Carbon-13 Magnetic Resonance. XVII.[1a] Pyrimidine and Purine Nucleosides

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Abstract: The natural abundance carbon-13 nuclear magnetic resonance spectra of 29 nucleosides, including those naturally occurring, are described. The spectra may be divided into two regions: that due to the nitrogen base (−37–33 ppm) and that due to the ribose moiety (37–89 ppm). The relative invariance of the ribose carbon shifts with nitrogen base has enabled their assignment independent of base. Total assignments are also given for the carbon atoms in the pyrimidine and purine bases. The observed shifts for the naturally occurring nucleosides correlate at least qualitatively with MO parameters derived for the parent bases. Consequently, it is apparent that the electronic structure of the nucleosides is reflected in the carbon-13 shifts. Furthermore, the shifts correlate reasonably well with the known chemical reactivity of these molecules. A cautionary note is added to prevent misconceptions relating to the simplicity of the factors contributing to the carbon-13 chemical shift.

Recent advances in the theory and instrumentation of nuclear magnetic resonance have made this technique most promising for studies of bioorganic and even biopolymer molecules. Since the pioneering work of the Jardetzkys,[2] much progress has been made in the area of proton magnetic resonance (pmr) studies on the pyrimidine and purine bases, nucleosides, and nucleotides.[3,4] The few protons on the pyrimidine or purine rings generally lead to well-resolved and easily interpretable pmr spectra which are dependent upon concentration, pH, and temperature, as well as other factors.[4] The pmr technique is limited in this area due to the need for sites in the molecular framework to be proton bearing.

Carbon-13 magnetic resonance spectroscopy (cmr) affords an opportunity to study in detail the structure of the molecular framework, with only the exception of the heteroatom sites. The carbon-13 spectra of the unsubstituted bases purine[5] and pyrimidine[6] have been determined. The spectrum of purine[5] provided the first example of the analysis of carbon-13 spectra of biologically important molecules and the ordering of the chemical shifts C-2, C-8, and C-6 differed from that noted for the corresponding directly bonded protons (6,2,8).[7] A gross correlation of the carbon-13 shift data with theoretical estimates of charge density was observed suggesting that pmr shifts are somewhat less reliable in considerations of the relation between shift and charge. More recently correlation of the carbon-13 shifts with total charge has been shown to be more reliable.[8] Further, the variation in the ordering of the carbon-13 and proton shifts has been attributed to a ring-current effect.[9] Carbon-13 studies on pyrimidine and its protonated cations emphasize a need to consider bond order in addition to total charge features.[6]

The very recent developments in instrumental techniques, in particular, noise decoupling of carbon-13 spectra,[10,11] have enabled application of the cmr method to larger bioorganic and related molecules. Thus, additivity relationships have been derived for the methylcyclohexanes,[12] perhydronaphthalene, anthracene, and phenanthrene systems,[13] which participate in the basic structure of many steroids. Similar additivity relationships have been derived for the amino acids[14] and detailed studies including total analyses of the carbon-13 shifts in a variety of steroids,[15] terpenes,[10] and carotenoid[16] systems have been made.

In the present study we are primarily concerned with the general application of cmr spectroscopy in the area of nucleoside and nucleotide chemistry. We have previously reported the carbon-13 shifts observed in the naturally occurring nucleosides.[17] Herein we describe the natural abundance carbon-13 spectra of 29 nucleosides and their assignment in terms of the observed spectral variations on modifying the substituent groups.

For relatively symmetrical and low molecular weight molecules selective proton decoupling techniques can be used to facilitate spectral assignment.[18] In the present study, however, although the proton resonance in most

(1) (a) Previous paper in this series: A. J. Jones, P. D. Gardner, D. M. Grant, W. M. Litchman, and V. Boekelheide, *J. Amer. Chem. Soc.*, **92**, 2395 (1970); (b) Colorado State University, the experimental work described in this paper was carried out at the University of Utah; (c) University of Utah.

(2) C. D. Jardetzky and O. Jardetzky, *J. Amer. Chem. Soc.*, **82**, 222 (1960); C. D. Jardetzky, *ibid.*, **82**, 229 (1960); *ibid.*, **83**, 2191 (1961).

(3) S. I. Chan and J. H. Nelson, *ibid.*, **91**, 168 (1969), and references therein.

(4) J. H. Prestegard and S. I. Chan, *ibid.*, **91**, 2843 (1969), and references therein.

(5) R. J. Pugmire, D. M. Grant, R. K. Robins, and G. W. Rhodes, *ibid.*, **87**, 2225 (1965).

(6) R. J. Pugmire and D. M. Grant, *ibid.*, **90**, 697, 4232 (1968).

(7) S. I. Chan, M. P. Schweizer, P. O. P. Ts'o, and G. K. Helmkamp, *ibid.*, **86**, 4182 (1964).

(8) W. Adam, A. Grimison, and G. Rodríquez, *Tetrahedron*, **23**, 2513 (1967).

(9) O. Jardetzky, private communication.

(10) F. J. Weigert, M. Jautelat, and J. D. Roberts, *Proc. Natl. Acad. Sci. U. S.*, **60**, 1152 (1968).

(11) R. R. Ernst, *J. Chem. Phys.*, **45**, 3845 (1966); A. J. Jones, D. M. Grant, and K. F. Kuhlmann, *J. Amer. Chem. Soc.*, **91**, 5013 (1969). The present authors would like to thank Scott W. Collins for building the phase-modulating noise generator used in the present experiments.

(12) D. K. Dalling and D. M. Grant, *ibid.*, **89**, 6612 (1967).

(13) D. K. Dalling, Ph.D. Thesis, University of Utah, Salt Lake City, Utah, 1969.

(14) W. J. Horsley and H. Sternlicht, *J. Amer. Chem. Soc.*, **90**, 3738 (1968); W. J. Horsley, H. Sternlicht, and J. Cohen, *ibid.*, **92**, 680 (1970).

(15) H. J. Reich, M. Jautelat, M. T. Messe, F. J. Weigert, and J. D. Roberts, *ibid.*, **91**, 7445 (1969).

(16) J. B. Grutzner and J. D. Roberts, private communication, see also, M. Jautelat, J. B. Grutzner, and J. D. Roberts, *Proc. Natl. Acad. Sci. U. S.*, **65**, 288 (1970).

(17) A. J. Jones, M. W. Winkley, D. M. Grant, and R. K. Robins, *ibid.*, **65**, 27 (1970).

(18) A. J. Jones, T. D. Alger, D. M. Grant, and W. M. Litchman, *J. Amer. Chem. Soc.*, **92**, 2386 (1970).

