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Carbon-13 Nuclear Magnetic Resonance: Naturally Occurring Nucleosides*

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Abstract. The natural-abundance carbon-13 nuclear magnetic resonance spectra of the naturally occurring pyrimidine and purine nucleosides have been determined and completely analyzed. The carbon-13 chemical shifts obtained provide information on the electronic structure and qualitatively at least correlate well with the known chemical reactivity of these molecules. The carbon-13 shifts are in general far more informative than proton shifts for structural analysis of the nucleosides.

A variety of physicochemical techniques have been applied to the problems of electronic features and of structural determination in biologically important molecules. In the area of nucleoside chemistry, perhaps the most useful technique to date has been proton magnetic resonance (pmr) spectroscopy.[1] However, the limitations of this technique are dependent on the need for all sites of the molecular framework to be proton bearing. Carbon-13 magnetic resonance spectroscopy affords an opportunity to study in detail the structure of the molecular framework, with only the exception of the hetero-atom sites. Further, the greater carbon-13 chemical shift range enables relatively small differences in structure to be detected.

We have determined the natural abundance carbon-13 spectra (15.1 MHz) of the naturally occurring nucleosides, uridine (U), thymidine (T), cytidine (C), deoxycytidine (dC), adenosine (A), deoxyadenosine (dA), guanosine (G), deoxyguanosine (dG), inosine (I), deoxyinosine (dI), and xanthosine (X), using proton noise decoupling techniques[2] in conjunction with a time averaging device for spectral accumulation. Spectra were determined for solutions in dimethylsulfoxide (internal standard) while dimethylformamide was required to remove overlapping of the DMSO peak with the C-2 peak in the deoxyderivatives. The molar concentrations used are given for each compound in Table 1. On the average, 150 accumulations at 10 Hz per second sweep rate were recorded. Examples of the spectra obtained are given in Figure 1. The observed chemical shifts are summarized in Table 1.

The observed resonances for all the compounds studied clearly separate into (*a*) those due to the sugar-carbons (+37 to +89 ppm) and (*b*) those due to the carbon atoms of the pyrimidine or purine base (−36 to +33 ppm). Complete

TABLE 1. *Carbon-13 chemical shifts in naturally occurring nucleosides.**

Nucleosides	Carbon Positions				
	C-2	C-4	C-5	C-6	C-8
Uridine (2.7 *M*)	-23.8_8	-36.2_0	$+25.4_6$	-13.6_8	. . .
Thymidine† (2.7 *M*)	-23.0_6	-36.3_7	$+18.0_2$	$-\ 8.8_0$	. . .
Cytidine (2.6 *M*)	-28.4_2	-38.1_7	$+32.8_5$	-14.2_9	. . .
Deoxycytidine (2.6 *M*)	-28.4_9	-38.4_4	$+32.6_7$	-13.9_3	. . .
Adenosine (1.5 *M*)	-24.9_2	-21.5_4	$+\ 8.1_5$	-28.5_9	-12.6_2
Deoxyadenosine (1.5 *M*)	-25.1_2	-21.5_0	$+\ 8.0_9$	-28.6_1	-12.4_4
Guanosine (0.6 *M*)	-26.0_6	-23.8_2	$+10.9_8$	-29.2_5	$-\ 8.3_5$
Deoxyguanosine (0.5 *M*)	-26.1_8	-23.4_8	$+10.8_5$	-29.6_3	$-\ 8.1_3$
Inosine (1.0 *M*)	-20.6_9	-18.4_3	$+\ 3.1_8$	-29.1_6	-11.3_9
Deoxyinosine (1.0 *M*)	-19.9_9	-17.7_6	$+\ 3.3_2$	-28.8_4	-10.8_1
Xanthosine (*ca.* 0.2 *M*)	-30.4_2	-23.8_3	$+11.2_5$	-35.9_7	$-\ 8.9_5$
Uridine (2.7 *M*)	$+39.4_2$	$+57.4_2$	$+53.6_6$	$+42.5_8$	$+66.3_0$
Thymidine† (2.7 *M*)	$+43.4_7$	$+88.1_0$	$+56.8_7$	$+40.1_0$	$+66.1_7$
Cytidine (2.6 *M*)	$+38.4_1$	$+57.9_6$	$+53.4_0$	$+43.1_6$	$+66.6_5$
Deoxycytidine (2.6 *M*)	$+41.9_4$	$+87.9_0$	$+56.7_5$	$+39.9_4$	$+65.8_8$
Adenosine (1.5 *M*)	$+39.3_3$	$+56.7_6$	$+53.8_8$	$+41.5_6$	$+65.8_9$
Deoxyadenosine (1.5 *M*)	$+42.9_8$	$+88.0_6$	$+56.2_9$	$+39.3_6$	$+65.6_0$
Guanosine (0.6 *M*)	$+41.2_7$	$+56.9_8$	$+53.6_6$	$+42.1_3$	$+66.3_4$
Deoxyguanosine (0.5 *M*)	$+44.6_7$	$+88.1_0$	$+56.6_9$	$+39.8_3$	$+65.6_5$
Inosine (1.0 *M*)	$+39.7_3$	$+57.2_8$	$+53.3_7$	$+41.8_8$	$+66.2_4$
Deoxyinosine (1.0 *M*)	$+44.1_7$	$+88.3_0$	$+57.1_4$	$+39.7_9$	$+66.2_8$
Xanthosine (*ca.* 0.2 *M*)	$+38.6_7$	$+57.0_0$	$+53.7_0$	$+41.2_6$	$+66.3_5$

