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Studies on Nucleosides and Nucleotides. LXXXVIII.[1] Purine Cyclonucleosides. XLIII. ^{13}C NMR Spectra of 2′-Substituted 2′-Deoxyadenosines. Substituent Effects on the Chemical Shifts in the Furanose Ring System

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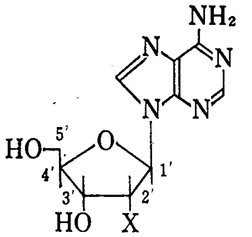
^{13}C nuclear magnetic resonance spectra of various 2′-substituted 2′-deoxyadenosines are presented. The relative substituent chemical shifts of each sugar carbon are analyzed in terms of a substituent electronegativity parameter and compared with the data for substituted cyclohexanes. The relative substituent chemical shifts of $C_{2'}$ and $C_{4'}$ are controlled mainly by the inductive effect of the substituent. Those of $C_{1'}$ and $C_{3'}$ cannot be interpreted by inductive effect only. Some effect which is perturbed by the presence of a *cis*-substituent seems to be operating. A good linear correlation was observed between the substituent chemical shift of $C_{2'}$ and the N conformer population in the furanose puckering equilibrium.

Keywords——^{13}C NMR; 2′-substituted 2′-deoxyadenosine; substituent effect; chemical shift; furanose puckering

The ^{13}C nuclear magnetic resonance (NMR) technique has been widely applied to nucleosides,[2] nucleotides,[3] oligonucleotides,[4] and polynucleotides.[4,5] However, little is known about the substituent effects on chemical shifts in nucleoside systems. We have already studied the effects of 8-substituents in purine nucleosides[6] and nucleotides,[7] and the effects of phosphate substitution.[7-9] On the other hand, a variety of 2′-substituted 2′-deoxypurine-nucleosides,[10,11] dinucleoside monophosphates[12,13] and homopolymers[14-16] containing these residues were synthesized in our laboratory and their physical and biological properties were examined. Poly(2′-azido-2′-deoxyinosinic acid) and poly(2′-fluoro-2′-deoxyinosinic acid) showed high interferon-inducing activity when complexed with poly(cytidylic acid); these are the first examples among non-ribo double-stranded complexes.[17,18] It was also found that the sugar puckering conformation of 2′-substituted 2′-deoxyadenosines depends greatly on the substituent, and that the N conformer (3′-*endo*) population is dependent on the electronegativity of the substituent.[19] These findings may be helpful in understanding the origin of the structural difference between DNA and RNA.

In this paper, we present ^{13}C NMR spectra of 2′-substituted 2′-deoxyadenosines and discuss the substituent effects on the chemical shifts of sugar carbons, from which we could derive information on the conformation of the sugar moiety.



1: $X=H$ **4**: $X=OCH_3$ **7**: $X=Cl$
2: $X=OH$ **5**: $X=N_3$ **8**: $X=Br$
3: $X=NH_2$ **6**: $X=F$ **9**: $X=I$

Fig. 1. 2′-Substituted 2′-Deoxyadenosines

Experimental

The methods for the preparation of compounds 3, 5—9 and arabinofuranosyladenine (ara-A) have been reported elsewhere.[11,20] Compound 4 was synthesized according to the published procedure.[21]

^{13}C NMR spectra were recorded with complete ^1H decoupling on a Hitachi R-22-CFT spectrometer (22.63 MHz, ambient probe temperature 32—35°C) operating in the Fourier transform mode with a Hitachi HITAC-10II computer. Samples of 0.2 M solution in DMSO-d_6 were measured in 8 mm tubes with dioxane (0.5%, v/v) as an internal reference. Typical conditions for the FT measurement were as follows: spectral width (200 ppm); data points (8K); pulse angle (60°); acquisition time (0.8 s); pulse delay time (2.2 s); number of transients (2000). All carbon chemical shifts were measured relative to the internal reference (dioxane) and converted to the TMS scale using the experimentally determined relationship: $\delta_{TMS}=\delta_{dioxane}+66.29$ ppm (0.5% dioxane and TMS in DMSO-d_6).

The substituent electronegativity values were taken from reference 22 except for those of methoxy[23)] and azido[24)] groups. In the case of the methoxy group, the value of the ethoxy group obtained from the data for diethylether was used.