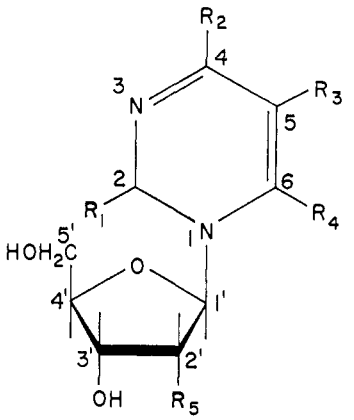

Figure 1. Structures of the pyrimidine nucleosides studied: uridine (U), R_1 = carbonyl; R_2 = R_5 = OH; R_3 = R_4 = H; 4-thiouridine, R_1 = carbonyl; R_2 = SH; R_3 = R_4 = H; R_5 = OH; 2,4-dithiouridine, R_1 = thio; R_2 = SH; R_3 = R_4 = H; R_5 = OH; 5-hydroxyuridine, R_1 = carbonyl; R_2 = R_3 = R_5 = OH; R_4 = H; thymidine (T), R_1 = carbonyl; R_2 = OH; R_3 = CH_3; R_4 = R_5 = H; 4-thiothymidine, R_1 = carbonyl; R_2 = SH; R_3 = CH_3; R_4 = R_5 = H; cytidine (C), R_1 = carbonyl; R_2 = NH_2; R_3 = R_4 = H; R_5 = OH; deoxycytidine (dC), R_1 = carbonyl; R_2 = NH_2; R_3 = R_4 = R_5 = H; 6-methylcytidine, R_1 = carbonyl; R_2 = NH_2; R_3 = H; R_4 = CH_3; R_5 = OH; 2-thiocytidine, R_1 = thio; R_2 = NH_2; R_3 = R_4 = H; R_5 = OH.

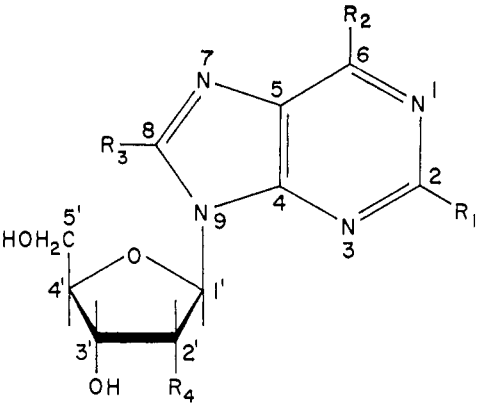

Figure 2. Structures of the purine nucleosides studied: nebularine (N), R_1 = R_2 = R_3 = H; R_4 = OH; 6-chloro-9-(β-D-ribofuranosyl)purine, R_1 = R_3 = H; R_2 = Cl; R_4 = OH; 2-amino-9-(β-D-ribofuranosyl)purine, R_1 = NH_2; R_2 = R_3 = H; R_4 = OH; 2,6-diamino-9-(β-D-ribofuranosyl)purine, R_1 = R_2 = NH_2; R_3 = H; R_4 = OH; adenosine (A), R_1 = R_3 = H; R_2 = NH_2; R_4 = OH; deoxyadenosine (dA), R_1 = R_3 = R_4 = H; R_2 = NH_2; 2-chloroadenosine, R_1 = Cl; R_2 = NH_2; R_3 = H; R_4 = OH; 6-N-methylamino-9-(β-D-ribofuranosyl)purine, R_1 = R_3 = H; R_2 = NHMe; R_4 = OH; guanosine (G), R_1 = NH_2; R_2 = R_4 = OH; R_3 = H; deoxyguanosine (dG), R_1 = NH_2; R_2 = OH; R_3 = R_4 = H; 6-thioguanosine, R_1 = NH_2; R_2 = SH; R_3 = H; R_4 = OH; inosine (I), R_1 = R_3 = H; R_2 = R_4 = OH; deoxyinosine (dI), R_1 = R_3 = R_4 = H; R_2 = OH; 6-thioinosine, R_1 = R_3 = H; R_2 = SH; R_4 = OH; xanthosine, R_1 = R_2 = R_4 = OH; R_3 = H; 6-thioxanthosine, R_1 = R_4 = OH; R_2 = SH; R_3 = H.

of the compounds are well defined, this technique could not be used due to the relative insolubility and asymmetry of the nucleosides. Carbon-13 spectra were consequently determined using noise decoupling,[11] an internal reference (most commonly dimethyl sulfoxide), and a time-averaging device. The total spectrum was thus determined after multiple scanning (average 150 scans at 10 Hz/sec sweep rate). Observation of the coupled spectra (after multiple scanning in some cases also provided an excellent means of distinguishing the quaternary carbon atoms from those bonded to protons. Figures 1 and 2 summarize the structures of the compounds studied.

Experimental Section

Carbon-13 spectra were determined using a Varian AFS-60 spectrometer in the manner described previously[18] with the exception that 2.5–3-kHz sweep widths were employed. Solutions of the nucleosides (0.2–2.7 *M*) in dimethyl sulfoxide were employed, though dimethylformamide was used to remove problems of overlapping of the DMSO peak with the C-2′ peak in the deoxy derivatives. Concentrations for each molecule discussed are given in the appropriate table. Spectra were accumulated on a Varian C-1024 time-averaging device usually over a period of 10–12 hr. All chemical shifts were calculated relative to the internal reference, dimethyl sulfoxide, and converted to the benzene scale. Proton spectra were determined using either Varian A60 or A56/60 spectrometers.

All of the nucleosides studied were prepared by procedures described in the literature and were purified and dried by conventional techniques prior to spectral determination.

Results

To simplify the problem of presenting the large quantity of data derived in the present study we have chosen to consider either the parent base or the naturally occurring nucleoside structure as a starting point. Thus we will consider the nucleosides under five main categories: (a) the uridines, (b) the cytidines, (c) the purines, (d) the adenosines, and (e) the guanosines. This categorization is based on structural resemblance and though it is appreciated that, for example, inosine is a chemical derivative of adenosine its structural resemblance to guanosine places it in category e. Categories a and b are of course derivatives of pyrimidine while c, d, and e are derivatives of purine.

The carbon-13 chemical shifts for the categories a–e are presented in Tables I–V, respectively. It is clear that the observed shifts for all the nucleosides studied separate, with only minor exceptions, into two regions. The resonances due to the ribose carbon atoms fall in the range 37–89 ppm and in general are relatively independent of the nitrogen base. The resonances due to the pyrimidine and purine carbon atoms on the other hand fall in the range −37–33 ppm unless the bases bear sulfur substituents. The large downfield shifts occurring on replacement of the oxygen atom of a carbonyl group by a sulfur atom provides the single most informative mechanism for assigning the carbonyl carbon atoms in all the systems studied.

Assignment of the Ribose Carbon Atoms. The apparent independence of the ribose carbon atom resonances to the nitrogen base (see Tables I–V) provides an opportunity to consider their assignments independent of the base. Thus, in the deoxyribose derivatives the line associated with C-2′ undergoes the expected upfield shift on exchange of the 2′-hydroxyl group for a proton. This ribose–deoxyribose effect is shown in the correlation diagram in Figure 3 for uridine (U) and thymidine (T).

