resonance by analogy with the data obtained for 9-methylpurine (XIII) and 7-methylpurine (XII) (vide supra). In good agreement with these data are the assignments for XVI which denote a downfield shift of 11.5 ppm for the C4 signal when compared with its position in XVIII and an upfield shift of −9.30 ppm for the C5 line (Figure 5 and Table V). The C6 resonance moves upfield −4.6 ppm because of a steric interaction between the β-D-ribofuranosyl moiety and the amino group and subsequent charge compression in the H_2N-C bond.[20] The C8 resonance position moves downfield 4.4 ppm while the C2 resonance position is nearly insensitive to the site of ribosylation on the heterocyclic moiety (N7 or N9). Assignments of 7-methyladenine (XV) and 9-methyladenine (XVII) were made by analogy with those of their nucleoside analogs (XVI and XVIII, see Figure 5 and Table V). A peri effect[21] shifts the methyl resonance in XV downfield (4.4 ppm) with respect to its position in XVII.

Hypoxanthine Derivatives. The off-resonance spectrum of inosine (XXI) revealed that the initial assignments[10] of C2 and C4 must be reversed. The correct order of the carbon resonances with respect to increasing field is: C6, C4, C2, C8, and C5.

As expected for 7-(β-D-ribofuranosyl)hypoxanthine (XX), one observes a downfield shift for the C4 signal (9.3 ppm) and an upfield shift for the C5 resonance (−9.8 ppm) with respect to inosine (Figure 5 and Table V). Steric interaction between the ribose moiety and the 6-oxo function causes the upfield shift (−2.7 ppm) of the resonance position of C6. The reverse assignments for C4 and C6 would not reflect any steric effect at C6. Furthermore, if the opposite assignments were made, the downfield shift for the C4 signal (5.7 ppm) would be inconsistent with the −9.8 ppm upfield shift observed for the C5 line with respect to inosine. The C2 resonance position was distinguished from the C8 signal by a selective proton decoupling experiment. In the case of XIX, methyl substitution at N7 produces effects similar to those observed for XX. The C2 and C8 signals are accidently degenerate (Table IV, see section IV-A for further explanation).

The coupled spectrum of 1-methyl-9-(β-D-ribofuranosyl)hypoxanthine (XXII) allowed us to assign the resonance positions of C2 and C8 from their long-range coupling patterns. The C2 resonance, at 148.8 ppm, is split into a doublet by its coupling with H2 (^1J = 207.8 Hz), then into a quartet by its coupling with the methyl group (^3J = 4.1 ± 0.2 Hz). The C-H coupling pattern of C8 exhibits a doublet of doublets, the first with H8 (^1J = 215.5 Hz) and the second with H1′ (^3J = 3.7 ± 0.6 Hz). Since the chemical shifts of C2 and C8 are quite similar to their corresponding values in inosine (XXI), then the resonance positions of C4, C5, and C6 can be assigned by analogy with the data obtained for these carbons in XXI.

Selective proton decoupling experiments distinguished the C2 signal (151.7 ppm) from the C8 resonance (142.3 ppm) in 6-methoxy-9-(β-D-ribofuranosyl)purine (XXIII). The C4 and C6 resonances were assigned to the singlets at 151.8 and 160.5 ppm, respectively. The reverse assignment would involve a downfield shift for the C4 signal of about 12 ppm (with respect to the corresponding position in inosine) which seems unlikely. Furthermore, our assignment of the C6 line is in agreement with the chemical shift C2 (163.1 ppm) in 2-methoxypyridine.[22] As usual, the upfield

