A conformational study of some adenosines by use of nuclear Overhauser effect

M. Ueyama and K. Tori, M. Ikehara* and M. Kaneko

Shionogi Research Laboratory, Shionogi & Co., Ltd., Fukushima-ku,
Osaka, 553 Japan

Received 17 May 1974

ABSTRACT

Conformations of 8-bromo-2'-Q-triisopropylbenzenesulfonyladenosine (1) and its 3'-Q-isomer (2) in solution have been determined by the use of intramolecular nuclear Overhauser effects in ^1H NMR spectroscopy. Compound 1 has been proved to have a conformation in which the adenosine and benzene rings are intramolecularly stacked and compound 2 an elongated non-stacked conformation in dimethylsulphoxide. The 5'-Q-acetyl derivative of 1 has also been found to adopt the intramolecularly stacked conformation in dimethylsulphoxide, but a non-stacked one in chloroform. Coupling constants observed are discussed in connection with the conformation of the ribose moiety. The ^{13}C NMR spectra have also been examined, but no effect which could be ascribed to the stacking phenomena was observed in the carbon chemical shifts.

8-Bromo-2'-Q- and 8-bromo-3'-Q-triisopropylbenzenesulfonyladenosines (1 and 2, respectively), which have been found to be suitable intermediates for the synthesis of 8,2'-S- and 8,3'-S-cyclonucleosides of adenosine, respectively,¹ have been reported to adopt molecular conformations different from each other in solution;² the conformation of the former retains a more folded form than that of the latter, as suggested from the ^1H chemical-shift difference between their 2-H signals, R_f values in TLC, and the difference in extinction coefficients in an acidic and a neutral solution.² Recently, it has been confirmed by X-ray analysis in crystals³ that the former has a conformation in which the adenine and benzene rings are intramolecularly stacked, whereas the latter adopts an elongated non-stacked conformation; and a CD spectral study of these isomers has suggested that they retain conformations similar to those in the solid state.³ We describe here some evidence for the conformations of 1, 2, and the 5'-Q-acetyl derivative of 1 (1a) in solution on the basis of intramolecular nuclear Overhauser effects (NOE),⁴ as frequently applied in conformational studies of nucleosides and nucleotides,⁵⁻¹¹ along with ^1H chemical shifts, and ^1H , ^1H spin coupling constants. The ^{13}C NMR spectra with full assignments are also

reported and discussed.

RESULTS AND DISCUSSION

Figure 1a shows the ^1H NMR spectrum of $\underline{1}$ in $\text{DMSO}-d_6$ at 100 MHz. Assignment of signals was aided by use of the results from double and triple resonance experiments and the values of chemical shifts. Figure 1b shows a result from double irradiation of the signal of H_2O included as an impurity; the signal intensities of OH protons exchanging with H_2O protons were decreased as the result of indirect spin saturation.¹² Irradiation of the signal at δ 4.56, probably due to 3'-H, caused a change in the signals at δ 5.31 and 5.98 (Fig. 1c). Thus, the signals due to 1'-H, 2'-H, 5'-OH, 4'-H, and 3'-OH have been assigned. The spectrum of $\underline{2}$ in $\text{DMSO}-d_6$ at 100 MHz is shown in Figure 2. The signals were assigned in a similar way. The ^1H NMR parameters were obtained by computer analysis using the LAOCN 3 program¹³ and/or by first-order analysis on the basis of the double and triple resonance experiments, although the signals being relatively broad did not allow the data to be determined very accurately. The results obtained are listed in Tables 1 and 2.

The signals arising from 2-H, benzene protons (Bz-H), methyl and methine protons of isopropyl groups ($i\text{-Pr-Me}$ and $i\text{-Pr-H}$), and NH_2 in $\underline{1}$ appear at higher fields than those due to the corresponding protons in $\underline{2}$. The result may be explained by the assumption that while in $\underline{1}$ the planes of the phenyl and adenine rings are

Fig. 1. ^1H NMR spectra of 8-bromo-2'-O-triisopropylbenzenesulfonyladenine (1) at 100 MHz in $\text{DMSO}-d_6$: (a) normal spectrum, (b) double-resonance spectrum irradiated at the water proton signal, and (c) double-resonance spectrum irradiated at the 3-H signal.