Model compounds were used to provide further assignments. Thus Figure 3 shows the correlation diagram for 1-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)thymine and 2,5′-anhydro-2′,3′-O-isopropylideneuridine. The observed resonances in the 2′,3′- unsaturated compound clearly distinguish C-2′ and C-3′ from C-1′, C-4′, and C-5′. The highest field

Table I. Carbon-13 Chemical Shifts in Pyrimidine Nucleosides.[a] The Uridines and Thymidines

Nucleoside	Carbon positions C-2	C-4	C-5	C-6	CH_3	C-1′	C-2′	C-3′	C-4′	C-5′
Uridine (2.7 *M*)	−23.88	−36.20	+25.46	−13.68		+39.42	+57.42	+53.66	+42.58	+66.30
4-Thiouridine (2.5 *M*)	−20.59	−62.57	+14.83	−8.39		+38.88	+57.95	+53.58	+42.47	+66.81
2,4-Dithiouridine (2.5 *M*)	−45.26	−58.47	+9.84	−7.02		+34.21	+58.91	+52.89	+42.87	+67.94
5-Hydroxyuridine (2.0 *M*)	−22.09	−33.18	+7.45	−5.05		+39.94	+57.18	+54.33	+42.66	+66.17
2,5′-Anhydro-2′,3′-isopropylidineuridine[b] (2.0 *M*)	−28.98	−42.62	+18.84	−15.29		+30.90	+43.37	+43.85	+46.51	+53.41
Thymidine (2.7 *M*)	−23.06	−36.37	+18.02	−8.80	+115.28	+43.47	+88.10	+56.87	+40.10	+66.17
4-Thiothymidine (2.7 *M*)	−20.20	−62.99	+9.71	−5.66	+110.67	+42.68	+88.60	+57.35	+40.70	+66.38
1-(2,3-Dideoxy-β-D-*glycero*-pent-2-enofuranosyl)thymine (2.0 *M*)	−23.58	−36.69	+18.21	−9.47	+115.16	+39.79	−7.57	+1.36	+38.33	+64.84
Phenyl-β-D-ribofuranoside[c] (1.5 *M*)						+22.21	+56.84	+52.86	+43.05	+64.76

[a] Shifts given in parts per million relative to benzene and positive values indicate higher field. [b] Isopropylidene carbons at +16.36, +102.08, and +103.63 ppm. [c] Phenyl ring carbons at −28.93, −1.75, +11.37, and +22.21 ppm.

Table II. Carbon-13 Chemical Shifts in Pyrimidine Nucleosides.[a] The Cytidines

Nucleoside	Carbon positions C-2	C-4	C-5	C-6	CH_3	C-1′	C-2′	C-3′	C-4′	C-5′
Cytidine (2.6 *M*)	−28.42	−38.17	+32.85	−14.29		+38.41	+57.96	+53.40	+43.16	+66.65
Deoxycytidine (2.6 *M*)	−28.49	−38.44	+32.67	−13.93		+41.94	+87.90	+56.75	+39.94	+65.88
6-Methylcytidine (2.5 *M*)	−28.82	−37.66	+31.99	−26.83	+107.65	+35.62	+57.39	+56.63	+42.36	+65.23
2-Thiocytidine (2.5 *M*)	−52.36	−32.56	+29.54	−14.15		+34.09	+59.17	+52.52	+43.42	+67.73

[a] Shifts given in parts per million relative to benzene and positive values indicate higher field.

line of this group is relatively unaffected by these changes, but on cyclization at C-5′ a downfield shift (−13 ppm) results. Hall and Johnson[19] also concluded that the hydroxymethylene resonances in hexapyranoses occur at highest field in this sugar group. The reorganization in the shifts of C-1′ to C-4′ in the cycloside structure presumably occur as a consequence of the geometrical changes produced by isopropylidenation.[20]

The invariance of the ribose shifts in these N-glycosides prompted study of an O-glycoside. The carbon-13 shifts for the ribose carbon atoms in phenyl-β-D-ribofuranoside are given in Figure 3. Only the lowest field line of the ribose group is perturbed (downfield, −18 ppm). The remaining lines are relatively unaffected and consequently the lowest field pentose line in the ribosides studied is assigned to C-1′.

In studies on the corresponding nucleotides (mono- and/or triphosphate groups at C-5′) Dorman and Roberts[21] have observed splitting of the C-5′ and C-4′ resonances by phosphorus. Thus it is clear that the C-4′ resonance which we have independently assigned in the ribosides is "crossed-over" in the deoxyribosides. That is, the C-1′ resonances in the deoxy compounds are shifted upfield 3–4 ppm from their position in the spectra of the ribosides. A similar β-substituent effect[22] would be expected at C-3′ and consequently the lower field lines of the pair associated with C-2′ and C-3′ is attributed to C-3′ in the ribosides. (This line also shifts upfield 3–4 ppm in the deoxyribosides.) It is also noteworthy that a γ-substituent effect[23] is evidenced at C-4′ (upfield 2–4 ppm) on addition of the 2′-OH group to the deoxyriboside.

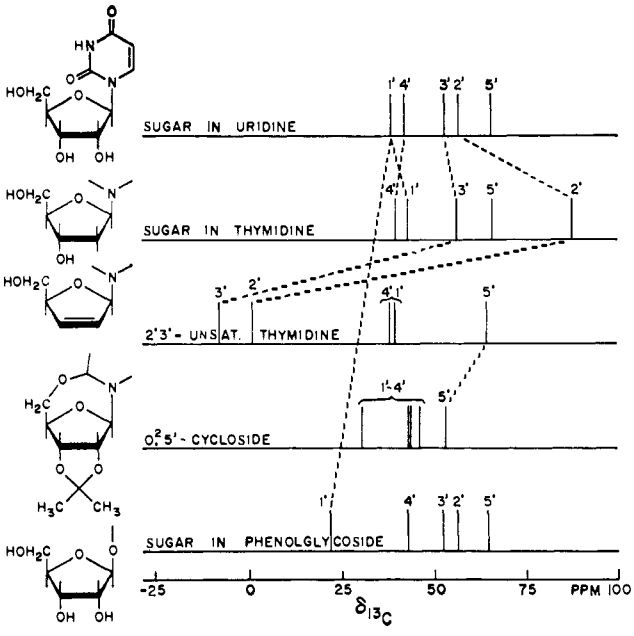

Figure 3. Chemical-shift correlations showing structural variations and assignments for the ribose carbons in the nucleosides.

The contrast provided by the ordering of the nuclei C-1′, C-4′, C-3′, C-2′, and C-5′ in the ribosides with that in the deoxyribosides (C-4′, C-1′, C-3′, C-5′, and C-2′), provides a fingerprint for identification.

(19) L. D. Hall and L. F. Johnson, *Chem. Commun.*, 809 (1969).
(20) I. L. Doerr, R. J. Cushley, and J. J. Fox, *J. Org. Chem.*, 34, 1390 (1969).
(21) D. E. Dorman and J. D. Roberts, *Proc. Natl. Acad. Sci. U.S.*, 65, 19 (1970). We are indebted to these authors for prepublication results.
(22) E. G. Paul and D. M. Grant, *J. Amer. Chem. Soc.*, 86, 2977 (1964).
(23) T. D. Brown, Ph.D. Thesis, University of Utah, Salt Lake City, Utah, 1966.

Table III. Carbon-13 Chemical Shifts in Purine Nucleosides.[a] The Purines

Nucleoside (or base)	Carbon positions C-2	C-4	C-5	C-6	C-8	C-1′	C-2′	C-3′	C-4′	C-5′
Purine[b]	−23.1	−25.9	+0.4	−15.9	−19.0					
Nebularine (1.5 *M*)	−23.38	−24.47	−6.62	−20.47	−17.87	+39.47	+57.10	+53.48	+41.68	+66.26
6-Chloro-9-(β-D-ribofuranosyl)-purine (1.6 *M*)	−21.74	−23.88	−3.64	−23.88	−17.84	+39.16	+57.49	+53.43	+42.05	+66.78
2-Amino-9-(β-D-ribofuranosyl)-purine (1.5 *M*)	−32.07	−26.37	+3.77	−22.21	−14.03	+40.10	+57.13	+53.70	+41.99	+66.24
2,6-Diamino-9-(β-D-ribo-furanosyl)purine (1.0 *M*)	−32.74	−24.07	+45.21	−29.08	+13.56	+39.51	+56.22	+53.50	+41.17	+65.17

[a] Shifts given in parts per million relative to benzene and positive values indicate higher field. [b] See ref 5.