Results and Discussion

The ^{13}C chemical shifts of 2′-substituted 2′-deoxyadenosines are presented in Table I. Assignments of ^{13}C signals were done mainly according to the work of Jones *et al.*[2)] except for the reversed assignment of $C_{2'}$ and $C_{3'}$ as pointed out by Mantsch *et al.*[5a)] The relative peak heights of signals and the results of partial decoupling experiments were taken into consideration for assignments. By the latter technique, the C_2, C_8 and $C_{5'}$ signals can be assigned easily.[6)] In addition, the $C_{1'}$ signal can be distinguished from the other sugar carbon signals because the H-1′ of nucleoside derivatives usually shows distinctly isolated signals at lower field with respect to the other sugar protons in ^1H NMR.[11)] The assignments for the closely located $C_{2'}$ and $C_{3'}$ signals of arabinofuranosyladenine may be reversed.

TABLE I. ^{13}C Chemical Shifts[a)] of 2′-Substituted 2′-Deoxyadenosines in DMSO-d_6

Compound	C(2)	C(4)	C(5)	C(6)	C(8)	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
2′-dA	152.30	148.88	119.27	156.01	139.47	83.91	39.50	70.91	87.93	62.13
A	152.27	149.03	119.29	156.05	139.86	87.93	73.42	70.60	85.83	61.67
Ara-A	152.24	149.18	118.01	155.71	140.19	83.55	75.64	75.04	83.97	60.78
2′-NH_2-dA	152.12	149.12	119.39	156.07	140.07	89.19	57.42	71.80	86.91	62.15
2′-OMe-dA	152.36	148.94	119.16	156.01	139.53	85.77	82.42	68.75	86.31	61.44
2′-N_3-dA	152.63	148.97	119.11	156.10	139.38	85.23	64.13	71.08	86.07	61.07
2′-F-dA[b)]	152.59	148.73	119.05	156.11	139.30	85.76	93.23	68.35	84.18	60.48
2′-Cl-dA	152.54	148.94	119.10	156.02	139.47	87.75	60.90	70.19	86.19	61.03
2′-Br-dA	152.54	149.00	119.15	156.07	139.53	88.23	52.87	70.19	86.43	61.20
2′-I-dA	152.57	149.03	119.12	156.11	139.56	89.86	31.28	71.21	86.50	61.62

a) Measured in ppm downfield from TMS.
b) ^{13}C-^{19}F coupling constants for C(1′), C(2′) and C(3′) were 34, 188 and 16 Hz, respectively.

Analysis with Substituent Electronegativity Parameters

The relative substituent chemical shifts ($\Delta\delta=\delta$ (nucleoside)$-\delta$(deoxyadenosine)) were plotted against the substituent electronegativity, χ, calculated by Dailey and Shoolery from ^1H NMR data for substituted ethane derivatives,[22)] as shown in Figs. 2 and 3. Good linear relationships between $\Delta\delta$ and χ exist in the cases of $C_{2'}$ ($r=0.86$, $n=9$) and $C_{4'}$ ($r=0.92$, $n=9$). For $C_{2'}$ a better correlation is obtained when the point of X=H is excluded ($r=0.93$, $n=8$). Correlations of lesser degree are also noted between the $\Delta\delta$'s of $C_{1'}$ $C_{3'}$ and $C_{5'}$ and χ ($r=0.73$, $n=8$; $r=0.68$, $n=8$; $r=0.73$, $n=9$ respectively). The slope of the least-squares line is positive for $C_{2'}$ and negative for the other sugar carbons. Thus the $\Delta\delta$'s of $C_{2'}$ and $C_{4'}$ are mainly controlled by the inductive effect of the substituents and the $\Delta\delta$'s of $C_{1'}$, $C_{3'}$ and $C_{5'}$ are partially controlled by the same effect. It should be noted that the point of X=H deviates completely from the least-squares line in the case of $C_{1'}$ (Fig. 3a). Therefore the $\Delta\delta$ of $C_{1'}$, which has a hydrogen *cis* to the substituent, may be controlled mainly by some effect which is absent in hydrogen, having only an *s* orbital electron, such as an electric field effect or resonance effect.