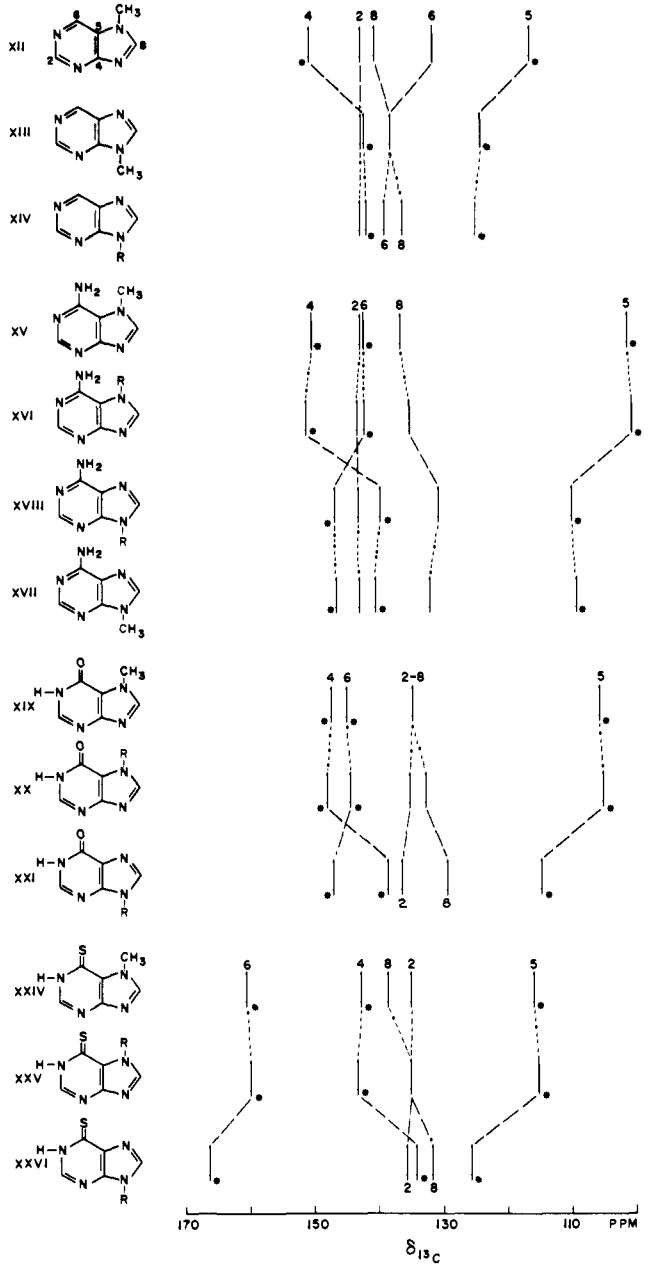
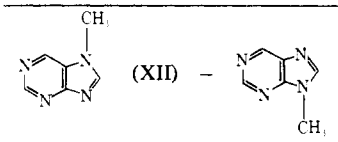
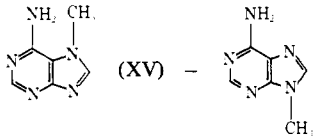
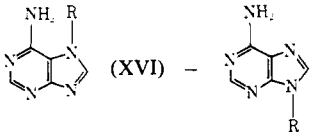
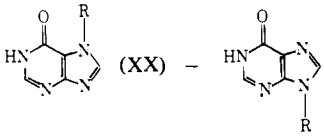
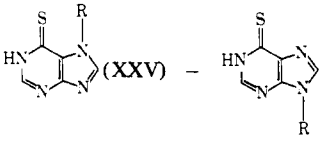


Figure 5. Correlation diagram of certain 7- and 9-substituted purine pairs (where R = β-D-ribofuranosyl). The ^{13}C chemical shifts are with respect to Me_4Si. The asterisks refer to quaternary carbon lines. The large dashed (— — —) lines connect signals of similar pairs, while the small dashed (- - -) lines connect signals from methyl to β-D-ribofuranosyl derivatives.

singlet was assigned to the C5 resonance.

