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Studies on Nucleosides and Nucleotides. LXXXI.[1] Carbon-13 Magnetic Resonance Spectra of 8-Substituted Purine Nucleotides. Effects of Various Phosphate Groups on the Chemical Shifts and Conformation of Nucleotides

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Carbon-13 magnetic resonance spectra of 8-substituted purine nucleotides and the corresponding parent nucleotides were measured. The 8-substituted derivatives included the 2′-, 3′-, 2′,3′-cyclic, 5′- and 3′,5′-cyclic phosphates of 8-bromoadenosine and the 5′-phosphates of 8-bromoguanosine, 8-methylinosine and 2-methylthio-8-methylinosine. All the 8-substituted nucleotides showed a characteristic upfield shift (−0.9 to −3.7 ppm) of the 2′-carbon with respect to the corresponding parent nucleotides. These results show that they take a *syn* conformation in aqueous solution to some extent. It was concluded from consideration of the sugar puckerings in the published PMR data that the 5′-phosphate of 8-bromoadenosine takes a more rigid *syn* conformation than the 2′-, 3′- and 2′,3′-cyclic phosphates. It is also suggested that 8-bromoadenosine has flexible glycosidic conformation similar to those for the latter compounds in water while in DMSO it adopts a more rigid conformation. The 5′-phosphates of the other 8-substituted nucleosides were also assumed to adopt a rigid *syn* conformation. The influences of various types of phosphate groups on the carbon chemical shifts are also discussed. Relatively large upfield shifts were observed for the C(4′) signal of the 8-substituted 5′-nucleotides which has been assumed to be a reflection of a high population of non-gg conformations about the C(4′)–C(5′) bond.

Keywords——C-13 NMR; 8-Br-GMP; 8-Br-AMP; 8-methyl-IMP; 2-thioethyl-8-methyl-IMP; chemical shift change

Introduction

Purine nucleosides and nucleotides with a bulky substituent at the 8-position show a characteristic downfield shift of the H(2′) signal upon 8-substitution.[3–6] We have examined Carbon-13 magnetic resonance (^{13}C–NMR) spectra of various 8-substituted purine nucleosides and found that all the 8-substituted derivatives show a characteristic upfield shift of the C(2′) upon 8-substitution with respect to the corresponding parent nucleosides.[7] These shifts of H(2′) and C(2′) are assumed to be associated with adoption of the *syn* conformation[8] where the lone pair electrons on N(3) come close to the C(2′)-H(2′) bond. CD studies also suggest that 8-substituted nucleosides and nucleotides take a *syn* conformation in aqueous solution.[3] X-Ray analyses have shown that 8-bromo-derivatives of adenosine and guanosine,[9] 8-bromo-2′,3′-*O*-isopropylideneadenosine[10] and 2-methylthio-8-methylinosine[11] take *syn*

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conformation in crystals. 2′-*O*- and 3′-*O*-triisopropylbenzenesulfonyl derivatives of 8-bromoadenosine also take a *syn* conformation in crystals[12] as well as in Me_2SO solution as indicated by nuclear Overhauser experiments.[13] Only a few papers have been published on the ^{13}C–NMR of mononucleotides[14–19] and they have mainly discussed ^{13}C–^{31}P coupling constants to estimate the torsion angle involving phosphorus atoms. Here we report on the ^{13}C–NMR properties of 8-substituted purine nucleotides and the corresponding parent nucleotides and discuss the effect of 8-substitution and phosphorylation on the chemical shifts and the conformation of the nucleotides.

Experimental

The sources of 8-substituted nucleosides have been given in our previous paper.[7] Sources of 8-substituted nucleotides and 8-bromo-isopropylideneadenosine are shown in the legends for Table I and II. The deuterated solvent, Me_2SO-d_6 (99.8%, ^2H) was obtained from CEA, France. Me_2SO-d_6 (for nucleosides) and H_2O (for nucleotides, sodium salts) were used as solvents. Dioxane (0.5%, v/v) was used as an internal standard. The natural abundance ^{13}C spectra were recorded on a Hitachi R-22 (22.63 MHz, ambient probe temperature 32—35°) spectrometer operating in the Fourier transform mode in connection with a Hitachi HITAC-1011 computer package. ^{13}C chemical shifts were obtained from noise-decoupled spectra using dioxane as internal reference. A chemical shift change on the downfield direction is expressed as a positive value and an opposite change is expressed as a negative value. The chemical shift of dioxane (0.5% in H_2O) signal relative to external tetramethylsilane was 67.11 ppm.