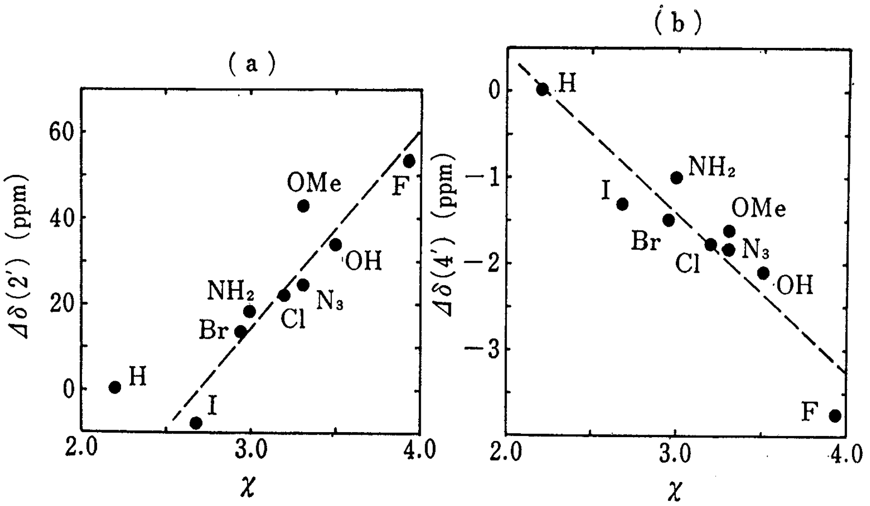


Fig. 2. Plots of the Relative Substituent Chemical Shift ($\Delta\delta$) of $C_{2'}$ (a) and $C_{4'}$ (b) *vs.* the Substituent Electronegativities (χ)

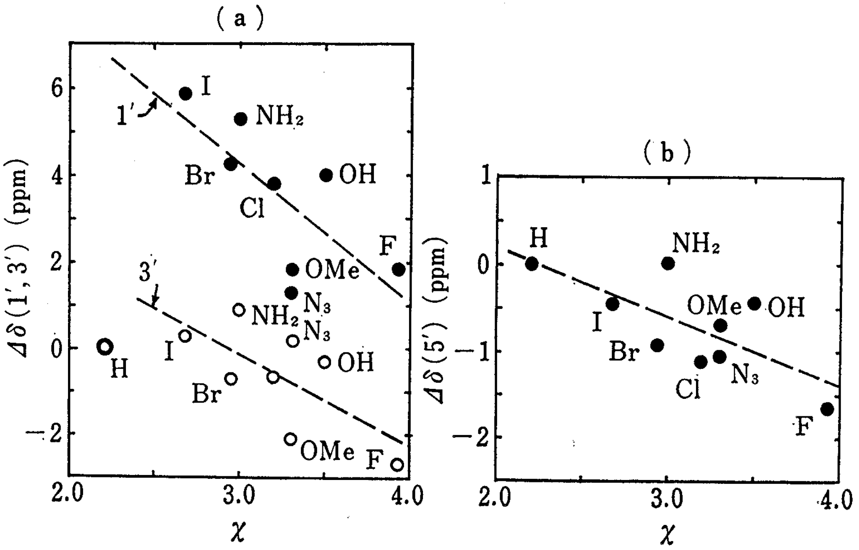


Fig. 3. Plots of the Relative Substituent Chemical Shifts ($\Delta\delta$) of $C_{1'}$ (a, ●) and $C_{3'}$ (a, ○) and $C_{5'}$ (b) *vs.* the Substituent Electronegativities (χ)

The $\Delta\delta$ of $C_{3'}$ should be controlled by the same effect. However, it seems that the presence of a hydroxyl group *cis* to the substituent cancels the deshielding effect by 4—5 ppm except for the case of $X = N_3$. The azido group may exert a specific anisotropic effect on $C_{1'}$. In this context, it is interesting to see the configurational effect at $C_{2'}$. The 2'-β-OH group in arabinofuranosyladenine causes a downfield shift (4 ppm) of $C_{3'}$, which has a proton on the same side of the sugar ring. In contrast, the 2'-α-OH group in adenosine causes a downfield shift (4 ppm) of $C_{1'}$ and a very small upfield shift (−0.3 ppm) of $C_{3'}$. Similar configurational effects of the OH group are observed in 2-methylcyclopentanol[25)] and 2-methylcyclohexanol systems where the downfield shift of $C_{2'}$ caused by the OH group seems to be cancelled by the *cis*-methyl group.[26)] This cancellation may be due to steric perturbation in a vicinally *cis*-substituted system which produces an upfield shift of the carbon signal.

Comparison with the Data for Substituted Cyclohexanes and *n*-Pentanes

Similar correlations were observed between the $\Delta\delta$'s of α, β and γ carbons and χ when the data for substituted cyclohexanes and *n*-pentanes were plotted in the same way. During