Table IV. Carbon-13 Chemical Shifts in Purine Nucleosides.[a] The Adenosines

Nucleoside (or base)	Carbon positions C-2	C-4	C-5	C-6	C-8	C-1′	C-2′	C-3′	C-4′	C-5′
Adenine (saturated solution)	−24.87	−23.62	+9.94	−27.88	−11.91					
Adenosine (1.5 *M*)	−24.92	−21.54	+8.15	−28.59	−12.62	+39.38	+56.78	+53.88	+41.56	+65.89
Deoxyadenosine (1.5 *M*)	−25.12	−21.50	+8.09	−28.61	−12.44	+42.98	+88.06	+56.29	+39.36	+65.60
2-Chloroadenosine (1.2 *M*)	−25.69	−22.78	+9.19	−29.06	−12.69	+39.61	+57.06	+53.68	+41.77	+65.98
6-N-Methylamino-9-(β-D-ribo-furanosyl)purine (1.4 *M*)	−25.10	−20.79	+7.61	−27.74	−12.29	+39.11	+56.56	+53.58	+41.41	+65.57

[a] Shifts given in parts per million relative to benzene and positive values indicate higher field.

Table V. Carbon-13 Chemical Shifts in Purine Nucleosides.[a] The Guanosines

Nucleoside	Carbon positions C-2	C-4	C-5	C-6	C-8	C-1′	C-2′	C-3′	C-4′	C-5′
Guanosine (0.6 *M*)	−26.06	−23.82	+10.98	−29.25	−8.35	+41.27	+56.98	+53.66	+42.13	+66.34
Deoxyguanosine (0.5 *M*)	−26.18	−23.48	+10.85	−29.63	−8.13	+44.67	+88.10	+56.69	+39.83	+65.65
6-Thioguanosine (0.5 *M*)	−25.78	−20.41	−0.89	−47.61	−10.96	+40.61	+57.11	+52.57	+42.09	+66.11
Inosine (1.0 *M*)	−20.69	−18.43	+3.18	−29.16	−11.39	+39.73	+57.28	+53.37	+41.88	+66.24
Deoxyinosine (1.0 *M*)	−19.99	−17.76	+3.32	−28.84	−10.81	+44.17	+88.30	+57.14	+39.79	+66.28
6-Thioinosine (1.0 *M*)	−17.60	−16.19	−7.78	−48.18	−13.42	+40.04	+57.65	+53.39	+42.09	+66.55
Xanthosine (0.2 *M*)	−30.42	−23.83	+11.25	−35.97	−8.95	+38.67	+57.00	+53.70	+41.26	+66.35
6-Thioxanthosine (0.2 *M*)	−29.92	−20.52	+1.26	−54.47	−8.94	+38.61	+56.86	+53.47	+41.48	+66.37

[a] Shifts are given in parts per million relative to benzene and positive values indicate higher field.

Assignment of the Pyrimidine Resonances. The assignment of the four carbon-13 resonances of the pyrimidine carbon atoms in uridine (U), thymidine (T), cytidine (C), and deoxycytidine (dC) may be made from model compounds and spectral comparisons. The chemical shifts are given in Tables I and II. The similarity in the pyrimidine carbon resonances in cytidine and deoxycytidine is indicative of independence of these shifts relative to the sugar fragment. Thus, the differences in the pyrimidine resonances of uridine and thymidine must result from the presence of the C-5 methyl group in the latter. Comparison of these spectra shows that the peak at +25.46 ppm in uridine is shifted downfield (−7.44 ppm) in thymidine. This shift is typical for a carbon atom directly substituted by a methyl group[12] and is consequently assigned to C-5. Further the peak at −13.68 ppm in uridine is shifted upfield (5.29 ppm). Such an upfield shift is typical of a β-substituent effect in alkenes,[24a] thus indicating that these resonances may be assigned to C-6 in uridine and thymidine, respectively. A correlation diagram showing these effects is given in Figure 4. A downfield substituent shift on the highest field pyrimidine carbon atom resonance in uridine is also noted in 5-hydroxyuridine.

(a) The Uridines. Substitution of sulfur for oxygen in the carbonyl group at C-4 in uridine provides a significant downfield shift[24b] (−26.4 ppm) for the lowest field line in the pyrimidine group. On this basis this line is attributed to C-4. The resonance due to C-5 is also shifted downfield while the two remaining lines attributed to C-2 and C-6 shift upfield to a lesser degree but by almost the same amount. The 2,4-dithio derivative provides a parallel effect for C-2 (−21.38 ppm) and C-4 (−22.27 ppm) as shown in Figure 4. Taken together these data indicate that the ordering of the lowest field lines is C-4 and C-2, respectively. It is also noteworthy that the resonance attributed to C-5 in these compounds moves downfield and that due to C-6 moves progressively upfield in the 2-thio and 2,4-dithio derivatives. Similar effects are also observed in 4-thiothymidine compared with thymidine, thus establishing C-4 at lowest field. The chemical shift order

(24) (a) R. A. Friedel and H. L. Retcofsky, *J. Amer. Chem. Soc.*, 85, 1300 (1963); S. W. Collins and D. M. Grant, unpublished data. (b) A similar though larger downfield shift (−53.4 ppm) has recently been observed between the carbonyl carbon in camphor and thiocamphor: P. V. Demarco, D. Dodrell, and E. Wenkert, *Chem. Commun.*, 1418 (1969).

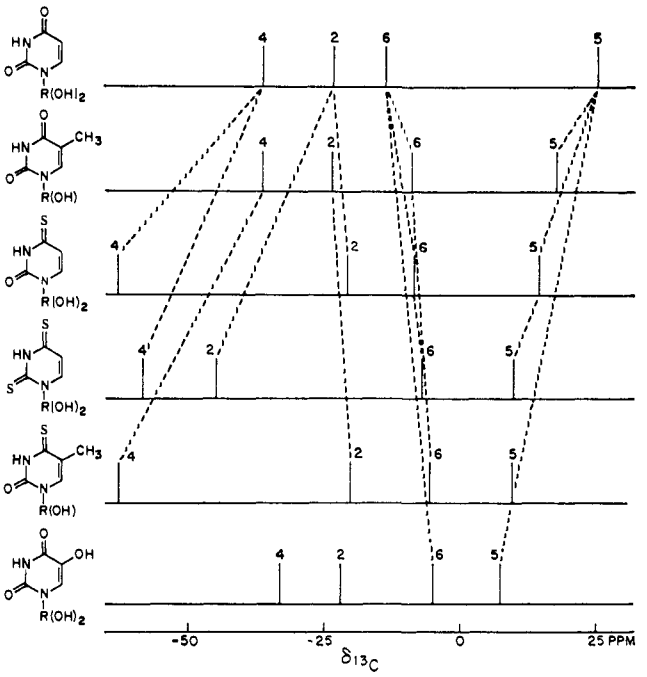

Figure 4. Chemical-shift correlations showing the structural variations and assignments in the uridines. The symbol $R(OH)_2$ denotes ribosides and R(OH) deoxyribosides.

C-4, C-2, C-6, and C-5 in the uridines is thus determined.