Mercaptopurine Derivatives. The data obtained for 9-(β-D-ribofuranosyl)purine-6-thione (XXVI) are consistent with earlier results from this laboratory.[10] When the site of ribosylation is changed from N9 (XXVI) to N7 (XXV), the differences between the chemical shifts are consistent with those for similar structural changes in the adenine (XVIII and XVI) or hypoxanthine (XXI and XX) nucleosides (Figure 5 and Table V). In XXV, the C6 resonance moves upfield (−6.4 ppm) as a result of steric interaction of the thione function with the ribose moiety. As expected, the magnitude of the chemical-shift changes (from XXV to XXVI) for C4 and C5 resonances is nearly identical but opposite in sign, 9.2 and −10.3 ppm for C4 and C5 signals, respectively. In XXV, the resonance positions for C2 and C8

Table V. Variations of Carbon-13 Chemical Shifts ($\Delta\delta$ in ppm) When the Site of Substitution for a Methyl or a β-D-Ribosyl (R) Group Changes from N7 to N9 in Purines

Structures (A – B)[a]	Aglycon ($\Delta\delta$)						Ribose ($\Delta\delta$)				
	CH_3	C2	C4	C5	C6	C8	C1'	C2'	C3'	C4'	C5'
 (XII) – (XIII)	2.3	0.2	8.5	−7.7	−6.7	2.3					
 (XV) – (XVII)	4.4	−0.2	9.9	−7.0	−4.1	4.5					
 (XVI) – (XVIII)		0.3	11.5	−9.3	−4.6	4.4	1.2	1.3	−1.9	0.3	−1.4
 (XX) – (XXI)		−1.4	9.3	−9.8	−2.7	3.4	1.7	0.7	−0.8	−0.4	−0.5
 (XXV) – (XXVI)		−0.5	9.2	−10.3	−6.4	3.5	1.2	1.2	−1.5	−1.2	−1.0

[a] (A – B) = (N7 substituted – N9 substituted).

are accidentally degenerate at 145.0 ppm because of a downfield shift (3.5 ppm) of C8 signal as compared with XXVI. When a methyl group is substituted at N7 (XXIV) instead of a β-D-ribofuranosyl moiety (XXV), the only noticeable change in the ^{13}C NMR spectrum occurs at the C8 resonance position which shifts downfield (3.3 ppm, see section IV-A).

In the 1-methyl-9-(β-D-ribofuranosyl)purine-6-thione (XXVII) off-resonance spectrum, the doublet at 148.5 ppm was assigned to the C2 signal by analogy with the chemical shift of this carbon (145.4 ppm) in 9-(β-D-ribofuranosyl)-purine-6-thione (XXVI). The remaining doublet (141.6 ppm) can then be assigned to the C8 resonance. As usual, the upfield singlet (135.8 ppm) was assigned to C5. The downfield singlet (177.4 ppm) belongs to C6 by analogy with XXVI. Therefore, the singlet at 142.0 ppm was assigned to the C4 resonance by default.

The chemical shifts for the lines in the 6-methylthio-9-(β-D-ribofuranosyl)purine (XXVIII) off-resonance spectrum were assigned by analogy with those shifts in 6-methylthiopurine (see the following paper in this issue[27]).

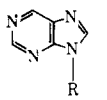
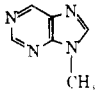
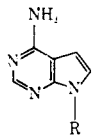
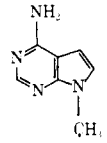
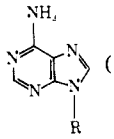
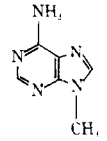
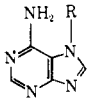
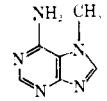
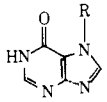
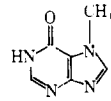
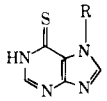
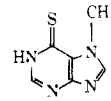
IV. Discussion