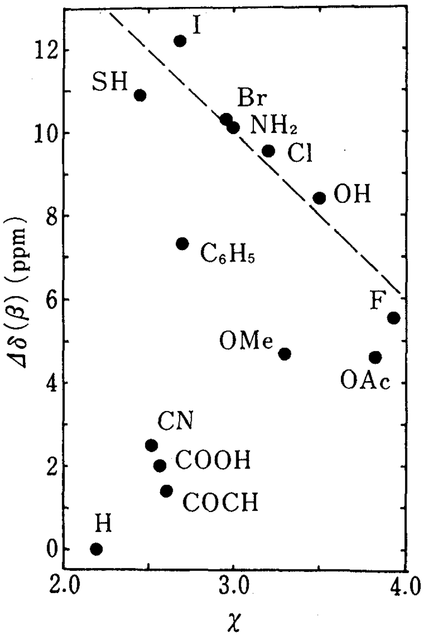


Fig. 4. Plots of the Relative Substituent Chemical Shifts ($\Delta\delta$) of the β-Carbon of Substituted Cyclohexanes *vs.* the Substituent Electronegativities (χ)

these analyses, it was noted that the points of $X=OCH_3$ and N_3 deviated significantly from the group of other substituents containing only one atom heavier than hydrogen (F, Cl, I, OH and NH_2), especially in the case of β-carbon including $C_{1'}$ and $C_{3'}$ of the substituted deoxyadenosines. This situation can be seen in the $\Delta\delta$ (β) *vs.* χ plot for the substituted cyclohexanes[27] which provide most points among the available systems (Fig. 4). Thus, relatively large upfield deviations for C_6H_5 and OCH_3 and further upfield deviation for a substituent containing a directly bound carbonyl or nitrile group from the least-squares line for substituents containing only one atom heavier than hydrogen are noted. The steric effect of C_6H_5 and OCH_3 groups may be responsible for the upfield deviation. Similar phenomena have been noted by Pehk and Lipman in the case of substituted adamantanes.[28] If one chooses only H, OH, NH_2, F, Cl, Br and I groups as substituents, the correlation factors for α, β and γ carbons with respect to χ are as follows (the point of hydrogen is excluded in the case of β-carbon): 2'-deoxyadenosine, 0.93, 0.93 (1') and 0.79 (3'), 0.95; cyclohexane; 0.98, 0.99, 0.97; *n*-pentane, 0.94, 0.82, 0.95.

Correlation between the $\Delta\delta$'s of Deoxyadenosines and Cyclohexanes

The $\Delta\delta$'s of $C_{2'}$, $C_{1'}$, $C_{3'}$ and $C_{4'}$ were plotted against the corresponding $\Delta\delta$'s of α-, β- and γ-carbons of cyclohexanes. As shown in Fig. 5 good linear correlations are observed in the

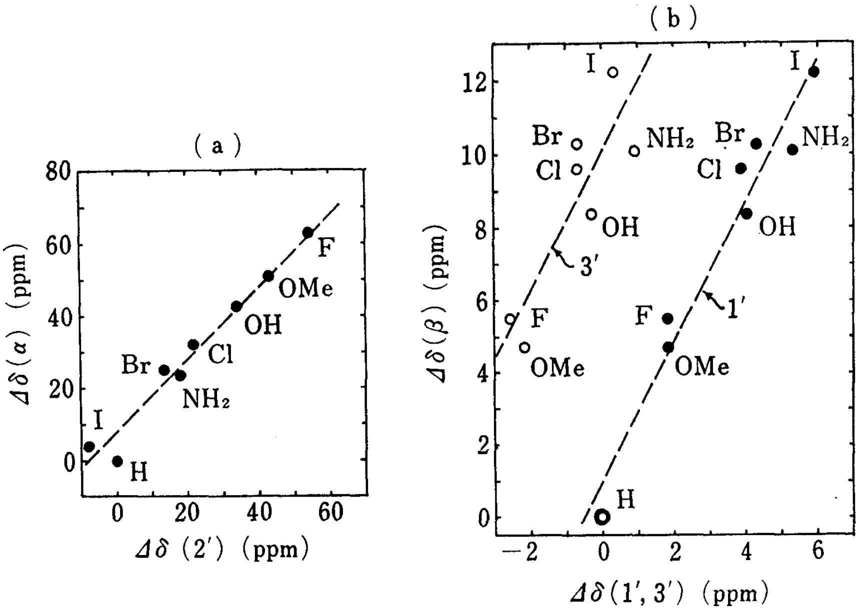


Fig. 5. Plots of the Relative Substituent Chemical Shifts ($\Delta\delta$) of $C_{2'}$ (a), $C_{1'}$ (b, ●) and $C_{3'}$ (b, ○) *vs.* the $\Delta\delta$'s of the Corresponding Carbons in Substituted Cyclohexanes

The points of $X=H$ for C_1' and C_3' are the same and appear at the positions of $\Delta\delta(\beta)=0$ and $\Delta\delta(1', 3')=0$.