Results and Discussion

The chemical shifts and ^{13}C–^{31}P coupling constants for various adenine nucleosides and nucleotides are presented in Table I. The chemical shifts and coupling constants for various guanine and hypoxanthine nucleosides and nucleotides are shown in Table II. Assignments of ^{13}C signals were made mainly according to the work of Jones *et al.*[20] except for a reversal of the C(2′) and C(3′) assignments as indicated by Mantsch *et al.*[15] and a reversal of the C(4) and C(2) assignments for inosine derivatives.[14] The relative peak heights of signals and the results of off-resonance experiments were taken into consideration for assignment as discussed in the previous paper.[7] In addition ^{13}C–^{31}P coupling constants and the effects of phosphate groups on chemical shifts were also taken into account for nucleotide spectra. For example the C(2′) and C(3′) signals of 8-bromoadenosine 3′-phosphate (8-Br-3′-AMP, IV) were assigned as shown in Table I in a reversed order to their appearance because the C(3′) signal has a slightly larger coupling constant and should show about a 2.5 ppm downfield shift with respect to the C(3′) chemical shift of the parent nucleoside in accordance with the properties of 3′-AMP signals.[19,20] For similar reasons the assignment of the C(4′) and C(3′) signals of adenosine 3′,5′-cyclic phosphate (3′,5′-cAMP, IX) by Lapper *et al.*[16,21] was reversed and the same principle was also applied to the case of the 8-bromo derivative (X). This problem will be discussed later.

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Table I. Carbon-13 Chemical Shifts[a] (ppm) and ^{13}C-^{31}P Coupling Constants (Hz)[b] for Various Adenine Nucleosides and Nucleotides

Compound[c]	C(2)	C(4)	C(5)	C(6)	C(8)	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
A	85.98	82.74	53.00	89.76	73.57	21.64	7.13	4.31	19.54	−4.62
2′-AMP (I)	85.71	81.85	52.26	88.71	74.07	21.08 (9)	9.55 (3.5)	4.01	18.58	−4.93
3-AMP (III)	85.70	81.61	52.33	88.70	73.91	21.88	7.32 (3)	6.78 (4)	18.88 (4.5)	−4.86
2′,3′-cAMP (V)	85.86	81.31	51.95	88.64	73.84	22.74 (3.5)	13.72	10.95	18.66 (3.5)	−5.31
5′-AMP (VII)	85.93	82.00	51.71	88.63	73.37	20.50	8.01	4.16	17.92 (9)	−2.93 (3.5)
3′,5′-cAMP (IX)	86.13	81.27	51.79	88.59	72.94	24.94	5.82 (8)	10.70 (4)	5.19 (4)	0.75 (7)
8-Br-A	86.04	83.52	53.36	88.80	60.74	24.10	4.80	4.50	20.33	−4.20
8-Br-2′-AMP[d] (II)	85.55	82.77	52.95	87.71	61.73	23.62 (8.5)	8.48 (3.5)	4.31	19.34	−4.39
8-Br-3′-AMP[d] (IV)	85.47	82.46	52.79	87.55	61.50	24.27	6.17 (3.5)	7.05 (5)	19.92 (5.5)	−4.32
8-Br-2′, 3′-cAMP[e] (VI)	85.93	82.62	52.33	87.39	60.73	23.89 (3.5)	12.87	10.86	18.65 (3.5)	−5.17
8-Br-5′-AMP[d] (VIII)	86.09	83.39	52.33	87.32	61.20	22.74	4.32	3.39	17.23 (7.5)	−2.78 (3.5)
8-Br-3′, 5′-cAMP[d] (X)	86.28	83.05	52.14	87.25	60.81	26.48	4.53 (8.5)	10.26 (4)	5.32 (3)	0.57 (7)
Isp-A (XI)	86.18	82.47	52.74	89.72	73.30	23.31	16.90	14.98	20.01	−4.74
8-Br-Isp-A[f] (XII)	86.46	83.46	53.00	88.68	59.96	24.70	15.59	15.29	20.80	−4.86

a) Measured from internal-dioxane (0.5%, v/v). Experimental error is about ±0.05 ppm.
b) Spectral resolution is 2—2.5 Hz.
c) 0.2M solution in DMSO-d_6 (nucleosides) or in H_2O (nucleotides, sodium salt, pH 7—8). All these nucleotides showed no significant change in the chemical shifts upon further increase of pH.
d) M. Ikehara and S. Uesugi, *Chem. Pharm. Bull.* (Tokyo), **17**, 348 (1969).
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Effects of 8-Substitution on Sugar Carbon Resonances

The chemical shift changes ($\Delta\delta$(Br) or $\Delta\delta$(Me)) of the sugar carbons of various nucleotides upon substitution at the 8-position are presented in Table III. The chemical shift changes for corresponding nucleosides are also included for comparison, but it should be noted that nucleoside spectra were taken in Me_2SO-d_6 and nucleotide spectra were obtained in H_2O.

8-Br-2′-AMP (II) and 8-Br-3′-AMP (IV) show nearly the same $\Delta\delta$ (Br)'s on each carbon as those of 8-Br-A except for C(2′). They show an upfield shift of C(2′), which is assumed to be characteristic of the *syn* conformation, but the absolute value of $\Delta\delta$ is about half that for 8-Br-A. 8-Bromoadenosine 2′,3′-cyclic phosphate (8-Br-2′,3′-cAMP, VI) and 8-Br-3′,5′-cAMP also show smaller upfield shifts of C(2′). On the other hand, 8-Br-5′-AMP (VIII) shows a larger upfield shift (−3.69 ppm) of C(2′) than that (−2.34 ppm) of 8-Br-A. Other 5′-phosphate derivatives also show relatively large upfield shifts (−2.9——3.2 ppm) on C(2′).

