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Nuclear Magnetic Resonance Spectroscopy: ^{13}C Spectra of Some Common Nucleotides*

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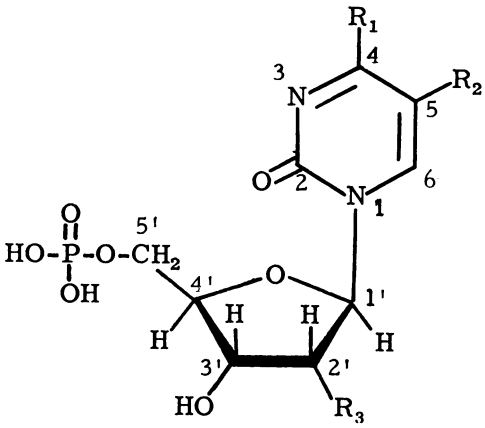
Abstract. Natural-abundance ^{13}C nuclear magnetic resonance spectra of ten common nucleotides in neutral, aqueous solutions have been determined and interpreted. The spectra of two of these substances were also determined in acidic solutions, and several of the carbon chemical shifts were found to depend markedly on pH. Within the limited range of concentrations employed, there were observed no carbon chemical-shift changes which could be ascribed to base-stacking or base-pairing phenomena.

Proton magnetic resonance (pmr) spectroscopy has yielded much information regarding conformation and association through base-stacking of nucleosides and nucleotides.[1] The few protons on the purine or pyrimidine rings generally lead to well-resolved and easily interpretable pmr spectra which are dependent upon concentration of nucleotide, pH of the solution, temperature, as well as other factors.[2] In some cases, however, the paucity of protons on the purine ring limits the sites at which conformational and/or base-stacking effects can be observed. For example, with guanosine-5′-monophosphate (GMP) there is only one proton (H-8) on the purine ring, and thus the effect of inter- and intramolecular interactions at other sites of the guanine ring cannot be observed.

In the interest of generalizing the applicability of nuclear magnetic resonance (nmr) spectroscopy for study of these phenomena, we have examined the ^{13}C magnetic resonance (cmr) spectra of ten nucleotides (Figs. 1, 2, and 3). The chemical shifts for the carbon nuclei of these substances are tabulated in Table 1. The spectral data were gathered at 15.1 MHz with the digital-frequency sweep spectrometer described previously,[3] using 1–2 *M* aqueous solutions and 1 per cent (v/v) *p*-dioxane as internal standard. The protons were decoupled by noise modulation at 60 MHz.[4]

The resonances of the ribose carbons fell within the range of 100 to 130 ppm,[5] and were relatively independent of the identity or substitution of the purine or pyrimidine moiety. The highest field peak characteristically appeared as a broadened singlet, though in some instances a poorly resolved doublet ($J \sim 4$ Hz) could be observed. Because the hydroxymethylene carbon resonances of pyranoses are known to occur at least 5 ppm toward higher field than those of the remaining carbon nuclei,[6, 7] and because phosphorylation of an alcohol appears to have only a small effect on the chemical shift of the α carbon,[8] this