The resonances for the ribose carbon atoms in the 2,4-dithio derivative of uridine are considerably modified, in particular at C-1′, compared with other compounds. Similar perturbations of the ribose C-1′ atom shift have been noted for 2,6-disubstituted pyrimidines,[25] though their origin is not completely understood.

(b) The Cytidines. The structural similarity at C-5 and C-6 in cytidine and uridine suggests that the high-field pair of the pyrimidine resonances in cytidine be assigned to C-6 and C-5, respectively, in order of increasing field. Confirmation of this assignment is obtained from the spectrum of 6-methylcytidine in which the lower field line of this pair moves downfield (−12.54 ppm) while the higher field line is relatively unaffected. These effects, though of different magnitude, are analogous to those observed in uridine and thymidine.

2-Thiocytidine was studied to establish the ordering of C-2 and C-4. A downfield shift as observed in the thiouridines and 4-thiothymidine was obtained. If C-2 is the lowest field line (−38.77 ppm) this downfield shift corresponds to a change of −14.19 ppm, whereas the resonance at −28.42 ppm would be shifted downfield (−23.94 ppm.) This latter value corresponds to that observed in the uridines. Thus the order of the nuclei C-4, C-2, C-6, and C-5 is established in the cytidines. Figure 5 provides a summary correlation diagram of these assignments.

Assignment of the Purine Resonances. (c) The Purines. The correlation diagram, Figure 6, shows the carbon-13 shifts (see also Table III) for the base purine and a variety of substituted purine nucleosides. It is clear that substitution of the parent base by a ribose moiety results in considerable modification of the chemical shifts in the base. Thus the ordering of the shifts

(25) A. J. Jones, to be published.

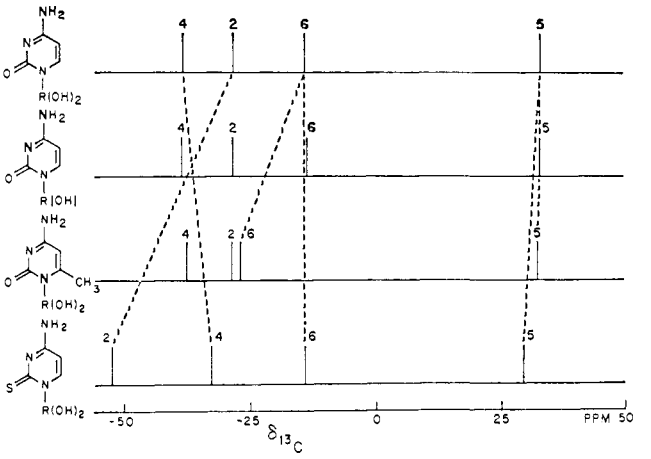

Figure 5. Chemical-shift correlations showing the structural variations and assignments in the cytidines. The symbol $R(OH)_2$ denotes ribosides and R(OH) deoxyribosides.

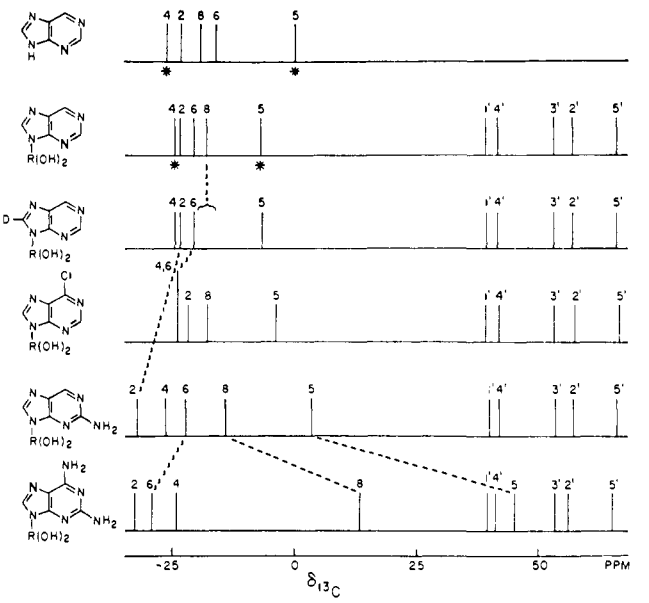

Figure 6. Chemical-shift correlations showing the structural variations and assignments in the purines. Asterisk indicates lines observed in the coupled spectra. The symbol $R(OH)_2$ denotes ribosides and R(OH) deoxyribosides.

in nebularine (N) must be established by independent methods.

In the coupled spectrum of nebularine (N) only the highest and lowest field lines remain, indicating that these lines be assigned to the quaternary atoms C-4 and C-5. Establishment of the ordering of these shifts must await the synthesis of a purine labeled with carbon-13 in either position 4 or 5. However, the highest field line is tentatively assigned to C-5 by analogy with the assignment in purine[5] which was based upon theoretical considerations. Further, the analogy to the shifts in the spectra of the pyrimidines should be noted. This argument has been extended throughout the series of purine nucleosides c, d, and e studied.

The resonance due to C-8 in nebularine was readily established by observation of the "washing out" of this line in the spectrum of 8-deuterionebularine. The two remaining lines at −23.38 and −20.47 ppm, due to C-2

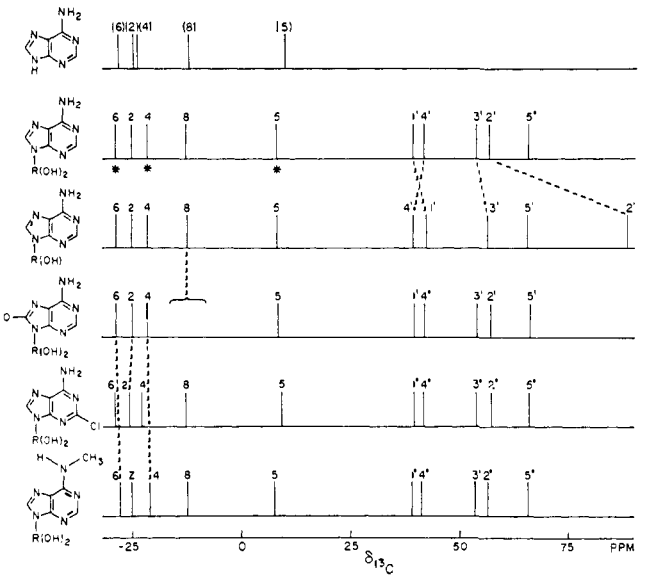

Figure 7. Chemical-shift correlations showing the structural variations and assignments of the adenosines. Asterisk indicates lines observed in the coupled spectra. The symbol $R(OH)_2$ denotes ribosides and R(OH) deoxyribosides.