cases of $C_{2'}$ ($r=1.00$, $n=8$) and $C_{1'}$ ($r=0.97$, $n=8$). The $C_{3'}$ (Fig. 5b) and $C_{4'}$ (not shown here) show somewhat lower correlations, $r=0.86$, $n=7$ and $r=0.83$, $n=8$, respectively. Thus the $\Delta\delta$ values of $C_{1'}$, which has a proton on the same side of the sugar as the substituent, are affected in a similar manner to those of the β-carbon of substituted cyclohexane (note that the least-squares line passes through the point of X=H). However, the $\Delta\delta$ of $C_{3'}$, which has an OH group on the same side is affected in a somewhat different way. It should be noted that the least-squares line for $C_{3'}$, which has about the same slope as that for $C_{1'}$, is shifted upfield by about 4 ppm relative to that for $C_{1'}$. This phenomenon has already been noted in the earlier analysis based on substituent electronegativity. It should also be noted that the slopes of the least-square lines for $C_{1'}$ and $C_{3'}$ are approximately 2. This means that the carbons of the deoxyadenosines resonate at the higher field than those of the cyclohexanes, which may be due to the more crowded structure of the sugar system of deoxyadenosines. A relatively poor correlation was observed in the case of $C_{4'}$ and may be due to the difference in the γ-effect[29] between the two ring systems. It has been observed that the γ-effects of CH_3 and OH group in a cyclopentane ring are smaller than those in a cyclohexane ring and is depend on the presence of other substituents on the ring.[25]

Correlation between $\Delta\delta$ of C_2, and N Conformer Population

In a previous paper,[18] we reported that the N conformer (3′-*endo*) population in the furanose puckering equilibrium of deoxyadenosines is linearly correlated to an inductive parameter ι proposed by Inamoto and Masuda.[30] A good linear correlation is also observed between the N conformer population and the substituent electronegativity (χ): $r=0.84$, $n=9$ (including X=H); $r=0.96$, $n=8$ (excluding X=H). When the N conformer population was plotted against $\Delta\delta$ of $C_{2'}$, the best correlation found so far was observed as shown in Fig. 6: $r=0.93$, $n=9$ (including X=H). Therefore we can say that the factors which determine N conformer population are almost the same as those which determine carbon chemical shift. Those factors may be mainly the electron density at $C_{2'}$ which determines the polarity of the C-X bond, and in part, the bulkiness of the substituent. The substituent ^{13}C chemical shift can be a good measure of these combined effects of the substituent on the furanose conformation.

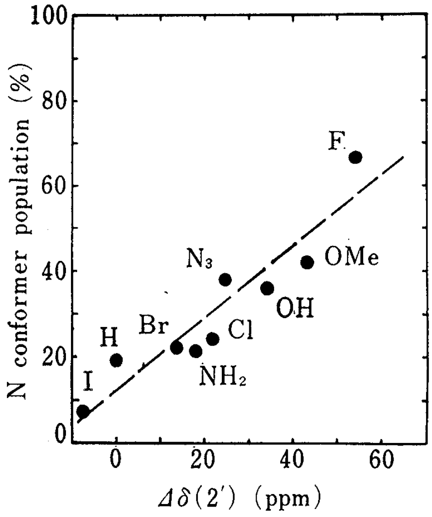


Fig. 6. Plots of the N Conformer Populations in Furanose Puckering Equilibrium *vs.* the $\Delta\delta$'s of $C_{2'}$

Conclusions

It is known that substituent effects on ^{13}C chemical shifts are strongly dependent on the steric arrangement of the substituent and the carbon observed.[31] Some empirical parameters describing the ^{13}C chemical shift effects associated with geometric features have been proposed.[32,33] These studies were done mainly on six-membered ring systems. In the present paper, we examined the effect of 2′-substituents on the ^{13}C chemical shifts of the five-membered furanose ring system. This system is far more complex than the simple cyclohexane or cyclopentane system[25] because it has four substituents including the base, OH and CH_2OH on a tetrahydrofuran ring. It was found that substituent effects on $C_{2'}$ and $C_{4'}$ are mainly controlled by the electronegativity of the substituents and those on $C_{1'}$ and $C_{3'}$ are partially determined by the same parameter. These $\Delta\delta$'s must be affected by the orientation of the 2′-substituent, which is determined by the puckering form of the furanose ring. However, the sugar puckering conformation is also determined by the electronegativity of the substituent.[19] Therefore, the effects of substituent electronegativity may include an inductive contribution through bonds and a geometrical contribution. These two contributions are indistinguishable in the present system. Further accumulation of data on the nucleoside systems seems to be needed before these effects can be fully understood.

After the completion of this work, a paper containing ^{13}C NMR data on 2′-substituted 2′-deoxyuridines was published.[34]

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