A. Validity of the Assumption That the Sets of α- and β-Substituent Parameters Are Similar for Substituents on Either the 7- or 9-Position of Purine Derivatives. A series of adenine derivatives with a β-D-ribofuranosyl moiety or a methyl group at N7 or N9 was chosen to test this assumption. The changes ($\Delta\delta$, Table VI) observed on the C5 (α_{N_7} = −1.5 ppm) and C4 (β_{N_7} = 0.9 ppm) chemical shifts for N7 substituted adenines (XVI − XV) are similar to those obtained on C4 (α_{N_9} = −0.7 ppm) and C5 (α_{N_9} = 0.9 ppm) for N9 adenine derivatives (XVIII − XVII). Although the purine ring is not symmetrical, the above data show that the set of α- and β-substituent parameters depends little on the position (N7 or N9) of substitution. Therefore, it is possible to neglect the differences between the α_{N_7} and α_{N_9} or β_{N_7} and β_{N_9} values on a first approximation basis. Further support of this assumption is provided by the comparison of other β-D-ribofuranosyl and methyl pairs (Table VI). This allowed one to calculate a very consistent set of α and β parameters for the substitution of a β-D-ribofuranosyl moiety vs. a methyl group (Table X). It is noteworthy that the largest substitution effect, i.e., β-D-ribofuranosyl vs. methyl (Table VI), is observed at C6(C8) in pyrrolo[2,3-*d*]pyrimidines and at the similar position, C8, in purines. This suggests that some interaction exists between the heterocyclic aglycon and the β-D-ribofuranosyl moiety. This interaction possibly reflects a preferential anti conformation for these nucleosides, thus causing the steric interaction at C6(C8) or C8. Support for this assumption is given by similar chemical-shift differences observed for C8 resonances of the purine aglycon regardless of the attachment of the ribose moiety (N7 or N9).[23]

B. Validity of Using Certain Pyrrolo[2,3-*d*]pyrimidines to Evaluate the α- and β-Substituent Parameters. Effects of substitution of a β-D-ribofuranosyl moiety vs. a methyl group on the bridgehead carbon chemical shifts ($\Delta\delta$) are similar in N9 adenine derivatives (XVIII − XVII) and their pyrrolo[2,3-*d*]pyrimidine analogs [V − IV (Table VI)]. These data justify our attempt to evaluate α and β parameters through a study of pyrrolo[2,3-*d*]pyrimidines (heterocycles and nucleosides).

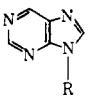
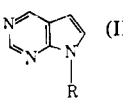
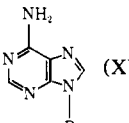
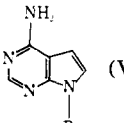
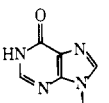
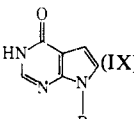
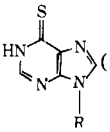
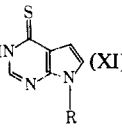
This assumption is further supported by data in Table VII and comparison of Figures 3 and 4. They indicate that the effects of substitution of a nitrogen atom for a methine group at C5(N7) in pyrrolo[2,3-*d*]pyrimidines appear mainly additive because they are not very dependent on the nature of the substituent at the C4(C6) position. As expected, such effects are very important for carbons adjacent to

Table VI. The Effects on the Aglycon ^{13}C Chemical Shifts ($\Delta\delta$, in ppm) Caused by Substitution of a β-D-Ribofuranosyl Moiety (R) for a Methyl Group in the Five-Membered Ring of 4-Aminopyrrolo[2,3-*d*] pyrimidine and Certain Purines

Structures (A – B)[a]	Pp C2 / Pu C2	C4 / C6	C4a / C5	C5	C6 / C8	C7a / C4
 (XIV) –  (XIII)	0.4	0.9	0.8 (β)		−1.9	−0.3 (α)
 (V) –  (IV)	−0.3	~0	0.9 (β)	1.5	−2.2	−0.4 (α)
 (XVIII) –  (XVII)	0.1	0.3	0.9 (β)		−1.3	−0.7 (α)
 (XVI) –  (XV)	0.5	−0.2	−1.5 (α)		−1.3	0.9 (β)
 (XX) –  (XIX)	0.4	−0.5	−0.7 (α)		−1.9	0.7 (β)
 (XXV) –  (XXIV)	0.3	−0.6	−0.6 (α)		−3.3	0.7 (β)

[a] (A – B) = (β-D-ribofuranosyl derivatives – methyl derivatives).