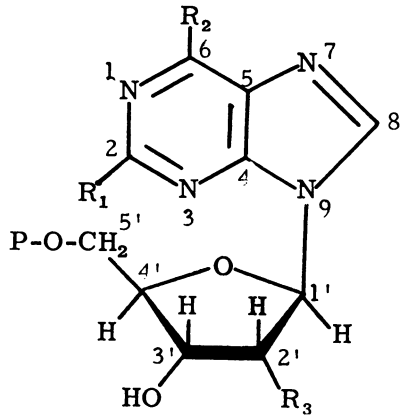


FIG. 1.—UMP:
$R_1 = R_3 = OH$, $R_2 = H$
TMP:
$R_1 = OH$, $R_2 = CH_3$, $R_3 = H$
CMP:
$R_1 = NH_2$, $R_2 = H$, $R_3 = OH$
dCMP:
$R_1 = NH_2$, $R_2 = R_3 = H$
AMP:
$R_1 = H$, $R_2 = NH_2$, $R_3 = OH$, $P = PO_3H_2$
dAMP:
$R_1 = R_3 = H$, $R_2 = NH_2$, $P = PO_3H_2$
ATP:
$R_1 = H$, $R_2 = NH_2$, $R_3 = OH$, $P = P_3O_9H_4$
GMP:
$R_1 = NH_2$, $R_2 = R_3 = OH$, $P = PO_3H_2$
dGMP:
$R_1 = NH_2$, $R_2 = OH$, $R_3 = H$, $P = PO_3H_2$
IMP:
$R_1 = H$, $R_2 = R_3 = OH$, $P = PO_3H_2$.

resonance was assigned to carbon 5′. The position of this peak is relatively invariant throughout the series studied, with the sole exception of adenosine-5′-triphosphate (ATP). Apparently, the triphosphate group is slightly more deshielding than the monophosphate group. Only one other resonance of the spectrum of each pentose fragment appeared to be split by the phosphorus nucleus ($J \sim 8$ Hz), and this resonance could be assigned to C-4′ on the basis that in phosphate esters the phosphorus-carbon coupling is often larger at the β carbon than at the α carbon.[8] The other low-field pentose carbon resonance was assigned to the anomeric carbon (1′),[7] and it is noteworthy that the chemical shift of this resonance is the one of the pentose carbons which is most dependent upon the identity of the pyrimidine or purine fragment.

The two remaining pentose resonances may be assigned by comparison of the spectra of ribose and deoxyribose nucleotides. For the deoxy compounds, the resonances of carbons 1′, 4′, and 5′ can be assigned as above. Of the remaining peaks, the upfield one (~153 ppm) is attributed to the methylene carbon, C-2′, leaving the resonance at about 121 ppm for C-3′. The C-1′ resonances of the deoxyribose nucleotides are 3-4 ppm upfield from their positions in the spectra of the ribonucleotides, and it is to be expected that the C-3′ resonances will show shifts similar in sign, if not in magnitude. On this basis, the resonances near 118 ppm of the spectra of the ribonucleotides are assigned to C-3′, leaving those near 122.5 ppm to be attributed to C-2′.