Fig. 2. ^1H NMR spectrum of 8-bromo-3'-O-triisopropylbenzenesulfonyl-adenosine (2) at 100 MHz in DMSO-d_6 .

Table 1. Proton chemical shifts (δ)

	<u>1</u>	<u>1a</u>		<u>2</u>	<u>3</u>	<u>4</u>
	DMSO-d ₆	DMSO-d ₆	CDCl ₃	DMSO-d ₆	DMSO-d ₆	DMSO-d ₆
2-H	8.00	8.00	8.10	8.13	8.14	—
Bz-H	7.18	7.19	7.10	7.35	—	7.32
<u>i</u> -Pr-Me	1.05	1.03	1.09	1.23		
	1.13	1.12	1.16		—	1.23
	1.18	1.19	1.22	1.31		
<u>i</u> -Pr-H	2.90	2.89	2.86	2.99		2.98
	3.86	3.85	3.95	4.20	—	4.09
NH ₂	7.55	7.51	6.14	7.61	7.53	—
1'-H	6.10	6.04	6.18	5.89	5.86	—
2'-H	6.10	6.07	6.32	5.47	5.10	—
3'-H	4.56	4.75	5.01	5.24	4.23	—
4'-H	4.07	4.14	4.28	4.20	4.00	—
5'-H	3.51	4.08	4.28	3.44	—	—
5"-H	3.70	4.39	4.46	3.69	—	—
2'-OH	—	—	—	6.06	—	—
3'-OH	5.98	6.06	---	—	—	—
5'-OH	5.31	—	—	5.68	—	—
5'-OAc	—	1.90	2.01	—	—	—

situated face to face so that the anisotropic shielding effect of the rings is exerted upon their protons, in 2 the phenyl-residue plane is not fixed. Further the fact that the i-Pr-Me signals appear as three magnetically non-equivalent doublets in 1 and as two doublets (ortho and para) in 2 also supports the locally packed conformation of 1 and free rotation of the benzene residue in 2. The ¹H NMR spectra of 8-bromoadenosine (3) and ethyl 2,4,6-triisopropylbenzenesulfonate (4) have been taken in DMSO-d₆ for the sake of comparison. The chemical shifts of 2-H, Bz-H, i-Pr-Me, and i-Pr-H in 3 and 4 were found to be similar to those obtained with 2. The spectrum obtained with a 1:1 mixture of their solutions in DMSO-d₆ was the same as those of the separate compounds. Thus, the unusual situation appears to occur only with 1.

Table 2. Proton coupling constants, J (± 0.2 Hz)

	<u>1</u>	<u>1a</u>	<u>2</u>
	DMSO-d ₆	DMSO-d ₆	DMSO-d ₆
J _{1',2'}	6.7	4.2	4.8
J _{2',3'}	4.9	4.0	5.3
J _{3',4'}	2.8	3.7	5.0
J _{2',2'-OH}	—	—	—
J _{3',3'-OH}	5.8	6.7	—
J _{4',5'}	~4.2	3.5	5.5
J _{4',5''}	~4.0	2.9	5.9
J _{5',5''}	-11.5	-12.1	-12.7
J _{5',5'-OH}	7.1	—	—
J _{5'',5'-OH}	4.5	—	—

