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Protonation Sites of Adenine Derivatives. I. Nuclear Magnetic Resonance Investigation of Adenine N-3 Derivatives in Dimethyl Sulfoxide-d_6

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The protonation sites of adenine N-3 derivatives in dimethyl sulfoxide (DMSO)-d_6 were investigated on the basis of proton nuclear magnetic resonance (PMR) and carbon-13 nuclear magnetic resonance (^{13}C NMR) spectral measurements. The spectra of the protonated and non-protonated adenine N-3 derivatives showed that, upon protonation: 1) the chemical shift of the H-8 proton moved further downfield than that of the H-2 proton, and 2) the signal of NH_2 protons was split into a doublet at 30°C and showed a broad line at 70°C, and 3) the signal of the C-5 carbon moved further upfield than that of the C-4 carbon. The findings indicate that adenine N-3 derivatives are protonated at the N-7 site.

Keywords——PMR spectra; adenine N-3 derivatives; ^{13}C NMR spectra; protonation site; chemical shift; NH_2 protons.

The biological importance of purine bases, which are constituents of nucleic acids, is well known. When desoxyribonucleic acid (DNA) is exposed to mutagenic and carcinogenic alkylating agents such as N-methyl-N′-nitrosourea and N-methyl-N′-nitro-N-nitrosoguanidine, one of the major alkylation products is 3-methyladenine.[1] The protonation site of adenine derivatives has so far been investigated mainly by X-ray crystallography. The results showed that the protonation sites were N-1 for adenine[2] and adenine N-9 derivatives,[3] N-9 for adenine N-1 derivatives,[4] and N-3 and N-9 for adenine N-7 derivatives.[5] However, the protonation site of adenine N-3 derivatives is unknown.

This paper deals with the protonation site of adenine N-3 derivatives as determined by proton nuclear magnetic resonance (PMR) and carbon-13 nuclear magnetic resonance (^{13}C NMR) spectroscopy of adenine derivatives in solution in DMSO-d_6.

Results and Discussion

1. Assignment of the H-2 and H-8 Protons of Adenine N-3 Derivatives

An attempt to identify the peaks due to the H-2 and H-8 protons of adenine N-3 derivatives in the PMR spectra of 3-benzyladenine(I) and 3-benzyl-8-bromoadenine(II) in DMSO-d_6 was made. Figure. 1 reveals that the spectra of I and II are strikingly similar except for the peak at 7.80 ppm.

This observation is to be expected since the NH_2, H-2 and CH_2R (R: $-C_6H_5$) protons, but not the H-8 proton, are connected to the pyrimidine structure. The H-2 and H-8 protons of I are responsible for the peaks at 8.60 and 7.80 ppm, respectively. This assignment of protons of adenine N-3 derivatives makes the H-8 proton more shielded than the H-2 proton. In the case of adenine N-9 derivatives,[6] the position of the H-2 proton signal was at higher field than that of the H-8 proton, whereas the H-2 proton was at lower field in adenine.[7]

2. Protonation Site in Adenine N-3 Derivatives as judged by PMR Spectroscopy

2-1. Signals of H-2 and H-8 Protons——The magnitudes ($\Delta\delta$) of the chemical shift differences of the non-exchanging protons of protonated and non-protonated adenine N-3 derivatives are summarized in Table I.

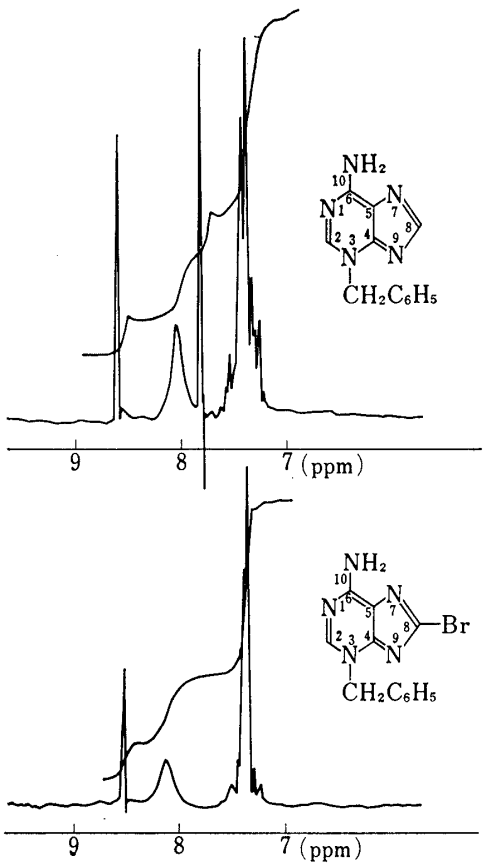

Fig. 1. PMR Spectra of 3-Benzyladenine (Upper) and 3-Benzyl-8-bromoadenine (Lower)

Chemical shifts were measured from TMS in DMSO-d_6 (0.2 M) at 25—30°C.