We can assume three possible explanations for the relatively small upfield shift in 8-bromoadenine nucleotides other than the 5′-phosphate derivative: 1) they can take an *anti* conformation more easily than 8-Br-5′-AMP; 2) their sugar puckering is different to that in 8-Br-5′-AMP and is not favorable for the upfield shift caused by N(3); 3) the corresponding adenine nucleotides are already in the *syn* conformation. The sugar puckering,[17,22–25] and

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TABLE II. Carbon-13 Chemical Shifts[a] (ppm) and ^{13}C-^{31}P Coupling Constants (Hz)[b] for Various Purine Nucleosides and Nucleotides

Compound[c]	C(2)	C(4)	C(5)	C(6)	C(8)	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
G	87.30	84.96	50.37	90.42	69.25	20.14	7.37	4.07	18.88	−4.86
5′-GMP (XIII)	87.47	84.77	49.40	92.33	70.90	20.26	7.63	4.08	17.80 (7)	−2.98 (5)
8-Br-G	87.02	85.64	51.18	89.00	54.66	23.37	4.07	4.07	19.53	−4.32
8-Br-5′-GMP[d] (XIV)	87.05	85.97	50.05	91.06	57.14	23.23	4.43	3.89	17.45 (7.5)	−2.63 (4)
I	79.46	81.85	58.11	90.19	72.32	21.17	7.80	3.96	19.25	−5.03
5′-IMP (XV)	80.11	82.09	56.91	92.23	73.22	20.87	8.22	4.14	18.17 (8)	−3.09 (4)
8-Me-I	78.40	82.36	56.97	89.61	81.88	21.94	5.58	4.02	19.60	−4.61
8-Me-5′-IMP[e] (XVI)	78.77	83.08	55.69	91.36	84.94	21.52	5.09	3.53	17.47 (9.5)	−2.70 (3)
2-SMe-I	90.43	82.15	54.81	91.33	71.72	21.05	7.56	3.96	19.13	−4.97
2-SMe-5′-IMP[f] (XVII)	93.13	82.88	53.58	93.73	72.34	20.97	7.79	4.07	17.62 (8)	−2.76 (3)
2-SEt-8-Me-I	89.60	82.94	53.76	90.32	81.45	22.23	4.85	3.65	18.69	−4.56
2-SMe-8-Me-5′-IMP[g] (XVIII)	91.85	83.21	52.27	91.19	83.75	22.00	4.86	3.36	16.19 (8)	−2.01 (4)

a) Measured from internal dioxane (0.5%, v/v).
b) Spectral resolution is 2—2.5 Hz.
c) 0.2 M solution, except for 2-SEt-8-MeI (0.12 M) and 8-Me-5′-IMP (0.1 M), in DMSO-d_6 (nucleosides) or in H_2O (nucleotides sodium salt pH, 7—8).
d) M. Ikehara, I. Tazawa, and T. Fukui, *Chem. Pharm. Bull.* (Tokyo), **17**, 1019 (1969).
e) T. Fukui, unpublished work. Methyl group at −52.46 ppm.
f) A. Yamazaki, I. Kumashiro and T. Takenishi, *Chem. Pharm. Bull.* (Tokyo), **16**, 338 (1968). Methylthio group at −53.17 ppm.
g) T. Fukui, unpublished work. Methylthio and methyl groups at −53.00 ppm.

TABLE III. Chemical Shift Changes ($\Delta\delta$(Br) or $\Delta\delta$(Me)) of the Sugar Carbons Upon 8-Substitution[a]

Compound	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
8-Br-A	2.46	−2.33	0.19	0.79	0.42
8-Br-2′-AMP	2.54	−1.07	0.30	0.76	0.54
8-Br-3′-AMP	2.39	−1.15	0.27	1.04	0.54
8-Br-2′,3′-cAMP	1.15	−0.85	−0.09	−0.01	0.14
8-Br-5′-AMP	2.24	−3.69	−0.77	−0.69	0.15
8-Br-3′,5′-cAMP	1.54	−1.29	−0.44	0.13	−0.18
8-Br-Isp-A	1.40	−1.30	0.32	0.80	−0.11
8-Br-G	3.23	−3.30	0.00	0.65	0.54
8-Br-5′-GMP	2.97	−3.20	−0.19	−0.35	0.35
8-Me-I	0.77	−2.22	0.06	0.35	0.42
8-Me-5′-IMP	0.65	−3.13	−0.61	−0.70	0.39
2-SET-8-Me-I[b]	1.18	−2.71	−0.31	−0.44	0.41
2-SMe-8-Me-5′-IMP	1.03	−2.93	−0.71	−1.43	0.75

a) $\Delta\delta=\delta$ (8-substituted compound)—δ(parent compound). Positive value represents a downfield shift.
b) 2-SMe-I was assumed to be the parent compound.