In order to confirm the above fact, further NOE experiments were carried out for most pairs of proton signals in the spectra of 1 and 2 in DMSO-d₆. Table 3 shows the data. Although the calculation of molecular-conformer distributions by a computer fit of intramolecular NOE results from multiple experiments have already been reported,⁵ the present data are not sufficient to employ it rigorously. However, a qualitative interpretation of NOE results has frequently proved useful for structure analysis. The most important interactions in the present compounds are those between the 2-H and i-Pr-Me and between the NH₂ and i-Pr-Me. Both NOE interactions were clearly observed in 1, but no NOE was detected in 2 (see Table 3). These results further confirm the above conclusions that the adenine and benzene moieties are stacked in the molecule of 1 similarly as observed in the solid state,³ and that there is no stacked form in the molecule of 2. Observation of the NOE [5'-OH]→2-H supports that the conformation around the glycosidic bond of both molecules is *syn*, with an intramolecular hydrogen-bond between the 5'-O atom of the ribose ring and the 3-N atom of the base. The conformations determined for 1 and 2 are illustrated in Figure 3 with the relevant NOE interactions observed. It should be noted that these conformations are stable on the NMR time scale, only representing the time-averaged structures.

Fig. 3. Molecular conformations and NOE values for **1** and **2** in DMSO- d_6 (i-H $\rightarrow$ j-H indicates that an NOE was observed on the j-H signal when the i-H frequency was saturated).

Table 3. The NOE values (increases in integrated signal intensities, %)

Signal irradiated	Signal observed	Compound			
		<u>1</u> DMSO-d ₆	<u>1a</u> DMSO-d ₆	CDCl ₃	<u>2</u> DMSO-d ₆
<u>i</u> -Pr-Me	Bz-H	15	18	18	18
<u>i</u> -Pr-Me	2-H	6	7	0	0
<u>i</u> -Pr-Me	3'-H	10	0	0	0
<u>i</u> -Pr-Me	3'-OH	7	-	-	-
<u>i</u> -Pr-Me	NH ₂	7	5	0	0
<u>i</u> -Pr-H ^a	Bz-H	4	4	6	4
2'-H	3'-H	3	<u>c</u>	~0	<u>c</u>
3'-H	2'-H	<u>b</u>	6	5	<u>c</u>
5'-H	2-H	0	<u>b</u>	<u>b</u>	2
5'-OH	2-H	4	-	-	10
5'-OAc	2-H	-	7	0	-

^a One of the signals appearing at the lower field was irradiated.

b Not observed.

c Undeterminable.

NOE experiments have also been carried out on the 5'-O-acetyl derivative (1a) in DMSO-d₆ and CDCl₃; the results obtained are listed in Table 3. The data obtained in DMSO-d₆ are very similar to those obtained on 1 in DMSO-d₆, showing NOE interactions characteristic of a stacked conformation around the glycosidic bond. Thus it is inferred that 1a adopts a conformation analogous to that of 1. In CDCl₃, however, neither the NOE [i-Pr-Me] → 2-H, nor any other NOE supporting stacking between the adenine and benzene rings, nor an NOE identifying the syn glycosidic bond were observed in spite of observation of the strong NOE [i-Pr-Me] → Bz-H (see Table 3). The conformational populations in CDCl₃ appear to be less weighted toward a syn conformation.

As a conclusion, the hydrogen bond between the 5'-O and 3-N atoms is not responsible for stabilizing the stacked form. Thus, the above results suggest that interaction of the nucleoside with the medium may be the ultimate determinant for

the conformation of the molecule in solution. In molecule 1, the stacking of hydrophobic groups probably occurs, the hydrophilic hydroxyl group projecting outwards in DMSO- d_6 ; whereas the molecule as a whole becomes hydrophobic in $CDCl_3$. On the other hand, steric interactions may prevent such a change in conformation for molecule 2.