The $\Delta\delta$ values for the H-2, H-8 and CH_2R (R:–H, $-C_2H_5$, $-C_3H_7$ and $-C_6H_5$) protons were 0.36—0.49, 0.84—0.90 and 0.09—0.13 ppm, respectively. The $\Delta\delta$ values for the chemical shifts of the non-exchanging protons in adenine and adenine N-9 derivatives in response to protonation are listed in Table II.

The $\Delta\delta$ values for the H-2, H-8 and CH_2R protons were 0.35—0.50, 0.39—0.50 and 0.11—0.17 ppm, respectively. It is clear from a comparison of the $\Delta\delta$ values for the H-2 and H-8 protons of adenine derivatives that the protonation site in adenine N-3 derivatives is different from those in adenine and adenine N-9 derivatives. In adenine and adenine N-9 derivatives, the $\Delta\delta$ values for the H-2 protons are approximately equivalent to those for the H-8 protons in spite of protonation at N-1.[2,3] On the other hand, in adenine N-3 derivatives the $\Delta\delta$ values for the H-8 protons are about twice those for H-2. The influence of protonation on the signal of the H-8 proton is larger than on that of the H-2 proton. On the basis of these observations, the protonation site in adenine N-3 derivatives is considered to be N-7 or N-9, adjacent to the H-8 proton.

2-2. Signals of NH_2 Protons——The PMR spectra of I and 3-benzyladenine·HCl(Ia) were measured at 30°C. The NH_2 signal of I was a broad line as shown in Fig. 1. On the other hand, that of Ia was split into a 0.15 ppm doublet owing to protonation at N-7 or N-9. A split signal can be explained on the basis of nonequivalence of the two amino protons which results from hindered rotation about the C_6–N_{10} bond owing to its partial double bond char-

Table I. Effect of Protonation on the Proton Chemical Shifts of Adenine N-3 Derivatives

Compound	H-2	H-8	$-CH_2R$
3-Methyladenine[a]	8.27	7.77	3.91
3-Methyladenine·HCl[a]	8.73	8.61	4.00
$\Delta\delta$	−0.36	−0.84	−0.09
3-Propyladenine	8.35	7.77	4.17
3-Propyladenine·HCl	8.83	8.67	4.30
$\Delta\delta$	−0.48	−0.90	−0.13
3-Butyladenine	8.37	7.78	4.20
3-Butyladenine·HCl	8.83	8.65	4.30
$\Delta\delta$	−0.46	−0.87	−0.10
3-Benzyladenine	8.60	7.80	5.54
3-Benzyladenine·HCl	9.09	8.69	5.67
$\Delta\delta$	−0.49	−0.89	−0.13

δ values are negative, indicating downfield shifts owing to protonation. Chemical shifts are in parts per million from TMS in DMSO-d_6 (0.2 M) at 25—30°C.

a) The spectra of 3-methyladenine and 3-methyladenine · HCl were taken at 60°C.

Table II. Effect of Protonation on the Proton Chemical Shifts of Adenine and Adenine N-9 Derivatives

Compound	H-2	H-8	$-CH_2R$
Adenine	8.15	8.12	
Adenine·HCl	8.62	8.60	
$\Delta\delta$	−0.47	−0.48	
9-Propyladenine	8.08	8.11	3.97
9-Propyladenine·HCl	8.58	8.61	4.14
$\Delta\delta$	−0.50	−0.50	−0.17
9-Butyladenine	8.11	8.14	4.05
9-Butyladenine·HCl	8.57	8.60	4.16
$\Delta\delta$	−0.46	−0.46	−0.11
9-Benzyladenine	8.24	8.33	5.40
9-Benzyladenine·HCl	8.59	8.72	5.51
$\Delta\delta$	−0.35	−0.39	−0.11

δ values are negative, indicating downfield shifts owing to protonation. Chemical shifts are in parts per million from TMS in DMSO-d_6 (0.2 M) at 25—30°C.