Table VII. The Effects on All ^{13}C Chemical Shifts ($\Delta\delta$, in ppm) Caused by Substitution of a Nitrogen Atom for the C5 Methine Group in Certain Pyrrolo[2,3-*d*] pyrimidine Nucleosides

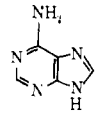
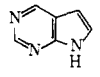
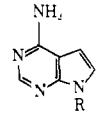
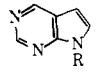
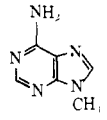
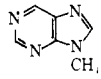
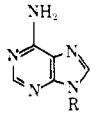
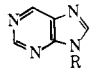
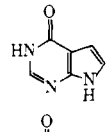
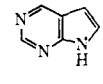
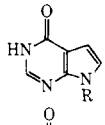
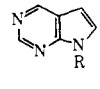
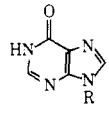
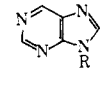
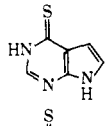
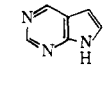
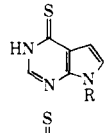
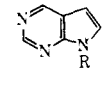
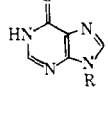
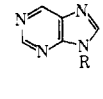
Structures (A – B)[a]	Aglycon ($\Delta\delta$) Pp C2 / Pu C2	C4 / C6	C4a / C5	C6 / C8	C7a / C4	Ribose ($\Delta\delta$) C1′	C2′	C3′	C4′	C5′
 (XIV) –  (II)	1.1	−1.3	14.9	17.7	0.3	0.8	−0.3	−0.3	0.5	−0.4
 (XVIII) –  (V)	1.3	−1.1	16.3	17.4	−0.3	0.4	~0	0.1	1.0	~0
 (XXI) –  (IX)	2.2	−1.7	16.0	17.7	0.5	0.5	−0.1	−0.2	0.6	−0.3
 (XXVI) –  (XI)	2.0	−0.5	14.8	17.2	0.7	0.8	−0.1	−0.3	0.5	−0.3

[a] (A – B) = (Pu – Pp).

the site of nitrogen substitution with average values of about 15.5 and 17.5 ppm. In spite of the remote location of C2, its resonances exhibit a downfield shift of about 1.6 ppm, which reflects a long-range inter-ring effect. This phenomenon has been previously observed in protonation studies of purine.[24] It is interesting to note that nitrogen substitution slightly influences the resonance positions of the β-D-ribofuranosyl carbons with the largest perturbations observed at C1′ and C4′.

The nearly additve effect of a nitrogen atom in the five-membered ring of the heterocyclic aglycon is further supported by the data in Table VIII. This table summarizes the substitution effects when an amino, oxo, or thione function replaces a hydrogen atom on the pyrimidine portion of the

Table VIII. The Effects on All ^{13}C Chemical Shifts ($\Delta\delta$, in ppm) Caused by Substitution of an Amino, Oxo, or Thione Function for a Hydrogen at C4 in Certain Pyrrolo[2,3-*d*] pyrimidines or at C6 in Certain Purines