glycosidic conformations of various types of adenine nucleotides in aqueous solution have been investigated by PMR analysis. Davies and Danyluk have extensively investigated the sugar and phosphate conformations of all common nucleoside 2′,3′- and 5′-phosphates.[22,23] According to their conclusions 2′-AMP, 3′-AMP and 5′-AMP are in almost the same sugar puckering equilibria with the S-type (C(2′)-endo or C(3′)-exo) conformer (60—70%) favored. Son and Chachaty have reported also that these three nucletides have very similar sugar

puckerings.[26] They also showed that 2′-AMP and 3′-AMP take a predominantly *syn* conformation (about 70%) while 5′-AMP takes a predominantly *anti* conformation (about 70%) from nuclear Overhauser effect experiments. The 2′-, 3′- and 5′-phosphates of 8-bromoadenosine could also be in similar sugar puckering equilibria, though the equilibrium ratios can be different to those for the corresponding adenine nucleotides. If this is the case, we can exclude the second explanation descrined earilier for the small upfield shift of C(2′) in 8-Br-2′-AMP and 8-Br-3′-AMP. PMR studies on 8-bromoadenosine derivatives by Sarma *et al.*[5] indicate that 8-Br-5′-AMP shows only a slight bias in favor of the S-type conformation while 8-Br-A shows almost 2:1 preference for the S-type conformation in aqueous solution similar to the cases of adenosine and 5′-AMP. If 8-Br-2′-AMP and 8-Br-3′-AMP have similar sugar puckerings to that of 8-Br-A favoring the S-type (2′-endo) conformation, which is favorable for shielding of C(2′) by N(3), the second explanation can be safely eliminated. It is unlikely that 8-Br-2′-AMP and 8-Br-3′-AMP take predominantly the N-type (3′-endo) conformation because their coupling constants between H(1′) and H(2′) (6 Hz and 7 Hz, respectively)[27] are almost identical to that (6 Hz) of 8-Br-5′-AMP in PMR. Therefore, the first and the third explanations are most plausible.

As to adenosine 2′,3′-cyclic phosphate (2′,3′-cAMP V), PMR and ^{13}C–NMR studies by Lapper and Smith indicate that its conformational equilibrium tends toward the 2′-endo (3′-exo) conformer.[17] Lanthanide probe experiments by Fazakerley and Wolfe indicate that the ribose conformation of 2′,3′-cAMP at pH 2.5 is best described as a rapid equilibrium of 2′-endo(3′-exo) and 3′-endo(2′-exo) conformations in a ratio of approximately 2 to 1 which is very similar to those proposed for the other adenine nucleotides.[28] Therefore the same conclusion as described for 2′- and 3′-phosphate derivative may also be valid for 8-Br-2′,3′-cAMP (VI).

On the other hand, it has been shown by PMR analysis that 3′,5′-cAMP has a rigid phosphate and ribose ring structure and the ribose ring is best described as 3′-endo-4′-exo which is consistent with that found in the solid state by X-ray crystallography.[29] This situation with rigid 3′-endo-4′-exo sugar puckering for 3′,5′-cAMP is entirely different to that for the other adenine nucleotides where a more flexible equilibrium between 2′-endo and 3′-endo conformers favors 2′-endo puckering to a certain extent. Schweizer and Robins[4] have reported that bromine substitution at the 8-position of 3′,5-cAMP produces a larger change (0.51 ppm) of the H(3′) chemical shift than that (0.31 ppm) of the H(2′) shift in the PMR. This may be a reflection of 3′-endo-4′-exo sugar puckering in 3′,5′-cyclic phosphate derivatives. The chemical shift change of H(2′) for the 3′,5′-cyclic phosphate derivative is rather smaller than that (0.43 ppm)[3] for 8-Br-A in Me_2SO. In the ^{13}C–NMR, only a small upfield shift (−0.44 ppm) is observed for C(3′) and it is smaller that (−1.29 ppm) of C(2′). This discrepancy between the PMR and ^{13}C–NMR results for H(3′) and C(3′) may be explained by a difference in the origins of the downfield shift in PMR and the upfield shift in ^{13}C–NMR (at least partially) and/or differences in distances between the nuclei examined and N(3). In the case of 3′,5′-cyclic phosphate derivative, the relatively small upfield shift of C(2′) may be attributed to the second explanation described earlier. Recently Fazakerly *et al.* suggested from lanthanide-ion probe experiments that 8-methylthioadenosine 3′,5′-cyclic phosphate takes a predomonantly *syn* conformation (about 80%) while 3′,5′-cAMP has slight preference for the *anti* conformation.[28]

As to 8-bromoadenosine itself, PMR data in Me_2SO[4,6,9] suggest it mainly takes 2′-endo conformation which is expected to be favorable for an upfield shift of the C(2′) signal. The data of upfield shift (−2.34 ppm) for 8-Br-A is considerably smaller than that (−3.69 ppm) for 8-Br-5,-AMP. This differences may also be explained by either the first and/or third

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explanation. Analysis of the adenosine conformation in Me_2SO by the nuclear Overhauser effect has suggested that it favors the *syn* conformation over the *anti* conformation (in about a 2:1 ratio)[30] and this situation is very similar to those suggested for 2′-AMP and 3′-AMP by the same technique.[26] It has been indicated by Jordan and Niv that 8-bromoadenosine takes less of a *syn* conformation in D_2O than in Me_2SO-d_6.[6] Their PMR data show that the downfield shift of H(2′) for 8-BrA in D_2O is 0.26 ppm while the $\Delta\delta$ in Me_2SO-d_6 is 0.48 ppm. The PMR data in D_2O reported by Sarma *et al.* showed that the downfield shift (0.28 ppm) for 8-Br-5′-AMP at pH 8. Therefore we can conclude that in aqueous solution 8-Br-A has a glycosidic conformational equilibrium which contains a considerable proportion of *anti* (or at least non-*syn*) conformations.