An assignment of the resonances of the pyrimidine carbons of uridine-5′-

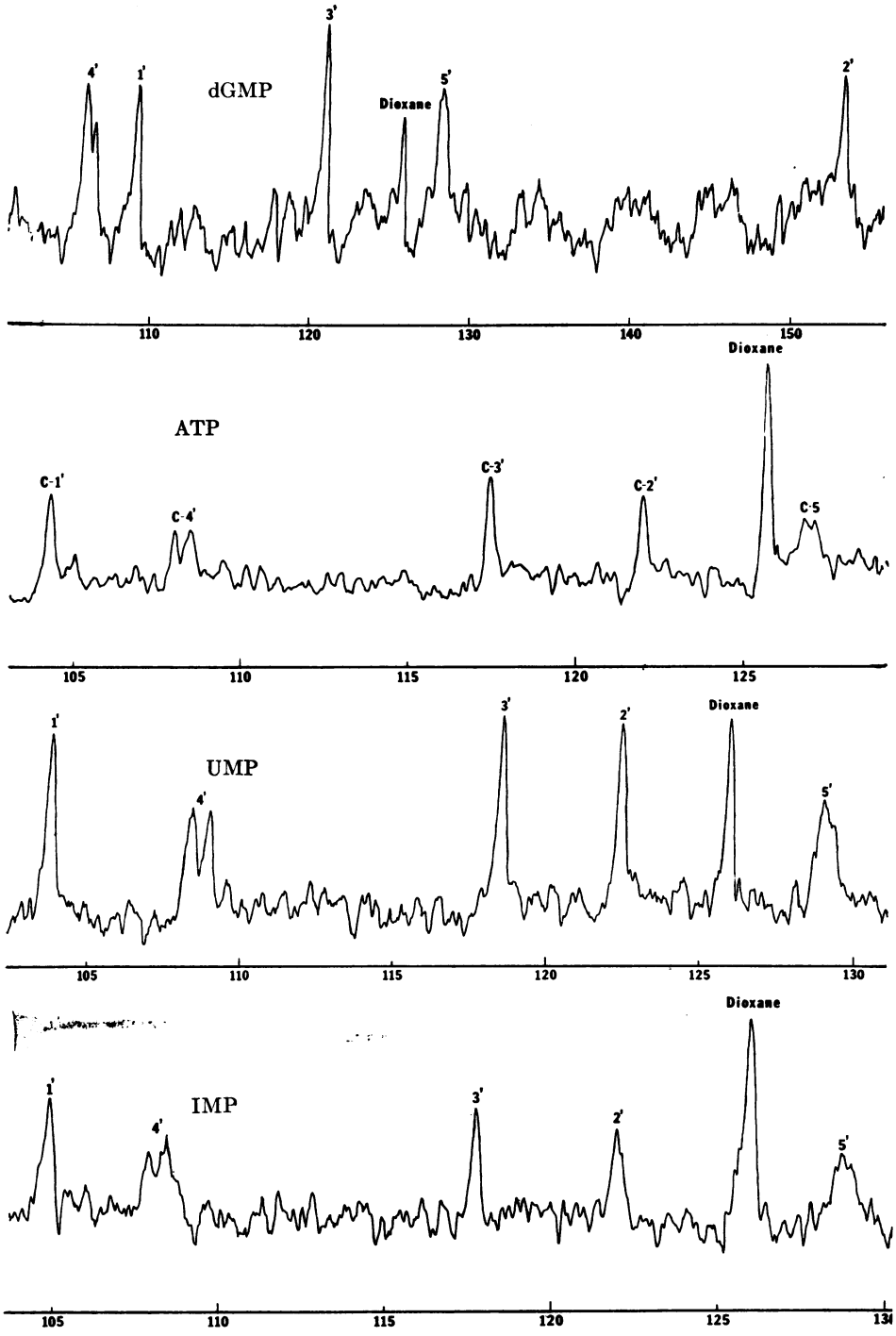


FIG. 2.—Representative ^{13}C spectra of some pentose moieties of nucleotides in aqueous solution. dGMP: pH = 7, 1091 scans, 40 Hz/second; ATP: pH = *ca.* 2.5, 134 scans, 20 Hz/second; UMP: pH = 7.8, 170 scans, 20 Hz/second; IMP: pH = 7.6, 75 scans, 20 Hz/second.