Structures (A – B)[a]				Aglycon ($\Delta\delta$)							Ribose ($\Delta\delta$)				
			Pp C2	C4	C4a	C5	C6	C7a	C1'	C2'	C3'	C4'	C5'
			Pu C2	C6	C5		C8	C4					
	(III) –		(I)	0.3	8.4	−15.9	−0.3	−5.7	−1.3					
	(V) –		(II)	0.3	7.8	−16.1	−0.8	−5.1	−1.2	1.0	−0.4	~0	−0.2	0.1
	(XVII) –		(XIII)	0.6	8.5	−14.7		−6.0	−1.4					
	(XVIII) –		(XIV)	0.4	8.0	−14.7		−5.3	−1.8	0.5	−0.2	0.4	0.3	0.5
			Av:	0.4	8.2	−15.4	−0.6	5.5	−1.4					
	(VIII) –		(I)	−7.5	9.8	−10.4	2.8	−6.7	−3.0					
	(IX) –		(II)	−7.1	8.9	−10.7	2.2	−6.4	−2.8	0.5	0.3	~0	~0	0.1
	(XXI) –		(XIV)	−6.0	8.6	−9.7		−6.4	−2.6	0.1	0.5	0.1	0.1	0.2
			Av:	−6.9	9.1	−10.3	2.5	−6.5	−2.8					
	(X) –		(I)	−8.0	27.4	1.8	5.0	−3.4	−7.6					
	(XI) –		(II)	−7.6	27.1	1.5	4.4	−3.6	−7.3	0.2	0.4	−0.1	~0	−0.1
	(XXVI) –		(XIV)	−6.7	27.9	1.3		−4.1	−7.0	0.2	0.6	~0	0.1	~0
			Av:	−7.4	27.5	1.5	4.7	−3.7	−7.3					

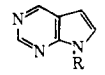
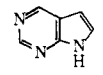
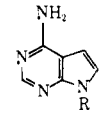
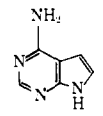
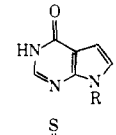
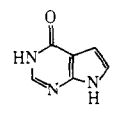
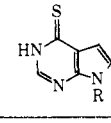
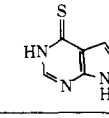
[a] (A – B) = [C(4)X·Pp – C(4)H·Pp] or [C(6)X·Pu – C(6)H·Pu], where X is an amino, oxo, or thione function.

pyrrolo[2,3-*d*]pyrimidine or purine aglycon. Data obtained from these two different ring systems are in very good agreement. It is important to notice that the adjacent bridgehead carbon resonances [C4a(C5) or C5] exhibit larger perturbations than the carbon bearing the substituent [C4(C6) or C6] when the substituent is an amino or oxo function. These carbons move approximately −15.4 and 8.2 ppm, respectively, for an amino substituent and about −10.3 and 9.1 ppm for an oxo group. A different pattern is observed for the thione derivatives. The resonances of the carbon [C4(C6) or C6] bonded to sulfur exhibit an important downfield shift of about 27.5 ppm, while the adjacent bridgehead carbon resonance position moves downfield about 1.5 ppm.

The upfield shifts observed for the C2 resonance when the C4(C6) or C6 carbon is bonded to an oxygen or sulfur atom come from the predominance of the lactam and thione forms in these derivatives (see the following paper in this issue[27]). It is interesting to point out again the long-range inter-ring effect of substitution at C4(C6) or C6 on the chemical shifts of C6(C8) or C8 in all the compounds studied. A similar phenomenon has been observed in ^1H NMR studies.[8] As expected, the β-D-ribofuranosyl carbon resonances are nearly insensitive to substitution in the pyrimidine ring.