According to the recently-published potential energy calculations by Govil *et al.*[31] 8-Br-adenosine with 2′-endo sugar puckering shows two broad energy minima, one in the *syn* and the other in the *anti* region. The CD spectrum of 8-Br-adenosine in aqueous solution gives further support to the existence of the *anti* conformation because it shows both negative and positive bands in the main absorption region (around 265 nm) whereas 8-Br-AMP shows only a positive band and 5′-AMP shows only a negative band in the same region.[3] In the case of 8-methyladenosine which shows a $\Delta\delta$ (Me) value of −1.4 ppm for C(2′) in Me_2SO,[7] the CD spectrum is nearly identical to that of adenosine in aqueous solution.[32]

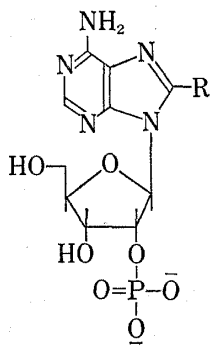
8-Bromo-2′,3′-*O*-isopropylideneadenosine (8-Br-Isp-A, XII) has a somewhat similar structure to that of 8-Br-2′,3′-cAMP. However it seems to adopt a planar sugar conformation as studied by PMR.[10] It shows a smaller upfield shift (−1.30 ppm) than that (−2.34 ppm) for 8-Br-A in the same solvent (Me_2SO). This difference can be explained either by a difference in sugar puckering or by the fact that 2′,3′-*O*-isopropylideneadenosine (XI) itself takes a predominantly *syn* conformation as concluded from the nuclear Overhauser effect experiments.[30] 8-Br-2′,3′-cAMP which has a larger content of 2′-endo puckering and a significantly smaller upfield shift (−0.85 ppm) may take an *anti* conformation more frequently in aqueous solution when compared with 8-Br-Isp-A in Me_2SO.

As discussed above, the relatively small upfield shift of C(2′) signal in 8-Br-2′-AMP, 8-Br-3′-AMP, 8-Br-2′,3′-cAMP and 8-bromoadenosine may be attributed to their flexible glycosidic conformation (the first explanation) and/or predominant *syn* conformation of the corresponding adenosine derivatives (the third explanation). As to the third explanation, the degree of upfield shift of C(2′) caused by a change of glycosidic conformation in adenosine derivatives can be estimated from the data for 5′-AMP (predominantly *anti*) and adenosine (predominantly *syn*). As readily obtained from the $\Delta\delta$ (P) in Table V, the estimate is −0.88 ppm. When this value is added to the $\Delta\delta$ (Br)'s for Br-A, 8-Br-2′-AMP, 8-Br-3′-AMP and 8-Br-2′,3′-cAMP, the corrected $\Delta\delta$ (Br's, the measure of *syn* conformation population, are −3.22, −1.95, −2.03 and −1.73 ppm, respectively. The absolute values for 8-Br-2′-AMP, 8-Br-3′-AMP and 8-Br-2′,3′-cAMP are still significantly smaller than that (3.69 ppm) for 8-Br-5′-AMP. Therefore, the first explanation may be also valid at least for these nucleotides.

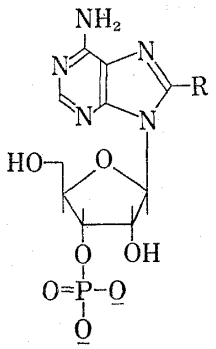
As to the other purine nucleotides, 8-bromoguanosine 5′-phosphate (XIV) and 2-substituted 8-methylinosine 5′-phosphate (XVII) show nearly the same $\Delta\delta$ values as those for the corresponding nucleotide while 8-Me-I shows a smaller upfield shift than that of 8-Me-5′-IMP (XVI) as observed in the case of the 8-bromoadenosine derivative. In the former cases, the *syn* conformation may be fully stable already at the nucleoside level. This situation is easily understood because guanosine itself favors non-*anti* conformation more strongly than

29) K. Waternpaugh, J. Dow, L.H. Jensen, and S. Furberg, *Sciences*, **159**, 206 (1968).
30) P.A. Hart and J.P. Davis, *Jerusalem Symp. Quantum Chem. Biochem.*, **5**, 297 (1973).
31) G. Govil, C. Fish, F.B. Howard, and H.T. Miles, *Nucleic Acid Res.*, **4**, 2573 (1977).
32) M. Ikehara, W. Limn, and T. Fukui, *Chem. Pharm. Bull.* (Tokyo), **25**, 2702 (1977).

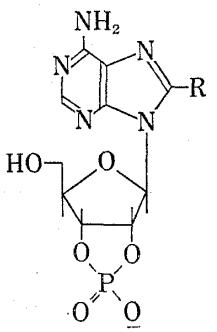
adenosine and inosine as indicated by the nuclear Overhauser effect experiments in Me_2SO.[30] These nucleosides are also expected to be more conformationally flexible in water than in Me_2SO because 8-Br-guanosine shows a smaller downfield shift ($\Delta\delta$(Br)) of H(2′) in PMR[6] and a smaller band in the main absorption region in the CD spectrum[3] compared with those for 8-Br-5′-GMP.