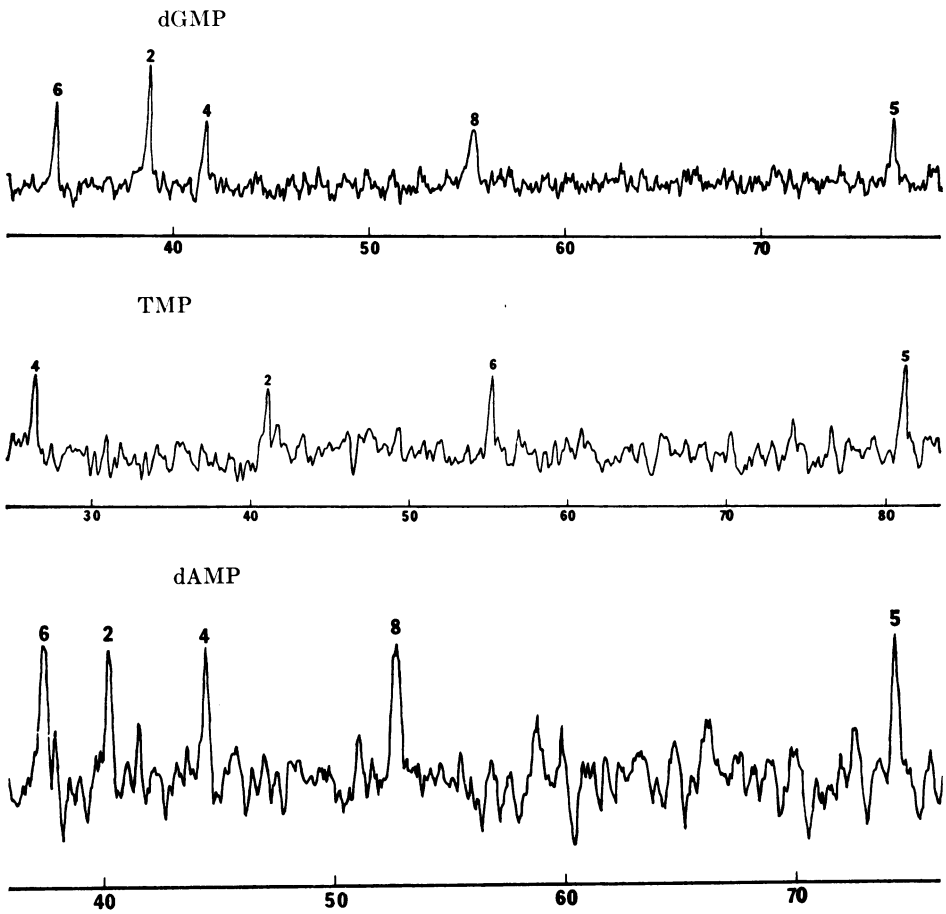


FIG. 3.—Representative ^{13}C spectra of pyrimidine and purine moieties of some nucleotides in aqueous solution. dGMP: pH = 7, 400 scans, 10 Hz/second; TMP: pH = 7, 170 scans, 40 Hz/second; dAMP: pH = 7, 454 scans, 40 Hz/second.

monophosphate (UMP), thymidine-5′-monophosphate (TMP), cytidine-5′-monophosphate (CMP), and deoxycytidine-5′-monophosphate (dCMP) is similarly possible by comparison of spectra. From the spectra of CMP and dCMP it is obvious that the pyrimidine carbon resonances are independent of whether the pentose fragment is ribose or deoxyribose. Thus, the differences in the pyrimidine spectra of UMP and TMP must result from the presence of the C-5 methyl group in the latter. Comparison of these spectra shows that the peak at 89.9 ppm in the spectrum of UMP is shifted downfield by almost 9 ppm by the methyl group. A downfield shift of this magnitude is typical of a carbon which is directly substituted by a methyl group,[9, 10] and this resonance is accordingly assigned to C-5. The peak at 50.4 ppm in the spectrum of UMP, however, appears about 5 ppm toward higher field with TMP. Such an upfield

TABLE 1. *^{13}C Chemical shifts in common nucleotides.*[b]

Nucleotide	C-2	C-4	C-5	C-6	C-8
UMP	40.7	26.3	89.9	50.4	...
TMP[c]	41.0	26.3	81.1	55.1	...
CMP	35.0	26.3	96.0	50.7	...
CMP[d]	43.7	32.8	96.1	48.7	...
dCMP	35.1	26.5	96.0	50.7	...
IMP	45.8	44.3	69.4	34.2	52.7
AMP	40.1	44.1	74.5	37.5	52.6
dAMP	40.3	44.4	74.4	37.5	52.7
ATP	40.2	44.2	74.5	37.7	52.9
ATP[e]	46.6	42.3 or 44.6	74.4	44.6 or 42.3	50.4
GMP	38.9	41.5	76.8	34.2	55.2
dGMP	38.7	41.6	76.8	34.0	55.4