C. α- and β-Substituent Parameters Related to the Bridgehead Carbons of Purine Derivatives. The α- and β-substituent parameters derived from substitution of a β-D-ribofuranosyl moiety vs. a hydrogen are determined from comparison of chemical shifts of certain 7-(β-D-ribofurano-

Table IX. The Effects on the Aglycon ^{13}C Chemical Shifts ($\Delta\delta$, in ppm) Caused by Substitution of a β-D-ribofuranosyl Moiety (R) for a Hydrogen (H) at N7(N9)[a] in Certain Pyrrolo[2,3-*d*]pyrimidines

Compd (β-D-ribofuranosyl – hydrogen)	Aglycon ($\Delta\delta$)					
	C2(C2)[a]	C4(C6)	C4a(C5)	C5(N7)	C6(C8)	C7a(C4)
 (II) –  (I)	0.2	0.7	1.1 (β)	1.1	0.6	−0.5 (α)
 (V) –  (III)	0.2	0.2	1.0 (β)	0.6	1.3	−0.5 (α)
 (IX) –  (VIII)	0.6	−0.2	0.8 (β)	0.6	0.9	−0.3 (α)
 (XI) –  (X)	0.7	0.4	0.8 (β)	0.6	0.5	−0.2 (α)

[a] Reference 4.

Table X. Sets of α- and β-Substituent Parameters Relative to Bridgehead Carbons (C4 and C5) in Purine Derivatives (in ppm)

Nature of the substituent at N7 or N9 in purines	Substituent at C6							
	α parameter				β parameter			
	H	NH_2	=O	=S	H	NH_2	=O	=S
(β-D-Ribosyl – H)[a]	−0.5	−0.5	−0.3	−0.2	1.1	1.0	0.8	0.8
(β-D-Ribosyl – CH_3)[b]	−0.3	−0.9[d]	−0.7	−0.6	0.8	0.9	0.7	0.7
(CH_3 – H)[c]	−0.2	0.4	0.4	0.4	0.3	0.1	0.1	0.1

[a] Data from Table IX. [b] Data from Table VI. [c] (β-D-Ribosyl – H) – (β-D-Ribosyl – CH_3) = (CH_3 – H). [d] Average of three sets of data from Table VI.

syl)pyrrolo[2,3-*d*]pyrimidines with their parent heterocycles (Table IX). Similar sets of parameters can be obtained from the data in Table VI, i.e., for the substitution of a β-D-ribofuranosyl moiety vs. a methyl group. All these parameters are summarized in Table X. The values of the β parameters show that it is not necessary to correct the chemical shift of the β carbon when the labile hydrogen is replaced by a methyl group. However, a correction of about 0.9 ppm must be taken into account when a hydrogen is replaced by a β-D-ribofuranosyl moiety. Like the β parameters, the α parameters derived from the substitution of a β-D-ribofuranosyl moiety vs. a hydrogen are only slightly dependent on the nature of the substituent at the C6 position in the purine ring. However, this dependence for the α parameters is more crucial when a hydrogen is replaced by a methyl group.

V. Conclusion

The study of a wide variety of nucleosides by means of ^{13}C NMR spectroscopy has demonstrated certain additive relationships which occur in the heterocyclic aglycons. These relationships can be very useful in future structure identifications of pyrrolo[2,3-*d*]pyrimidines and purines. Substituent parameters for chemical shifts have been derived which illustrate the relationships between β-D-ribofuranosyl, methyl, and proton moieties. This information can be used not only to determine the site of N-alkylations[25] but, more important, to determine tautomeric populations in the imidazole portion of the purine ring system (see the following paper in this issue[27]). It should be pointed out that these parameters are specific for purines and cannot be used for the study of prototropic tautomerism in other heterocyclic ring systems.[26]

Acknowledgment. One of us (M.-T.C.) would like to thank NATO for partial financial support. This research was supported by Research Resource Grant RR-574 with NIH and by Research Contract No. NIH-N01-CM-23710 with DCT, NCI, NIH.