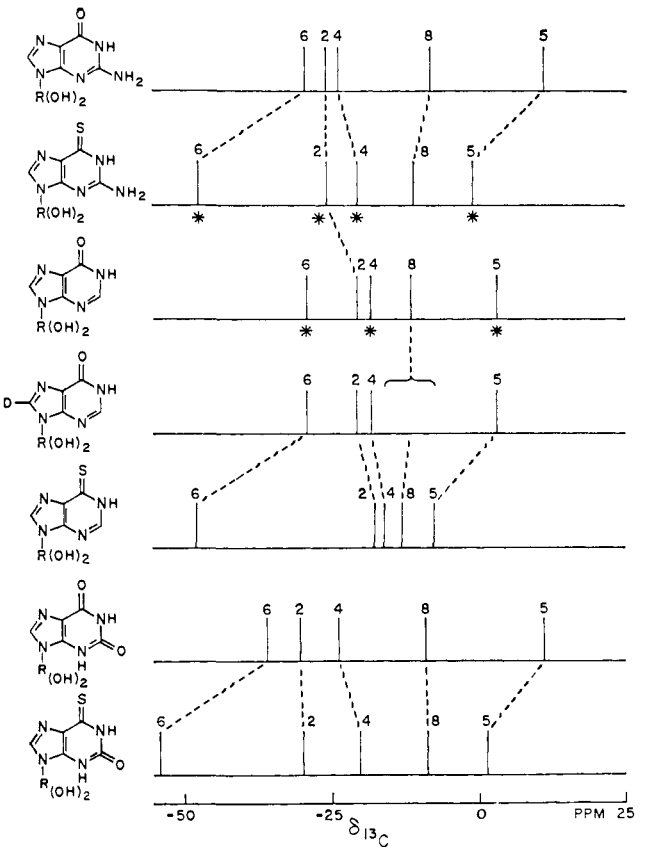

Figure 8. Chemical-shift correlations showing the structural variations and assignments in the guanosines. Asterisk indicates lines observed in the coupled spectra. The symbol $R(OH)_2$ denotes ribosides and R(OH) deoxyribosides.

and C-6, have been assigned using model compounds. Thus, in 6-chloro-9-(β-D-ribofuranosyl)purine one of the two unassigned lines in the parent molecule is shifted downfield (−3.41 ppm) while the other moves slightly upfield (0.59 ppm). The downfield shift is a typical substituent effect and suggests that the resonance at −20.47 ppm be assigned to C-6. The resonance due to C-2 has been assigned independently using the data from the spectrum of 2-amino-9-(β-D-ribofuranosyl)purine. This latter spectrum is quite complex in terms of the observed shifts compared with the parent. Nevertheless it is apparent that the position of substitution undergoes the greatest modification in electron density resulting in a shift of C-2 downfield (−8.69 ppm). C-6 remains relatively unaffected (downfield, −1.74 ppm). This downfield shift at C-2 in the 2-amino derivative is comparable to that observed in the 6-amino derivative (adenosine) (−8.12 ppm, downfield) at the 6 position, which has been assigned independently.

The chemical shifts for 2,6-diamino-9-(β-D-ribofuranosyl)purine are also presented in Table III and Figure 6. The shift at C-6 (downfield, −6.38 ppm) in this compound is somewhat attenuated compared with the effects of amino substitution at C-2 and C-6, independently. The resonances due to C-8 and C-5 on the other hand are shifted upfield (31.43 and 51.83 ppm) compared with those in nebularine. These shifts are remarkable and demonstrate participation of the amino group electrons in the aromatic system.

(d) The Adenosines. Figure 7 shows the correlation diagram for the carbon-13 spectra of adenine and a variety of adenosines, the chemical shifts of which are given in Table IV. In comparison with the changes in shifts noted between purine and nebularine there is remarkably little change between adenine and adenosine (A). The ordering of the carbon-13 shifts in adenine is not known and has not been determined in the present work due to the compound's relative insolubility. However, extrapolation of the shifts in adenosine may well be permitted on the basis of the observed similarities.

The coupled carbon-13 spectrum of adenosine exhibits three resonances attributable to the quaternary atoms C-4, C-5, and C-6. As indicated in the earlier discussion the highest field line is attributed to C-5. The "washing out" effect observed by deuterium substitution at C-8 in nebularine was also used in the present case and provided unequivocable assignment of the line at −12.62 ppm to C-8. The remaining proton-bearing atom C-2 is thus assigned by default to the resonance at −24.92 ppm.

Assignment of the remaining resonances due to the quaternary atoms C-4 and C-6 requires consideration of a variety of effects. It is clear that the higher field line (−21.54 ppm) of this pair is relatively independent of the substituent at C-6 but this line shows considerable dependence on the substituent at C-2. On the other hand the lower field line (−28.59 ppm) is relatively independent of the substituents at C-2 and C-6. Thus in 2-chloroadenosine the lower field resonance occurs at −29.06 ppm and in guanosine (to be discussed) at −29.25 ppm, while the higher field line is shifted downfield −1.3 and −4.5 ppm in these compounds, respectively. These observations are best reconciled if the low-field line is assigned to C-6 and the high field line to C-4 in adenosine. That is, the C-6 substituent effect is relatively invariant whereas substituents at C-2 result in shifts at C-4 through changes in electron

density *via* conjugative effects. It is unfortunate that in 6-N-methylamino-9-(β-D-ribofuranosyl)purine, also shown in Figure 7, the shifts at C-6 and C-4 are both upfield and identical in magnitude (0.75 ppm). These shifts, although identical, presumably occur as a consequence of different contributing factors. At C-6 the inductive effect of the N-methyl group presumably dominates while at C-4 the steric contribution appears to be the major term.

The ordering of the nuclei in adenosine (A) C-6, C-2, C-4, C-8, and C-5 in the direction of increasing field is established. It is suggested that this is also the ordering of the nuclei in the parent base adenine.

(e) **The Guanosines.** The carbon-13 shifts of this category are given in Table V and the correlation diagram for the compounds to be discussed in Figure 8. The coupled spectrum of guanosine (G) exhibits four resonances and hence provides a direct assignment of C-8, the only proton-bearing carbon in the purine ring. This is best observed in the coupled spectrum of 6-thioguanosine in which the carbon-13 shifts are better resolved and C-8 is only shifted downfield (−2.6 ppm) compared with the parent. The major changes observed on substitution by the thio group are for the lowest field line (downfield, −21.6 ppm) and the highest field line (downfield, −11.9 ppm), while the remaining lines shift upfield 0.3 and 3.4 ppm. These effects resemble those observed in the thiouridines and lead to the assignment of the lowest field line to C-6 and the highest field line to C-5.

Assignment of the resonance due to C-2 and C-4 in guanosine is obtained by comparison with the spectrum of inosine (I). Both of the remaining unassigned lines undergo a 5.4-ppm upfield shift in inosine. This difference must arise from the absence of the 2-amino group. The coupled spectrum of inosine shows that the high-field line of this pair is quaternary and consequently must be assigned to C-4. The carbon atom C-2 in guanosine is therefore assigned by default. Confirmation of the assignments in inosine was obtained from the observed "washing out" of the resonance due to C-8 in 8-deuterioinosine. Further, the lowest field line in the spectrum of inosine was confirmed as being due to C-6 from the downfield shift (−19.02 ppm) observed at this position in 6-thioinosine. The shifts of all other carbon atoms in 6-thioinosine paralled those described in 6-thioguanosine. The ordering of the nuclei C-6, C-2, C-4, C-8, and C-5, in the direction of increasing field in both inosine and guanosine is established.

We have also obtained the carbon-13 spectra of xanthosine (X) and 6-thioxanthosine, though with some difficulty due to the relative insolubility of these compounds in dimethyl sulfoxide. Only the shift due to C-6 in these compounds is assigned unequivocably but the resemblance of these spectra to those of inosine and guanosine suggests the general ordering of the chemical shifts given for xanthosine.