Nucleotide	C-1′	C-2′	C-3′	C-4′[f]	C-5′[g]
UMP	104.0	122.4	118.5	108.5	129.0
TMP[c]	107.7	154.0	121.3	106.6	128.5
CMP	103.4	122.8	118.2	109.2	129.2
CMP[d]	102.8	123.1	118.1	109.0	128.8
dCMP	106.9	153.2	121.6	106.6	128.9
IMP	104.9	121.9	117.7	108.3	129.0
AMP	105.5	122.0	118.0	108.3	129.0
dAMP	109.0	153.4	121.1	106.4	128.6
ATP	105.6	122.4	118.3	108.7	127.3
ATP[e]	104.5	122.3	117.7	108.5	127.2
GMP	105.5	121.9	118.2	108.4	128.6
dGMP	109.3	153.4	121.2	106.4	128.5

[a] In parts per million upfield from external CS_2. Experimental error ca. ± 0.2 ppm.
[b] Spectra determined in neutral aqueous solutions, except where noted.
[c] Methyl carbon chemical shift 180.8 ppm.
[d] At pH 2.5.
[e] At pH 2.3.
[f] Doublet, $J_{CP} \sim 8$ Hz.
[g] Poorly resolved doublet or broadened singlet, $J_{CP} \leq 4$ Hz.

shift is typical of a β-substituent shift in alkenes,[10] thus indicating that these resonances of the spectra of UMP and TMP may be assigned to carbons 6. The remaining two resonances are virtually unchanged in these two spectra.

The assignment of pyrimidine carbons may be completed by comparison of the cytosine and uracil moieties of CMP and UMP, respectively. These differ only in the substitution at C-4, and it might therefore be expected that the C-4 resonances of the two substances would differ more than the C-2 resonances. This suggests assignment of the resonances in the range of 35-41 ppm to C-4, leaving the peaks at 26.3 ppm to be assigned to C-2 by default. However, the work of Grant *et al.*[11] using pyrimidine nucleosides substituted at C-4 dictates the opposite assignment, and we accept their interpretation.

The assignment of the resonances of the spectra of the purine fragments can be initiated by the utilization of off-resonance proton decoupling,[8, 12] under which conditions only the quaternary carbon resonances remain uncoupled. The off-resonance proton decoupled spectrum of GMP allows the identification of the resonance of carbon 8, the only proton-bearing carbon in the purine ring. In the remainder of the spectrum of the guanine moiety of GMP, the peaks are

TABLE 2. *^{13}C Chemical shifts[a] in nucleotide mixtures.*

Mixture	Nucleotide	2	4	5	6	8
AMP-TMP	AMP	40.0	44.0	74.3	37.4	52.5
	TMP[b]	40.9	26.3	81.1	55.1	...
CMP-GMP	CMP	35.2	26.8	96.2	51.1	...
	GMP	38.7	41.2	76.7	33.9	55.1
dCMP-dGMP	dCMP	35.3	26.5	96.1	50.9	...
	dGMP	38.6	41.5	76.7	33.9	55.5
IMP-UMP	IMP	46.1	44.2	69.4	34.2	52.9
	UMP	40.9	26.7	90.2	50.6	...

Mixture	Nucleotide	1′	2′	3′	4′	5′
AMP-TMP	AMP	105.3	121.9	117.9	108.3	129.0
	TMP[b]	107.6	153.9	121.4	106.7	128.3
CMP-GMP	CMP	103.1	122.8	118.3	109.4	129.0[c]
	GMP	105.5	122.0	118.3	108.5	129.0[c]
dCMP-dGMP	dCMP	106.8	153.2	121.6	106.7[c]	128.6[c]
	dGMP	109.3	153.5	121.1	106.7[c]	128.6[c]
IMP-UMP	IMP	104.9	122.2	118.6	108.7[c]	129.1[c]
	UMP	104.1	122.6	117.8	108.7[c]	129.1[c]

[a] In parts per million upfield from external CS_2.
[b] Methyl carbon resonance at 180.8 ppm.
[c] These values are more approximate than the others reported.

of significantly lower intensity than those of the pentose carbons, with the sole exception of the resonance at 38.9 ppm. The latter peak has been assigned to carbon 2, the enhanced intensity of the peak probably resulting from a nuclear Overhauser enhancement[13] associated with the protons on the C-2 amine group.