* Shifts given in parts per million relative to benzene and positive values indicate higher field.
† Methyl carbon at $+115.2_8$ ppm.

assignments of these resonances have been made from studies of a variety of models compounds, many of which were synthesized to effect major changes in electron density at specific sites in the pyrimidine, purine, or ribose frameworks.[3] Assignment of the C-1′ and C-4′ carbons in the deoxycompounds depends upon prepublication results furnished by Dorman and Roberts[4] on the corresponding nucleotides where phosphorus couplings to C-4′ allow an assignment to be made.

We have plotted the observed chemical shifts in the pyrimidine and purine rings for the nucleosides described above against the π-charge densities derived

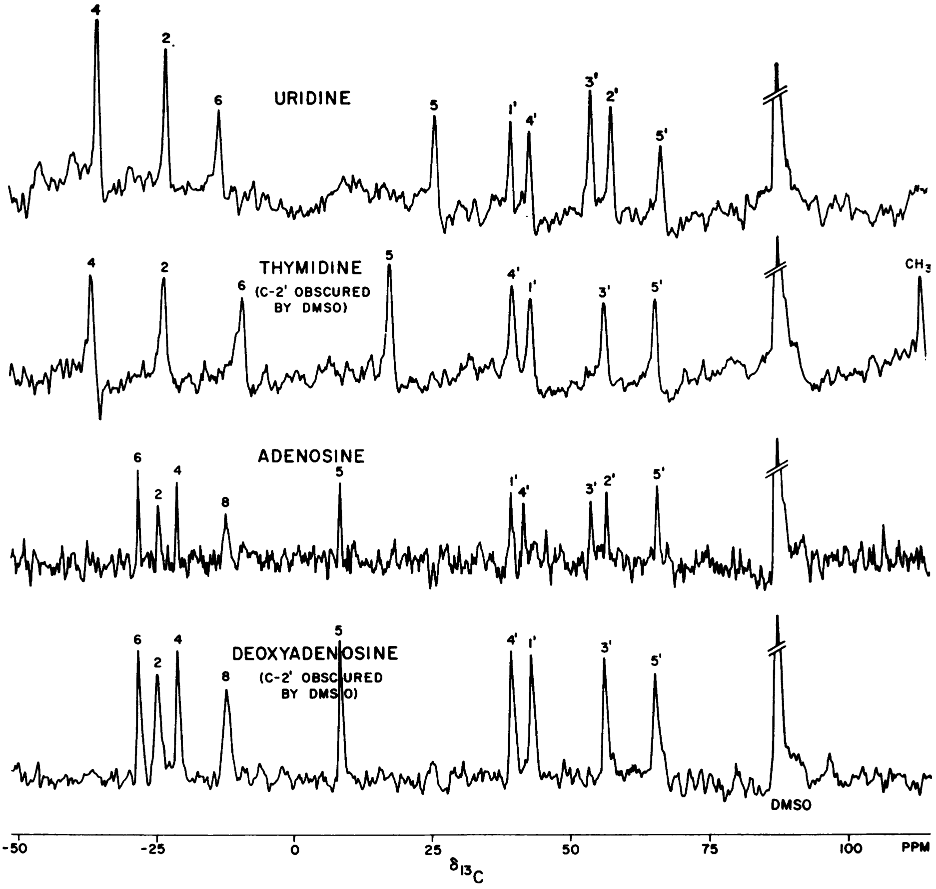

FIG. 1.—Typical carbon-13 magnetic resonance spectra of selected nucleosides.