It should be noted that throughout the discussion concerned with the assignment of the purine resonances the deoxy derivatives of the nucleosides have not been discussed. It should suffice to point out that these shifts are in general almost identical with those observed in the parent ribosides and consequently their ordering is taken to be the same.

Discussion

Attention has previously been drawn to the fact that in all the nucleosides thus far discussed the carbon-13 resonances may be divided into two regions, that due to the nitrogen heterocycle and that due to the ribose carbon atoms. In the present discussion we shall be primarily concerned with factors contributing to the observed carbon-13 shifts in the nitrogen heterocycles which occur over the region −37–33 ppm, unless the bases have thio substituents.

A comprehensive review of the theoretical studies on nitrogen heterocycles is beyond the scope of the present paper. However, it should be pointed out that linear correlations between proton chemical shifts and calculated π-electron densities have been established, as notably shown by the work of Schaefer and Schneider,[26] Veillard,[27] and more recently Lynch and Dou.[28] These papers also point out the significant contribution of ring currents and nitrogen atom magnetic anisotropy. Similar charge dependence has been observed for carbon-13 shifts in nitrogen heteroaromatic systems provided σ polarization[6,29,30] and total charge[6,8,30,31] terms are also considered.

The semiquantitative account of carbon-13 shifts provided by theoretical considerations[6,8,29–31] has been relatively successful and has led us to consider the contributing factors to the observed carbon-13 shifts in the naturally occurring nucleosides. A variety of theoretical treatments have been presented for the heterocyclic bases[32–36] and the derived parameters have been compared to the observed carbon-13 shifts in the corresponding nucleosides. Some justification for this comparison is gained from the observed similarities in the shifts in adenosine and adenine in addition to a variety of simple heterocyclic bases with and without ribose substituents.[37] The observed differences between the heterocyclic base carbon resonances in purine and nebularine would appear to be the exception. In this case tautomeric equilibrium between N-7 and N-9 in purine appears to play a major role in accounting for the observed carbon-13 shifts.[38]

The compilation of calculated MO parameters[32–35] presented here is by no means comprehensive but is rather taken as representative of a group in which π-electron effects have been considered. Both σ- and π-electronic contributions may be assessed from the recent calculations of Mely and Pullman.[36] Table VI provides a summary of π-electron densities calculated for the heterocyclic bases in the naturally occurring nucleosides taken from the literature.[32–35] The magnitudes and distributions of these calculated values are in relatively close correspondence between each calculation procedure used, but perhaps more significant is

(26) T. Schaefer and W. G. Schneider, *Can. J. Chem.*, **41**, 966 (1963).
(27) A. Veillard, *J. Chim. Phys.*, **59**, 1056 (1962).
(28) B. M. Lynch and H. J. Dou, *Tetrahedron Lett.*, 2627 (1965).
(29) P. C. Lauterbur, *J. Chem. Phys.*, **43**, 360 (1965).
(30) J. E. Bloor and D. L. Breen, *J. Amer. Chem. Soc.*, **89**, 6835 (1967).
(31) W. Adam, A. Grimison, and G. Rodrigues, *J. Chem. Phys.*, **50**, 645 (1969).
(32) A. Veillard and B. Pullman, *J. Theor. Biol.*, **4**, 37 (1963).
(33) J. I. Fernández-Alonso, *Advan. Chem. Phys.*, **7**, 1 (1964).
(34) T. A. Hoffmann and J. Ladik, *ibid.*, **7**, 94 (1964).
(35) J. Ladik and K. Appel, *Theor. Chim. Acta*, **4**, 132 (1966).
(36) B. Mely and A. Pullman, *ibid.*, **13**, 278 (1969).
(37) A. J. Jones, to be published.
(38) R. J. Pugmire, private communication.

Table VI. Charge Densities in the Naturally Occurring Nucleoside Bases and Carbon-13 Shifts in the Corresponding Nucleosides

Nucleoside base	Carbon position	$Q\pi$ a	b	c	d,e	d,f	$\delta^{13}C^g$
Uracil	2	0.729	0.777	0.849	0.728	0.782	−23.38
	4	0.743	0.794	0.849	0.754	0.795	−36.20
	5	1.031	1.244	1.236	1.033	1.062	+25.46
	6	0.992	0.852	0.880	0.940	0.944	−13.68
Thymine	2		0.777	0.851	0.728	0.782	−23.06
	4		0.793	0.853	0.756	0.795	−36.37
	5		1.229	1.193	1.044	1.064	+18.02
	6		0.862	0.907	0.927	0.943	−8.80
Cytosine	2	0.740	0.791	0.861	0.760	0.798	−28.42
	4	0.840	0.780	0.868	0.857	0.866	−38.17
	5	1.096	1.210	1.178	1.051	1.070	+32.85
	6	0.934	0.838	0.861	0.916	0.923	−14.29
Adenine	2	0.809	0.836	0.895	0.905	0.904	−24.92
	4	0.936	0.945	1.002	0.942	0.951	−21.54
	5	1.050	1.075	1.087	1.035	1.046	+8.15
	6	0.831	0.822	0.872	0.899	0.896	−28.59
	8	0.896	0.852	0.971	0.938	0.948	−12.62
Guanine	2	0.855	0.773	0.844	0.869	0.869	−26.06
	4	1.009	0.975	1.010	0.953	0.969	−23.82
	5	1.032	1.158	1.173	1.051	1.073	+10.98
	6	0.722	0.797	0.844	0.761	0.799	−29.25
	8	0.928	0.909	1.020	0.962	0.979	−8.35
Hypoxanthine (inosine)	2	0.853	0.784				−20.69
	4	1.002	0.980				−18.43
	5	1.014	1.124				+3.18
	6	0.724	0.798				−29.16
	8	0.916	0.898				−11.39
Xanthine	2	0.853	0.780				−30.42
	4	1.022	0.957				−23.83
	5	1.014	1.184				+11.25
	6	0.724	0.790				−35.97
	8	0.916	0.922				−8.95

[a] Reference 32. [b] Reference 33. [c] Reference 34. [d] Reference 35. [e] $\beta_{C,N} = 0.80\beta$; $\beta_{C,O} = 1.30\beta$. [f] $\beta_{C,N} = 0.90\beta$; $\beta_{C,O} = 2.00\beta$. [g] Chemical shift value observed in the corresponding nucleoside expressed in parts per million relative to benzene.

the apparent gross correlation of these values with the carbon-13 chemical shifts. Most striking is the correlation of the high-field shifts observed at C-5, which are clearly reflected by the high charge densities calculated at this position for all of the compounds under consideration. As a consequence it is presumed that the carbon-13 shifts reflect the high electrophilic reactivity of the C-5 position, particularly in the pyrimidine nucleosides.