The C-2 and C-8 resonances of adenosine-5′-monophosphate (AMP), adenosine-5′-triphosphate (ATP), deoxyadenosine-5′-monophosphate (dAMP), and inosine-5′-monophosphate (IMP) can also be identified by off-resonance proton decoupling. The relatively unshifted peaks in the range 52.6-55.4 ppm are shown by off-resonance decoupling to bear a directly attached proton in all the purine nucleotides, and these resonances are assigned to carbon 8. This identifies by elimination the C-2 resonances of AMP, ATP, dAMP, and IMP. Of the three remaining resonances, all of which arise from quaternary carbons in every purine nucleotide, the highest field is assigned to carbon 5 by analogy to the spectra of the pyrimidines. The general observation that this peak is the least intense and most easily saturated of the spectrum may reflect a generally lower efficiency of relaxation for the nucleus of C-5. The lower field of the two remaining, unassigned peaks is assigned to C-6, which leaves the remaining peak to be attributed to C-4. This assignment requires that the chemical shift of the C-6 resonance is dependent upon the identity of the directly attached functional group (i.e., hydroxyl vs. amine group) and is independent of substitution at C-2. The C-4 resonance, on the other hand, seems independent of the C-6 substituent, being shifted only when C-2 is substituted.

Although these assignments are to some degree tentative, they are supported by some preliminary investigations of the pH dependence of carbon chemical shifts for two of these substances (see Table 1). For CMP, acidification changes only the chemical shift of carbon 1′ in the spectrum of the pentose fragment, while all the carbon resonances of the cytosine ring, except that of C-5, are

shifted. The resonances of carbons 1′ and 6, which are adjacent only to the basic nitrogen 1, are shifted downfield on acidification. The resonances of carbons 2 and 4, which are adjacent to the more weakly basic nitrogen 3, are shifted upfield by 6 to 8 ppm. From Table 1 it is seen that similar changes occur for ATP. Thus, again with the sole exception of C-5, those carbons adjacent to more weakly basic nitrogens undergo upfield shifts upon acidification, while those attached to the basic N-9 tend to be shifted slightly downfield. However, even these data leave an unsatisfactory uncertainty in the differentiation of carbons 4 and 6 in the purine system, and a secure assignment of these resonances would require study of substituted derivatives which are not presently available.

Preliminary experiments designed to determine the effect of base destacking[1] on the cmr spectra gave negative results. The spectrum of UMP, for example, was essentially unchanged when a 2 *M* solution was diluted to 1 *M*. Other examples showed little, if any, concentration dependence. Probably our failure to observe concentration effects resulted from the limited range of concentrations over which it is now practicable to determine in a reasonable period natural-abundance cmr spectra. Attempts to measure chemical shifts in solution of concentration equal to or less than 0.25 *M* were not successful.

Base pairing is known to be important in the maintenance of the helical structure of deoxyribonucleic acids (DNA).[14, 15] It seemed possible, therefore, that the mixture of nucleotides containing bases which are known to interact strongly in DNA would show noticeable changes in the chemical shifts of the carbons of the respective nucleotides. The results (Table 2), however, show the mixtures AMP-TMP, CMP-GMP, and dCMP-dGMP have cmr shifts which are virtually identical with those determined for the separate nucleotides. A mixture of IMP and UMP, which was examined as a control, shows the same result. The failure to observe larger changes may be the result of preferential hydrophobic, base-stacking self-association of the purine bases in these concentrated solutions.[16]

Study of nucleotides by natural-abundance cmr spectroscopy is clearly feasible, and as future advances in instrumentation permit enhanced sensitivity in the detection of ^{13}C resonance signals, it seems likely that effects of concentration, pH, and temperature on the carbon chemical shifts of nucleotides could afford significant aid to the understanding of inter- and intramolecular interactions in these substances.

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[5] All chemical shifts reported here are measured in parts per million upfield from external carbon disulfide. On this scale, the chemical shift of 1% (v/v) *p*-dioxane in water is 126.0 ppm.

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