for these systems by Fernandez-Alonso[5] using the Pauling-Wheland method. The results are shown in Figure 2. The similarity of this plot and others of its kind is remarkable considering the complexity of the molecules considered. The majority of points associated with proton-bearing carbon atoms correlate reasonably well with the 160 ppm per electron relationship (*solid line*) proposed by Spiesecke and Schneider.[6] However, the quaternary carbon atoms C-4 and C-5 in the purine nucleosides form a separate group along with the C-5 carbon in uridine and thymidine. It is apparent that a parallel correlation line (*dashed*) could be drawn through these latter points. Similar observations have been made in previous theoretical studies where correlations between molecular orbital parameters and carbon-13 shifts exist.[7] The difference noted between the quaternary and C-H bonded carbon atoms has been attributed to either the σ-bond polarization of the C-N bonds or to the neglect of overlap in the methods of calculation. There would seem to be considerable justification for invoking these arguments in the present case, in particular, corrections for the neglect of overlap implicit in the Pauling-Wheland method should be considered.

The high charge density at C-5 in all the compounds studied correlates

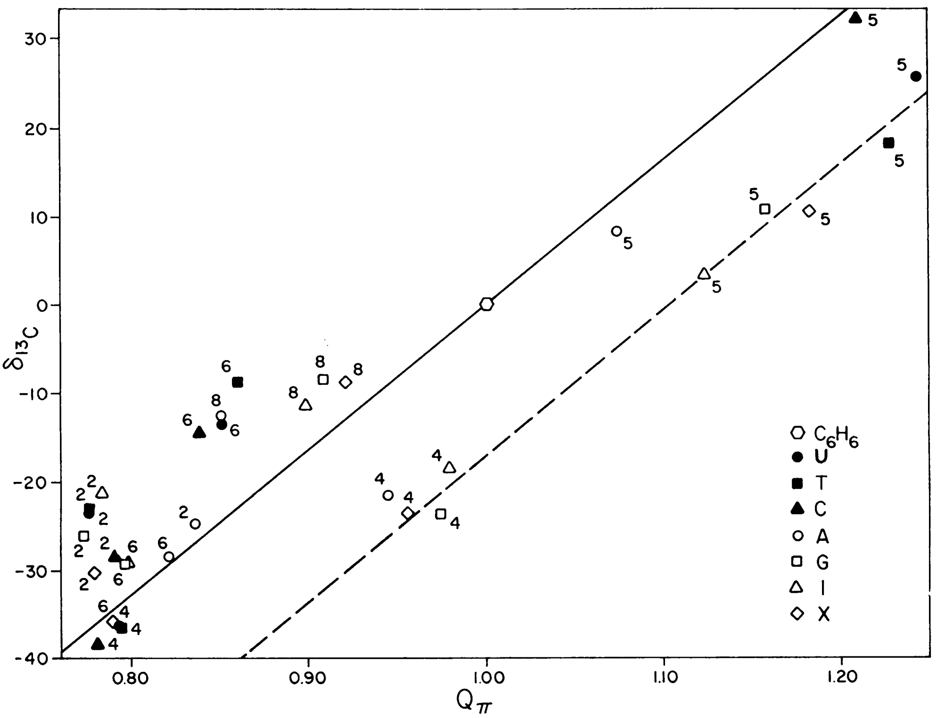

FIG. 2.—Carbon-13 shifts plotted against π-electron densities calculated by Fernandez-Alonso with the Pauling-Wheland method.

reasonably well with the carbon-13 shifts and consequently these shifts may be considered to reflect the high electrophilic reactivity of this position, particularly in the pyrimidine nucleosides. Further studies of these correlations are obviously necessary due to the uncertainties involved in the theoretical parameters and the observation that carbon-13 shifts are not simply related to π-charge.[8] It is clear, however, that the carbon-13 shifts do reflect the electronic structure and promise to be a valuable tool in the structural analysis of these biologically important molecules.

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[3] Details will be presented shortly in a full paper.

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