The coupling constants allow two important conclusions to be made regarding the rotamer population about the exocyclic $C_4'-C_5'$ bond, and the puckering of the ribose ring. Treatment using the Karplus relation has been extended¹⁴⁻¹⁶ to the 4' to 5'(5'')-vicinal couplings to yield estimates of the rotamer populations about the exocyclic $C_4'-C_5'$ bond. Consideration of the data on 1, 1a, and 2 leads to the conclusion that the hydroxymethyl group in 1 and 2 in DMSO- d_6 interconverts between the gauche-gauche, gauche-trans, and trans-gauche rotamers (Fig. 4) with a preference for the

Fig. 4. Newman projections for conformers about the $C_4'-C_5'$ bond.

gauche-gauche ($P_I = \text{Ca. } 0.8$), and that in 1a the acetoxymethyl group predominantly adopts the gauche-gauche conformation in DMSO- d_6 and interconverts between forms with preferences for the gauche-trans and the trans-gauche in $CDCl_3$. Considerably different values between the $J_{5',5''}\text{-OH}$ and $J_{5'',5'}\text{-OH}$ also indicate that there exists a preferred conformation about the C-O bond. These conclusions are reasonably explained by the formation of the hydrogen bond between the 5'-O atom of the ribose ring and the 3-N atom of the base moiety in 1 and 2. Both the $J_{2',\text{OH}}$ and $J_{3',\text{OH}}$ values are slightly greater than those observed and calculated¹⁷ for a freely rotating OH group. For the conformation of the ribose ring, qualitative models for the three dimensional structure of the furanose moieties have been presented by

using the Karplus treatment.¹⁴⁻¹⁶ Rationalization of the $J_{1',2'}$, $J_{2',3'}$, and $J_{3',4'}$ values in Table 2 according to Hruska, et al.¹⁴ lead us to conclude that the ribose residue of 1 and 2 in DMSO is preferentially in the C_{2'}-endo-C_{3'}-exo conformation, and that in 1a the ribose exists in an equilibrium between various puckered ring conformations. Thus, the conformation of the ribose residue seems to be unimportant in so far as mononucleoside conformation in solution is concerned.

Table 4. Carbon-13 chemical shifts (δ) in DMSO-d₆

	<u>1</u>	<u>1a</u>	<u>2</u>	<u>3</u>	<u>4</u>
C-2	152.4	152.6	152.3	152.3	-
C-4	149.8	149.8	149.7	149.9	-
C-5	119.3	119.1	119.6	119.8	-
C-6	155.0	154.9	155.2	155.0	-
C-8	126.8	126.7	127.1	127.1	-
C-1'	87.0	87.3	89.7	90.5	-
C-2'	76.6	76.9	69.0	71.0	-
C-3'	69.2	68.7	79.7	71.4	-
C-4'	86.1	81.5	83.6	86.8	-
C-5'	61.1	62.5	61.2	62.2	-
C-1"	154.1	154.2	153.8	-	153.6
C-2"	123.7	123.8	123.7	-	123.7
C-3"	150.3	150.2	150.1	-	150.1
C-4"	128.5	128.5	130.2	-	129.1
i-Pr-CH ₃					
(ortho)	24.0 24.3	23.9 24.3	24.4	-	24.4
(para)	23.1	23.1	23.3	-	23.3
i-Pr-CH					
(ortho)	29.2	29.2	29.3	-	29.0
(para)	33.4	33.3	33.4	-	33.5
COCH ₃	-	169.7	-	-	-
COCH ₃	-	20.3	-	-	-

Finally, the ^1H noise-decoupled natural-abundance ^{13}C FT NMR spectra of the above-mentioned compounds 1–4 have been examined. The chemical shifts obtained are shown in Table 4. The spectral assignments were made on the bases of chemical shift rules and signal intensity,¹⁸ along with the known data on analogous nucleosides.¹⁹ Although the magnetic non-equivalence in i-Pr-Me carbon in 1 and 1a was clearly observed, the chemical shifts of the adenine- and benzene-ring carbons are virtually not changed from 1 to 4. The results show that the stacking interaction examined here exerts no effect upon ^{13}C NMR spectra, as previous ^{13}C NMR experiments designed to determine the effect of base stacking gave only negative results.²⁰

The differences in chemical shifts of C-2' and C-3' between 1, 2, and 3 are explained by the substituent effect. The replacement of an OH group by a triisopropylbenzenesulfonyl group tends to deshield the α -carbon and shield the β -carbon, the trend resembling the result of acetylation.¹⁸