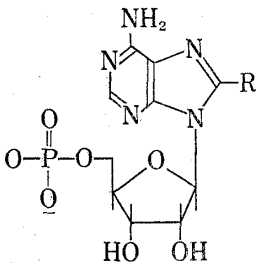
I : R=H
II : R=Br



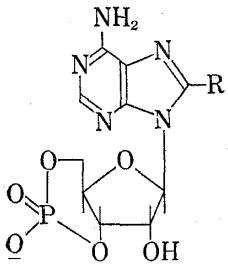
III : R=H
IV : R=Br



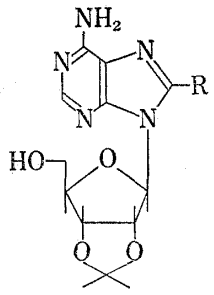
V : R=H
VI : R=Br



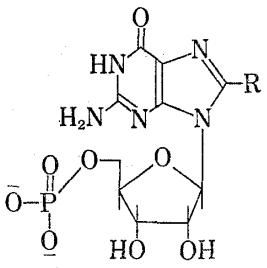
VII : R=H
VIII : R=Br



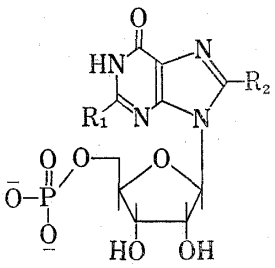
IX : R=H
X : R=Br



XI : R=H
XII : R=Br



XIII : R=H
XIV : R=Br



XV : R_1=R_2=H
XVI : R_1=H, R_2=CH_3
XVII : R_1=SCH_3, R_2=H
XVIII : R_1=SCH_3, R_2=CH_3

Chart 1

Another large shift change upon 8-substitution is commonly observed on C(1′). Previous studies on purine nucleosides with various substituents have shown that $\Delta\delta$ of C(1′) is dependent on the nature of the substituent.[7] It is noted that the compound with an additional cyclic structure to the sugar moiety shows relatively small downfield shift.

Effect of Phosphate Groups on Base and Sugar Carbon Resonance

The chemical shift changes ($\Delta\delta$(P)) upon phosphorylation of adenosine and 8-bromoadenosine are summarized in Table IV. Since carbon-13 spectra of the nucleosides are measured in Me_2SO-d_6, these $\Delta\delta$ (P) values also include solvent effects upon changing from Me_2SO to H_2O. Among $\Delta\delta$(P)'s on the base carbons, the upfield shift of C(6) is almost constant for all nucleotides here and, therefore, may be mainly due to the solvent effect. The $\Delta\delta$(P)'s of C(2) for adenine nucleotides are close to zero showing little dependence on the phosphate groups as well as solvents. Slightly more dependence on the phosphate groups is observed for the C(2) resonances of 8-bromoadenine nucleotides. In contrast to the C(2) resonances, the C(8) resonances for adenine nucleotides show a considerable dependence on the phosphate groups. These phenomena are consistent with the *syn* preference of the 8-bromoadenosine derivatives and *anti* preference of the adenosine derivatives. The C(8) resonances for 8-bromoadenine nucleotides also show a dependence on the phosphate groups but the pattern of

sequential $\Delta\delta$ (P) changes from the 2′-phosphate to the 3′,5′-phosphate is different to that for adenine nucleotides. The C(4) and C(5) resonances for both groups of nucleotides also show significant dependence on phosphate groups and the pattern of sequential $\Delta\delta$ (P) changes for each carbon is very similar for the adenine nucleotides and the 8-bromoadenine nucleotides.

Table IV. Chemical Shift Changes ($\Delta\delta$(P)) Upon Phosphorylation of Adenosine and 8-Bromoadenosine[a)]

Compound	C(2)	C(4)	C(5)	C(6)	C(8)	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
A	0	0	0	0	0	0	0	0	0	0
2′-AMP	−0.27	−0.89	−0.74	−1.05	0.50	−0.56	2.42	−0.30	−0.96	−0.31
3′-AMP	−0.28	−1.13	−0.67	−1.06	0.34	0.24	0.19	2.47	−0.66	−0.24
2′,3′-cAMP	−0.12	−1.43	−1.05	−1.12	0.27	1.10	6.59	6.64	−0.88	−0.69
5′-AMP	−0.05	−0.74	−1.29	−1.13	−0.20	−1.14	0.88	−0.15	−1.62	1.69
3′,5′-cAMP	0.15	−1.47	−1.21	−1.17	−0.63	3.30	−1.31	6.39	−14.35	5.37
8-Br-A	0	0	0	0	0	0	0	0	0	0
8-Br-2′-AMP	−0.49	−0.75	−0.41	−1.09	0.99	−0.48	3.68	−0.19	−0.99	−0.19
8-Br-3′-AMP	−0.57	−1.06	−0.57	−1.25	0.76	0.17	1.37	2.55	−0.41	−0.12
8-Br-2′,3′-cAMP	−0.11	−0.90	−1.03	−1.41	−0.01	−0.21	8.07	6.36	−1.68	−0.97
8-Br-5′-AMP	0.05	−0.13	−1.03	−1.48	0.46	−1.36	−0.48	−1.11	−3.10	1.42
8-Br-3′,5′-cAMP	0.24	−0.47	−1.22	−1.55	0.07	2.38	−0.27	5.76	−15.01	4.77

a) $\Delta\delta$=δ(nucleotide)—δ(nucleoside). Positive value represents a downfield shift.