References and Notes

(1) (a) Previous paper in this series: D. K. Dalling and D. M. Grant, *J. Am. Chem. Soc.*, **96**, 1827 (1974). (b) Presented in part at the Fourth International Congress of Heterocyclic Chemistry, Salt Lake City, Utah, July 1973, A-24. (c) All experimental work described in this paper was conducted at the University of Utah. (d) Laboratoire de Chimie-Physique. (e) University of Utah.
(2) For a recent review on tautomerism in purines, see B. Pullman and A. Pullman, *Adv. Heterocycl. Chem.*, **13**, 77 (1971).
(3) Since prototropic tautomerism is eliminated in the five-membered ring of pyrrolo[2,3-*d*]pyrimidines, the α and β parameters can be easily determined from a study of this ring system.
(4) The following abbreviations have been used: pyrrolo[2,3-*d*]pyrimidine, Pp; purine, Pu. We would like to point out that the pyrrolo[2,3-*d*]pyrimidine numbering is followed by the purine numbering in parentheses in order to facilitate a comparison of the data.
(5) M.-Th. Chenon and D. M. Grant, manuscript in preparation. The chemical shift of dioxane was shown to be independent of concentration in Me_2SO in the 0.5–5% v/v range, with respect to an external standard.
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(13) R. J. Pugmire, D. M. Grant, R. K. Robins, and G. W. Rhodes, *J. Am. Chem. Soc.*, **87**, 2225 (1965).
(14) R. J. Pugmire, D. M. Grant, L. B. Townsend, and R. K. Robins, *J. Am. Chem. Soc.*, **95**, 2791 (1973).
(15) (a) H. L. Retcofsky and R. A. Friedel, *J. Phys. Chem.*, **71**, 3592 (1967); (b) J. Riand, M.-Th. Chenon, and N. Lumbroso-Bader, *Tetrahedron Lett.*, 3123 (1974). In 4-aminopyrimidine, the carbon on which NH_2 substitution occurs moves downfield about 6 ppm, while the resonance position of the ortho carbon (C5) is shifted approximately −17 ppm to higher field, with respect to their resonance positions in pyrimidine. On the other hand, the substituent effect at the meta position (C6) is much smaller (ca. −2 ppm upfield).
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(17) L. F. Johnson and W. C. Jankowski, "Carbon-13 NMR Spectra", Wiley-Interscience, New York, N.Y., 1972, Spectrum 226.
(18) We have found the published assignments[19] for the carbon signals of

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_8 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.

(19) (a) T. R. Krugh, *J. Am. Chem. Soc.*, **95**, 4761 (1973); (b) P. Dea, G. R. Revankar, R. L. Tolman, R. K. Robins, and M. P. Schweizer, *J. Org. Chem.*, **39**, 3227 (1974).
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(22) U. Vögeli and W. von Philipsborn, *Org. Magn. Reson.*, **5**, 551 (1973).
(23) The anti conformation for certain 7-(β-D-ribofuranosyl)purines has been suggested by other workers, see: (a) R. P. Panzica, R. J. Rousseau, R. K. Robins, and L. B. Townsend, *J. Am. Chem. Soc.*, **94**, 4708 (1972), and references cited therein; (b) D. W. Miles, W. H. Inskeep, L. B. Townsend, and H. Eyring, *Biopolymers*, **11**, 1181 (1972).
(24) R. J. Pugmire and D. M. Grant, *J. Am. Chem. Soc.*, **93**, 1880 (1971).
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(26) Different sets of α and β parameters have been obtained from studies of indole derivatives [R. G. Parker and J. D. Roberts, *J. Org. Chem.*, **35**, 996 (1970)] and certain azole derivatives [J. Elguero, C. Marzin, and J. D. Roberts, *J. Org. Chem.*, **39**, 357 (1974)].
(27) Part XXVI: M.-Th. Chenon, R. J. Pugmire, D. M. Grant, R. P. Panzica, and L. B. Townsend, *J. Am. Chem. Soc.*, the following paper in this issue.

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

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Contribution from the CNRS, Laboratoire de Chimie-Physique, 94320 Thiais, France, and the Departments of Chemistry and Biopharmaceutical Sciences, University of Utah, Salt Lake City, Utah 84112. *Received September 5, 1974*

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