Figure 9 is a composite of plots of the observed chemical shifts in the pyrimidine and purine rings for the naturally occurring nucleosides against the π-charge densities derived by Veillard and Pullman,[32] Fernández-Alonso,[33] Hoffmann and Ladik,[34] and Ladik and Appel.[35] While the scatter of all of these plots leaves something to be desired, a correlation of the data does exist for most of the treatments. Furthermore, the general correlation roughly parallels the empirical (160 ppm/electron) relationship proposed by Spiesecke and Schneider.[39]

It is of further interest to compare the theoretical factors which make for differences in the plots shown in Figure 9. Fernández-Alonso[33] employed the Pauling-Wheland method which like the more recent CNDO-2 method completely neglects overlap. This feature is particularly clear if one compares this plot with similar plots where CNDO/2 MO parameters have been considered.[6,30] Thus the majority of points associated with proton-bearing carbon atoms correlate reasonably well with the 160 ppm/electron relationship.[39] However, the quaternary atoms C-4 and C-5 in the purine nucleosides form a separate group along with the C-5 in uridine and thymidine. It is apparent that a parallel correlation line (dashed) could be drawn through these latter points. This difference between quaternary and C–H-bonded carbon atoms has previously been attributed to either effects of carbon–nitrogen bond polarization[6,29] or to the neglect of everlap implicit in the method of calculation.[6,30] It is clear that the correlation of the observed shifts with the Fernández-Alonso charge densities can be interpreted in a similar manner. Some justification for this conclusion can be obtained from the correlations of the shifts with the charge densities of Hoffmann and Ladik[34] and Veillard and Pullman.[32]

Hoffmann and Ladik[34] derived their π densities using a simple HMO method, while Veillard and Pullman[32] used a Pariser–Parr SCF method. Both of these methods include overlap but the β integrals used by Veillard and Pullman[32] were limited. It is clear that in both cases the C-4 atoms in the purine nucleosides do not correlate as well as the other points in relation to the 160 ppm/electron line. The atom C-4 is centered between two nitrogen atoms and consequently it is suggested that the C–N bond polarization terms are probably amplified in this case. Some further improvement is observed in the correlation with the π charges derived by Ladik and Appel[35] using an SCF-CI method with variable values for β. The "best fit" is obtained for the parameters derived for $\beta_{C,N} = 0.90\beta$ and $\beta_{C,O} = 2.00\beta$ and is shown in Figure 9.

The most recent theoretical calculations on the

(39) H. Spiesecke and W. A. Schneider, *Tetrahedron Lett.*, 14, 468 (1961).

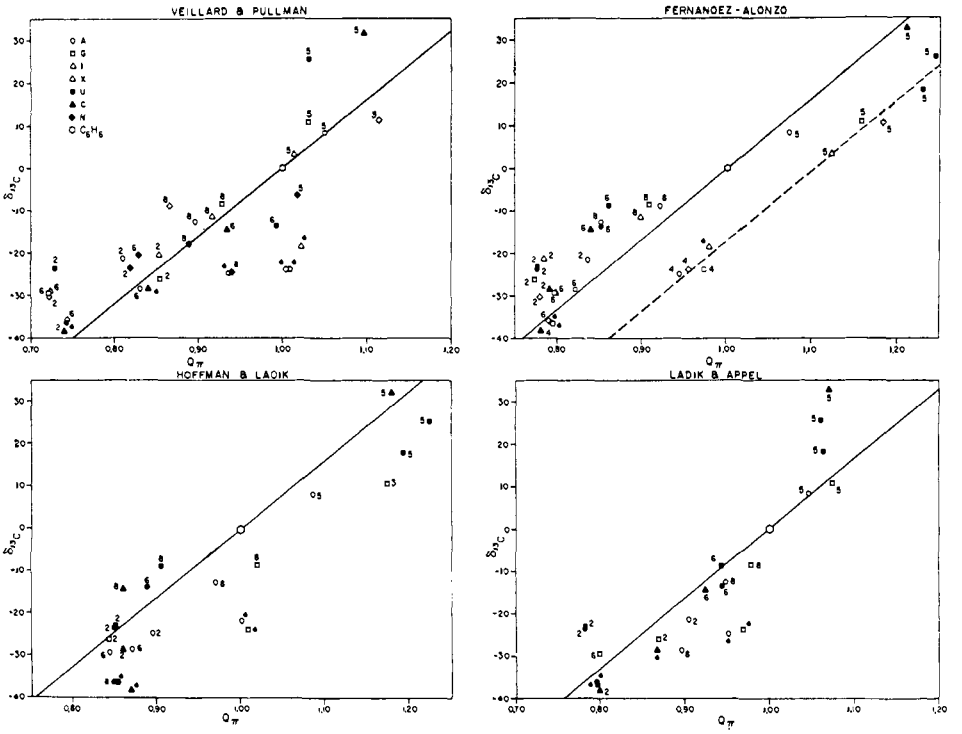

Figure 9. Observed carbon-13 chemical shifts for the naturally occurring nucleosides are plotted against the π-charge densities derived by Veillard and Pullman, Fernández-Alonso, Hoffmann and Ladik, and Ladik and Appel. The solid line indicates the 160 ppm/electron relationship.

heterocyclic bases in thymidine, cytidine, and adenosine are due to Mely and Pullman.[36] The π- and total-charge data derived by these workers are expressed in terms of Mulliken gross populations. A good correlation (135 ppm/electron) is obtained for the π-charge data in adenosine with the exception of C-4. However, inclusion of the data for thymidine and cytidine provides a considerable spread of points (Figure 10A). Consideration of total charge on the other hand provides the improved correlation shown in Figure 10B.

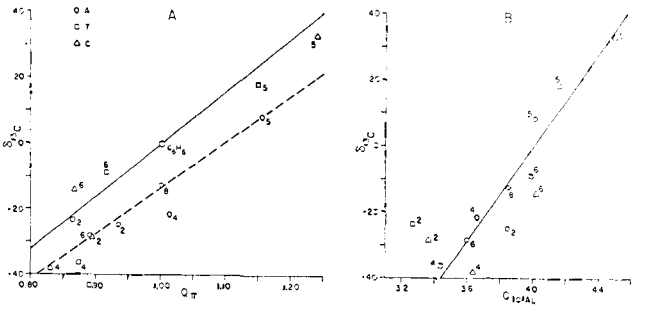

Figure 10. Observed carbon-13 chemical shifts for thymidine, cytidine, and adenosine are plotted against the π-gross population (A) and total gross population (B) derived by Mely and Pullman.

Conclusions

It is appreciated that the comparison we have made above between the theoretical parameters derived for the heterocyclic bases and the carbon-13 chemical shifts for the corresponding nucleosides is not strictly exact. Neither, perhaps, is the comparison between the various theoretical methods. However, it is clear that this comparison does provide at least an estimate of the reliability of the theoretical method in accounting for the important electronic features reflected in the carbon-13 chemical shifts.

The results indicate that the simple correlation of π charge with carbon-13 chemical shift data is inadequate and that a variety of additional contributing factors must be considered. These factors have been discussed in several more refined treatments[6,8,29-31] all of which are based on the theory of carbon-13 shifts derived by Karplus and Pople.[40] It is a consequence of the Karplus–Pople theory[40] that the emphasis in accounting for carbon-13 shifts has to date been placed upon the dominant charge-polarization features of the calculation, though it has become increasingly clear that variations in bond order and related overlap features[6,8,30,31] and average excitation energy terms[41] are important.

It is perhaps more important to point out that the present results emphasize the fact that carbon-13 chemical shifts do reflect the electronic structure of even relatively complex molecules and thus promise to be a valuable source of information regarding electronic and structural analysis in bioorganic molecules.

Acknowledgments. This work was supported by Research Grants No. GM-08521-09 and CA-08109-04 from the National Institutes of Health, U.S. Public Health Service.

(40) M. Karplus and J. A. Pople, *J. Chem. Phys.*, 38, 2803 (1963).

(41) A. J. Jones, D. M. Grant, J. G. Russell, and G. Fraenkel, *J. Phys. Chem.*, 73, 1624 (1969).