As to the sugar resonances, a pair of nucleotides with the same phosphate group show very similar $\Delta\delta$ (P) values and also very similar ^{13}C–^{31}P coupling constants on each carbon. These results suggest that adenine and 8-bromoadenine nucleotides have very similar sugar-phosphate conformations. The carbon at which the phosphate group is attached shows a downfield shift. The $\Delta\delta$ (P) value for the phosphomonoester dianion ranges from 1.7 to 2.5 in the case of adenine nucleotides. The $\Delta\delta$ (P) values for the 2′-phosphates and the 3′-phosphates are considerably larger than those for the 5′-phosphates. The $\Delta\delta$ (P) values on C(2′) and C(3′) for the phosphodiester monoanion of 2′,3′-cAMP are about 6.6 ppm. In the case of 3′,5′-cAMP, Lapper *et al.* initially assigned a signal which appears next to the C(1′) signal and has a coupling constant of 4.5 Hz as a C(4′) resonance and a signal which appears at higher field and has a coupling constant of 4.3 Hz as a C(3′) resonance.[16)] In that case, However, the $\Delta\delta$ (P) for C(3′) was only 0.88 ppm and the $\Delta\delta$ (P) for C(4′) was −8.84 ppm whereas C(5′) shows a downfield shift (5.37 ppm) similar to those observed on C(2′) and C(3′) in 2′,3′-cAMP. Since we find no reason why C(3′) should receive so much shielding as to cancel out the expected downfield shift, we have at present tentatively made the reversed assignment as shown in Table I.[33)] In this assignment, the $\Delta\delta$(P)'s on C(3′) and C(5′) in the 3′,5′-cyclic phosphate are about 5—6 ppm. The $\Delta\delta$ (P) on C(3′) is larger by about 1 ppm than that on C(5′) and this relationship is similar to that observed between the 3′-phosphates and the 5′-phosphates. Moreover an unusually large upfield shift on C(4′) and a considerable downfield shift on C(1′) are observed. The unusual upfield shift on C(4′) could be caused by close contact with the phosphate group as observed on examination of the molecule with a CPK model. A small upfield shift on C(4′) is also observed in the 5′-phosphates when compared with the 2′- and 3′-phosphates. The $\Delta\delta$(P) on C(4′) in 8-Br-5′-AMP is considerably larger than that in 5′-AMP. This phenomenon can be explained in terms of an increase in the population of tg or gt conformation about the C(4′)-C(5′) bond in which the phosphate group is closer to C(4′) than in the case of the gg conformation. PMR analysis by Sarma

33) This assignment was supported also by the data of 8,2′-anhydro-8-thio-9-D-arabinofuranosyladenine 3′,5′-cyclic phosphate (S. Uesugi, S. Tanaka, and M. Ikehara, *Europ. J. Biochem.*, in press.

et al. suggests that 8-Br-5′-AMP takes a predominantly tg or gt conformation (up to 80%) at pH 8 while 5′-AMP prefers a gg conformation (65%).[5]

The difference in the $\Delta\delta$(P)'s on each carbon for a 8-bromoadenine nucleotide and the corresponding adenine nucleotide reflects a difference in the effects of 8-bromo substitution on the 8-bromoadenine nucleotide and 8-bromoadenosine, because the $\Delta\delta$(P) of 8-Br-5′-AMP minus $\Delta\delta$(P) of 5′-AMP equals the $\Delta\delta$(Br) of 8-Br-5′-AMP minus $\Delta\delta$(Br) of 8-bromoadenosine. Therefore the 8-bromoadenine nucleotides which show a small upfield shift of C(2′) upon 8-substitution show larger $\Delta\delta$(P)'s on the same carbon upon phosphorylation when compared with those of the corresponding adenine nucleotide.

Chemical Shift Changes Upon 5′-Phosphorylation in Various 5′-Nucleotides

The $\Delta\delta$(P) values for various 5′-phosphate derivatives are presented in Table V. It should be noted again that these $\Delta\delta$(P) values also include a component reflecting the effect of solvent change from DMSO to H_2O. Almost the same upfield shifts (—1——1.5 ppm) are observed for C(5) in all the 5′-nucleotides. All the guanosine and inosine derivatives, which have a carbonyl oxygen at C(6), show a downfield shift of C(6) whereas the adenosine derivatives which have an amino group at C(6) show an upfield shift of the same carbon. These shifts may be mainly due to the solvent effect. All the 8-substituted 5′-nucleotides show a larger $\Delta\delta$(P) (downfield shift) of C(8) than those of the corresponding parent nucleotide. 2-SMe-5′-IMP (XVII) and its 8-methyl derivative exhibit relatively large shifts of C(2) while the other nucleotides show much smaller shifts of the same carbon. These differences may arise because of the variation in susceptibility of the carbon-substituent bonds to the effects of the solvent and phosphate group.