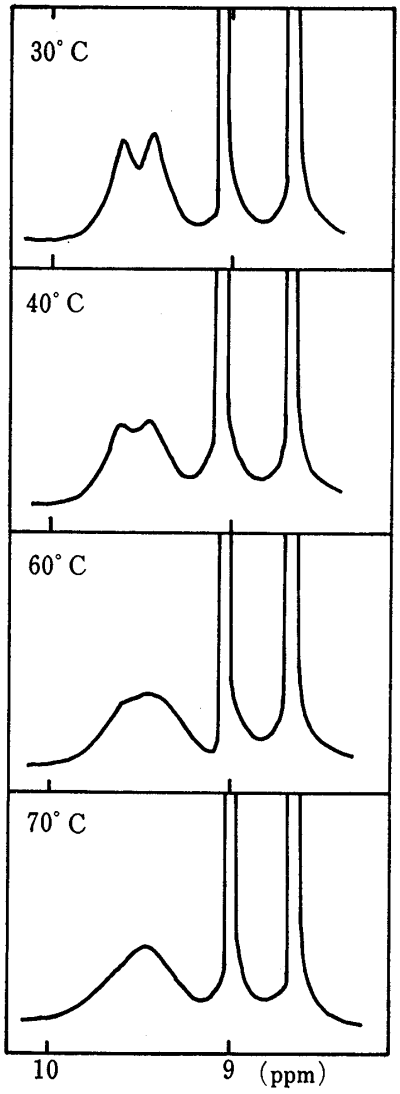

Fig. 2. Temperature Dependence of NH_2 Signals of 3-Benzyladenine·HCl

Chemical shifts were measured from TMS in DMSO-d_6 (0.2 M).

Table III. ^{13}C Chemical Shifts of 3-Benzyladenine·HCl(Ia), 3-Benzyl-8-bromoadenine (II), 3-Butyladenine·HCl (IIIa) and 3-Butyladenine (III)

Compound	C-5	C-2	C-4	C-8	C-6
Ia	110.89	144.72	147.71	148.66	153.96
II	121.56	143.78	150.16	139.45	153.57
IIIa	110.89	144.67	147.84	148.63	153.57
III	120.77	143.69	150.03	152.83	155.33

Chemical shifts are in parts per million with respect to TMS in DMSO-d_6 (0.2—0.5 M).

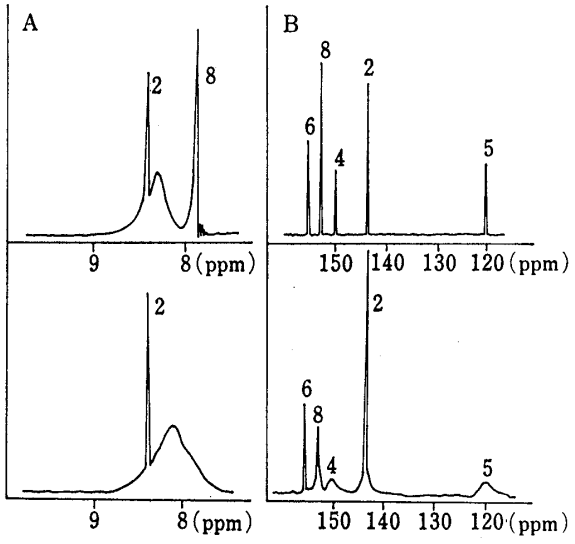

Fig. 3. Effect of Cu^{2+} on the PMR(A) and ^{13}C NMR(B) Spectra of 3-Butyladenine in DMSO-d_6

A: 0.2 M 3-butyladenine in the absence(upper) and in the presence(lower) of 2×10^{-4} M Cu^{2+}.
B: 0.5 M 3-butyladenine in the absence(upper) and in the presence(lower) of 2×10^{-4} M Cu^{2+}.

acter.[8] In order to reduce the rotational barrier, the solution temperatures of Ia was varied from 30°C to 70°C, and PMR spectra of Ia were measured (see Fig. 2).

The NH_2 signal of Ia is dependent on temperature, and gives rise to a broad singlet at 70°C. For comparison of the NH_2 signal of Ia with those of other protonated adenine derivatives, the PMR spectra of adenine·HCl, 1-methyladenine·HNO_3, 7-methyladenine·HCl and 9-benzyladenine·HCl were measured at 25—30°C. Their NH_2 signals appear as one broad line at the region of 8.9—9.7 ppm, and protonation occurs at N-1, N-3 or N-9.[2-5] These results further support the view that the protonation site in adenine N-3 derivatives is N-7 or N-9.

3. Protonation Site in Adenine N-3 Derivatives as judged by ^{13}C NMR Spectroscopy

3-1. Assignment of the ^{13}C NMR Lines——The chemical shifts for Ia, II, 3-butyladenine (III) and 3-butyladenine·HCl (IIIa) are shown in Table III.