EXPERIMENTAL

The ^1H NMR spectra were taken with a Varian HA-100 and an A-60A spectrometers using about 10% (w/v) degassed solutions in $\text{DMSO}-d_6$ and/or CDCl_3 containing about 1% of TMS as an internal standard. Double- and triple-resonance experiments were performed on the HA-100 spectrometer in the frequency-swept and TMS-locked mode with two Hewlett-Packard HP-200ABR audiooscillators and an HP-5212A electronic counter. The NOE experiments were carried out using a sweep rate of 1 Hz/sec for integrations. Accuracies are ± 0.01 ppm for chemical shifts and about $\pm 2\%$ for NOE. Spectral analyses were carried out using a CDC 6600 computer, and simulated spectra were produced on a CALCOMP plotter.

The ^{13}C FT NMR spectra were taken with a Varian NV-14 FT spectrometer in the ^1H noise-decoupled natural-abundance deuterium-locked mode at 15.09 MHz. Each spectrum was accumulated by about 80,000 transients. Accuracies are ± 0.1 ppm for chemical shifts (δ in ppm downfield from internal TMS).

REFERENCES

- 1 Ikehara, M. and Kaneko, M. (1970) Chem. Pharm. Bull. 12, 2401-2406
- 2 Ikehara, M. and Kaneko, M. (1970) Tetrahedron 26, 4251-4259
- 3 Fujihara, T., Fujiwara, T., Tomita, K., Kaneko, M., and Ikehara, M. (1972) Biochem. Biophys. Res. Commun. 46, 708-711
- 4 Anet, F. A. L. and Bourn, A. J. R. (1965) J. Amer. Chem. Soc. 87, 5250-5251
- 5 Schirmer, R. E., Noggle, J. H., Davis, J. P., and Hart, P. A. (1970) J. Amer. Chem. Soc. 92, 3266-3273

- 6 Hart, P. A. and Davis, J. P. (1971) J. Amer. Chem. Soc. 93, 753-760
- 7 Kainosho, M. and Kyogoku, Y. (1972) Biochemistry 11, 741-752
- 8 Davis, J. P. (1972) Tetrahedron 28, 1155-1165
- 9 Davis, J. P. and Hart, P. A. (1972) Tetrahedron 28, 2883-2891
- 10 Lüdemann, H.-D. and Goldammer, E. v. (1973) Z. Naturforsch. 28c, 361-369
- 11 Nanda, R. K., Tewari, R., Govil, G., and Smith, I. C. P. (1974) Can. J. Chem. 52, 371-375
- 12 Fung, B. M. and Stolow, R. D. (1967) Chem. Commun. 257-258, and references therein
- 13 Bothner-By, A. A. and Castellano, S. Program 111, Quantum Chemistry Program Exchange, Indiana University
- 14 Hruska, F. E., Grey, A. A., and Smith, I. C. P. (1970) J. Amer. Chem. Soc. 92, 214-215, 4088-4094
- 15 Blackburn, B. J., Grey, A. A., Smith, I. C. P., and Hruska, F. E. (1970) Can. J. Chem. 48, 2866-2870
- 16 Schleich, T., Blackburn, B. J., Lapper, R. D., and Smith, I. C. P. (1972) Biochemistry 11, 137-145
- 17 Davies, D. B. and Danyluk, S. S. (1970) Can. J. Chem. 48, 3112-3115
- 18 Levy, G. C. and Nelson, G. L. (1972) Carbon-13 Nuclear Magnetic Resonance for Organic Chemists, John Wiley & Sons, Inc., New York
- 19 Wenkert, E., Hagaman, E. W., and Gutowski, G. E. (1973) Biochem. Biophys. Res. Commun. 51, 318-322, and references cited therein
- 20 Dorman, D. E. and Roberts, J. D. (1970) Proc. Nat. Acad. Sci. U.S. 65, 19-26, and references cited therein

*Faculty of Pharmaceutical Sciences, Osaka University,
Toneyama, Toyonaka, Osaka, 560 Japan