TABLE V. Chemical Shift Changes ($\Delta\delta$ (P)) Upon 5′-phosphorylation of Purine Nucleosides[a]

Compound	C(2)	C(4)	C(5)	C(6)	C(8)	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
5′-AMP	−0.05	−0.74	−1.29	−1.13	−0.20	−1.14	0.88	−0.15	−1.62	1.69
8-Br-5′-AMP	0.05	−0.13	−1.03	−1.48	0.46	−1.36	−0.48	−1.11	−3.10	1.42
5′-GMP	0.17	−0.19	−0.97	1.91	1.65	0.12	0.26	0.01	−1.08	1.88
8-Br-5′-GMP	0.03	0.33	−1.13	2.06	2.48	−0.14	0.36	−1.08	−2.08	1.69
5′-IMP	0.65	0.24	−1.20	2.04	0.90	−0.30	0.42	0.18	−1.08	1.94
8-Me-5′-IMP	0.37	0.72	−1.28	1.75	3.06	−0.42	−0.49	−0.49	−2.13	1.91
2-SMe-5′-IMP	2.70	0.73	−1.23	2.40	0.62	−0.08	0.23	0.11	−1.51	2.21
2-SMe-8-Me-5′-IMP[b]	2.25	0.27	−1.49	0.87	2.30	−0.23	0.01	−0.29	−2.50	2.55

a) $\Delta\delta=\delta$(nucleoside 5′-phosphate)—δ(parent nucleoside). Positive value represents a downfield shift.
b) 2-SEt-8Me-I was assumed to be the parent nucleoside.

As to the sugar carbon resonances, all the 5′-nucleotides show relatively large shifts of C(5′) and of C(4′). The downfield shift of C(5′) ranges from 1.4 to 2.6 ppm. The upfield shift of C(4′) ranges from −1 to −3 ppm. The absolute value of $\Delta\delta$(P) for C(4′) for an 8-substituted derivative is always larger than that for the corresponding parent nucleotide. These phenomena may be explained by a change of the conformation about the C(4′)-C(5′) bond as discussed in the preceding section. Information on these commom trends of chemical shifts as discussed above, may be useful for the assignment of the signals of other purine nucleosides and 5′-nucleotides.

Conclusions

Our conclusions on the glycosidic conformation of 8-bromoadenosine derivatives derived from the discussion described above are: 1) 8-Br-5′-AMP takes a rigid *syn* conformation; 2) 8-Br-2′-AMP, 8-Br-3′-AMP and 8-Br-2′,3′-cAMP have considerable flexibility of rotation

about the glycosidic bond and can take an *anti* conformation in addition to the *syn* conformation, which is more favored; 3) in Me_2SO 8-Br-adenosine has a higher *syn* conformation content than do its 2′-, 3′- and 2′,3′-cyclic phosphate derivatives but in water the *syn* conformational content is reduced to the level of the above nucleotides.

From nuclear Overhauser experiments,[26,30] it is indicated that 2′-AMP, 3′-AMP and adenosine take predominantly a *syn* conformation while 5′-AMP takes an *anti* conformation. It seems that the presence of a phosphate group on the 5′-position reduces the population of a *syn* conformation which is preferred intrinsically by an adenosine residue. The role of a 5′-phosphate group in restriction of conformation of a nucleoside residue has been suggested by Sundaralingam *et al.* from X-ray crystallographic data.[34,35] These effects are assumed to be a consequence of the Coulombic interaction between the negatively charged phosphate group and the base. A similar interaction presumably arises between the 5′-phosphate and the 8-bromo substituent to eliminate the *anti* conformation in the case of 8-Br-5′-AMP. On the other hand, 8-Br-2′-AMP, 8-Br-3′-AMP, 8-Br-2′,3′-cAMP and 8-bromoadenosine may be allowed to take flexible glycosidic conformation containing more population of *anti* form.

8-Me-5′-IMP also has a more rigid *syn* conformation than that of 8-Me-inosine. However in the cases of 8-bromoguanosine and 2-alkylthio-8-methylinosine, which have an additional substituent at the 2-position, the nucleosides takes a *syn* conformation in Me_2SO as rigid as those of the 5′-nucleotides in water. The parent nucleosides of these derivatives (guanosine and 2-alkylthioinosine) may have an intrinsic preference for the *syn* conformation. These nucleosides are also expected to be more conformationally flexible in water than in Me_2SO.

As to the glycosidic conformations of 8-Br-3′,5′-cAMP and 8-Br-Isp-A, no clear conclusions can be drawn from the ^{13}C–NMR data only because they have unique sugar puckering conformations which apparently influence degree of shielding on C(2′).

The $\Delta\delta$(P) values presented in Table IV and V may be useful for the assignment of ^{13}C–NMR spectra of other purine nucleotides. A large $\Delta\delta$(P) value for C(4′) seems to reflect a high population of non-gg conformations about the C(4′)-C(5′) bond.

34) M. Sundaralingam, *Jerusalem Symp. Quantum Chem. Biochem.*, **5**, 417 (1973).
35) N. Yathindra and M. Sundaralingam, *Biopolymers*, **12**, 297 (1973).