Assignment of the ^{13}C NMR lines of adenine N-3 derivatives was made mainly by comparison with the spectra of II and III. The C-5 signal of III is assigned as that at 120.77 ppm, since the C-5 resonance lines of adenine, 7-methyladenine and 9-methyladenine[9] were found at the highest field. Proton decoupling and off-resonance spectra of III distinguished the C-2 and C-8 signals from the other signals. Assignment of the C-4 and C-6 resonances was accomplished by comparing their relative peak intensities, because the C-4 signal is expected to be smaller than the C-6 signal (there is a saturation effect due to the longer T_1 relaxation times associated with bridgehead carbons[10]). Uesugi and Ikehara[11] showed that the bromination of adenosine at N-8 causes a downfield shift of the C-2 signal by 0.05 ppm and an upfield shift of the C-8 signal by 12.78 ppm. Therefore, it is considered that the bromination of adenine N-3 derivatives at N-8 causes similar shifts of the C-2 and C-8 signals to those of 8-bromoadenosine. Namely, the C-2 signals of III and II are assigned to those at 143.69 and 143.78 ppm, and the C-8 signals to those at 152.83 and 139.45 ppm, respectively. These assignments were confirmed by the observation of selective broadening of the proton peak and the carbon lines of III in DMSO-d_6 upon addition of copper chloride (see Fig. 3).

As indicated by the selective broadening of the H-8 proton peak, complex formation occurs at the sites of imidazole structure. Therefore, the effect of Cu^{2+} in the ^{13}C NMR spectrum is observed in the C-4, C-5 and C-8 carbon lines but not the C-6 and C-2 lines. In fact, the signals at 152.83, 150.03 and 120.77 ppm become so broad that those at 150.03 and 120.77 ppm are no longer observable. On the other hand, the 143.69 ppm signal is not affected and that at 155.33 ppm is only slightly affected. From these observations, it is clear that the assignment of adenine N-3 derivatives is as shown in Fig. 3.

3-2. Effect of Protonation on the C-4 and C-5 Signals——Pugmire *et al.*[12] showed that protonation on the imidazole ring of purines causes a 7—12 ppm upfield shift of the resonance line of the bridgehead carbon atom adjacent to the protonation site with respect to its position in the non-protonated molecule, whereas the other bridgehead carbon atom is less affected. Thus, we checked the protonation site by determining the difference ($\Delta\delta$) in the chemical shifts of III and IIIa (see Table III). The $\Delta\delta$ values for the C-4 and C-5 carbons correspond to upfield shifts of 2.19 and 9.88 ppm, respectively. These results show that the C-5 carbon rather than the C-4 carbon is adjacent to the protonation site. Thus, it is clear that protonation of adenine N-3 derivatives occurs mainly at the N-7 site.

Experimental

PMR spectra were recorded with a Varian EM 360A (60 MHz) spectrometer equipped with an EM-3640 variable temperature unit and an EM-3630 lock/decoupler. The concentration of all adenine derivatives was 0.2 M in DMSO-d_6, and the chemical shifts were measured from TMS. The sample temperatures were recorded as the reading at which the EM-3640 variable temperature controller was set. ^{13}C NMR spectra were taken on JEOL PFT-100 (25 MHz) and Varian FT-80A (20 MHz) spectrometers using proton decoupling and off-resonance conditions. Melting points were measured with a Mettler FP 5/FP 51 combination.

Reagents——Adenine was purchased from Tokyo Kasei Co. and other reagents were obtained from

Wako Pure Chemical Industries, Tokyo. They were of reagent grade and were used without further purification. Treatment of adenine with benzyl bromide, propyl bromide, butyl bromide and methyl *p*-toluenesulfonate in dimethylformamide (DMF) furnished, after basification, the corresponding adenine N-3 derivatives.[13] 9-Propyladenine and 9-butyladenine were obtained through sublimation of an equimolar mixture of adenine and tetrapropylammonium or tetrabutylammonium.[14] 9-Benzyladenine was prepared by reaction of the sodium salt of adenine with benzyl bromide in DMF.[15] The hydrochlorides of adenine and adenine N-3 or N-9 derivatives were prepared from dilute HCl solution. 1-Methyladenine·HNO_3 was prepared from dilute HNO_3 solution after methylation of adenosine with methyliodide in dimethylacetoamide followed by hydrolysis with dilute HCl.[16] 7-Methyladenine and 7-methyladenine·HCl were gifts from Taisho Pharm. Co., Ltd.

3-Benzyl-8-bromoadenine (II)——A mixture of 5 g of 3-benzyladenine (I) and 5 g of N-bromoacetamide in 100 ml of $CHCl_3$ was heated at 60—70°C for 40 min on a water bath and then evaporated to dryness *in vacuo*. The residue was collected by filtration, and washed with acetone. The residue was dissolved in hot dilute MeOH–NaOH, and charcoal was added to the solution. The filtered hot solution was adjusted to pH 3—4 with dilute HCl. The crystals were dissolved in hot dilute EtOH–NaOH. The residue was collected by filtration and recrystallized from MeOH, mp 226°C. *Anal.* Calcd for $C_{12}H_{10}BrN_5$: C, 47.38; H, 3.32; N, 23.03. Found: C, 47.31; H, 3.23; N, 23.43.

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