CONFORMATION OF 1- AND 3-DEAZAADENOSINES IN SOLUTION AS STUDIED BY ^{1}H NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY (1)

Shigeru Kitano and Yoshihisa Mizuno
Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, 060 Japan
Masako Ueyama and Kazuo Tori*
Shionogi Research Laboratory, Shionogi & Co., Ltd., Fukushima-ku, Osaka, 553 Japan
Masako Kamisaku and Katsumi Ajisaka
Central Research Laboratories, Ajinomoto Co., Inc., Suzuki-cho, Kawasaki, 210 Japan

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SUMMARY: ^{1}H nuclear magnetic resonance spectra of 1- (II) and 3-deazaadenosines (III) together with adenosine (I) in dimethylsulfoxide have been examined. Features of coupling constants indicate that the furanose rings of I, II, and III have similar conformational preferences and that conformations about the 4'-C — 5'-C bond are preferentially gauche-gauche. Nuclear Overhauser effect and spin-lattice relaxation-time measurements demonstrate that II predominantly adopts the syn-conformation similar to that of I, whereas that of III has a greater anti (freely rotating) component. The results suggest that the syn-conformation in II as well as I is stabilized presumably through a hydrogen bond between the 3-N and 5'-hydroxyl group.

Nuclear magnetic resonance (NMR) spectroscopy involving multiple resonance and relaxation phenomena has been employed successfully to provide valuable information about the structure and conformation of biologically active substances including nucleosides and nucleotides (2-5). Conformation about the N-glycosidic bond of purine nucleosides in solution was thus shown to lie predominantly in the syn region (2,5), whereas some of them were found to assume the anti conformations (5,6); and only the derivatives of adenosine (I) in anti-conformation can be substrates for adenosine deaminase (6).

1-Deazaadenosine (II) showed the ability to act as a substrate or an activator for a number of enzymes, including cAMP-dependent protein kinase (7) and 5'-nucleotidase (8). 1-Deazakinetin ribofuranoside was found to exhibit potent cytokinin activity in

* To whom inquiries should be addressed.

the tobacco pitch callus bioassay (1). Furthermore, II shows potent antileukemic activity (9). In sharp contrast, 3-deazaadenosine phosphate derivatives were found to be poor (nearly inactive) substrates or activators in these enzyme systems. Moreover, it has been shown that III is inactive as an antileukemic reagent (9) and that 3-deaza-kinetin ribofuranoside is also ineffective as a cytokinin (1).

Whether this general trend arises for the same reasons in each pair is of course not clear. However, conformational comparison between each pair could shed light on the nature of this contrasting behavior toward the above enzyme systems. Thus, II and 3-deazaadenosine (III) are thought to be logical starting points of this conformational study of these interesting pairs of isomers.

In this communication are reported, in a preliminary form, N-glycosyl conformations of II and III together with that of I for comparison as studied by ^{1}H NMR spectroscopy including nuclear Overhauser effect (NOE) (10) and spin-lattice relaxation time (T_1) measurements.

MATERIALS AND NMR MEASUREMENTS

The preparation of II (11) and III (12) followed reported procedures. The purity of these samples was analytical grade. ^{1}H NMR spectra were taken with a Varian HA-100 and an XL-100-15 NMR spectrometer using about 10% (w/v) degassed solutions in [$^2H_6$]dimethylsulfoxide (DMSO) containing 1% TMS as an internal standard at ordinary probe temperature (ca. 30°). NOE experiments (10) were performed on the HA-100 in the frequency-swept and TMS-locked mode. T_1 measurements were carried out on the XL-100-15 operating at 100 MHz by the use of an inversion-recovery technique with 180°$-\tau$-90° pulse sequences in the pulse and Fourier transform mode.

RESULTS AND DISCUSSION

The NMR spectral parameters obtained in DMSO are listed in Table I. This observation may be taken as reflections that the furanose rings of I, II, and III have similar conformational preferences in the rapid equilibria (4,5) with approximately

Table I. Chemical Shifts, δ, Spin-coupling Constants, J, and Spin-lattice Relaxation Times, T_1

Proton	δ (±0.01)			T_1 (±0.5 sec)		
	I[a]	II[b]	III[b]	I	II	III
1-H	---	6.42d	---	---	0.50	---
2-H	8.18s	7.80d	7.79d	2.74	0.72	0.32
3-H	---	---	7.46d	---	---	0.32
8-H	8.37s	8.25s	8.72s	0.63	0.72	0.66
1'-H	5.93d	5.92d	5.98d	0.55	0.58	0.39
2'-H	4.66q	4.73q	4.33t	0.26	0.26	0.25
2'-OH	5.47d	5.36d	[c]	0.55	0.65	[c]
3'-H	4.21q	4.17m	4.19t	0.29	0.29	0.25
3'-OH	5.19d	5.13d	[c]	0.58	0.65	[c]
4'-H	4.02q	4.02q	4.07q	0.43	0.43	0.38
5'-H_A	3.60m	3.58brm	3.67brm	0.14	0.15	0.12
5'-H_B	3.72m	3.68brm	3.71brm			
5'-OH	5.44m	6.04brm	[c]	0.55	0.66	[c]
6-NH_2	7.35brs	6.45brs	8.59brs	0.11	0.13	0.09
		J (±0.2 Hz)				
1-H,2-H	---	5.8	---			
2-H,3-H	---	---	7.1			
1'-H,2'-H	6.1	6.4	5.9			
2'-H,3'-H	5.1	4.8	5.0			
3'-H,4'-H	3.0	2.6	3.0			
4'-H,5'-H_A	3.8	~3.5[d]	~3.5[d]			
4'-H,5'-H_B	3.3	~3.5[d]	~3.5[d]			
5'-H_A,5'-H_B	-12.2	~(-)12[d]	~(-)12[d]			
2'-H,2'-OH	6.4	6.2	[c]			
3'-H,3'-OH	4.7	4.2	[c]			

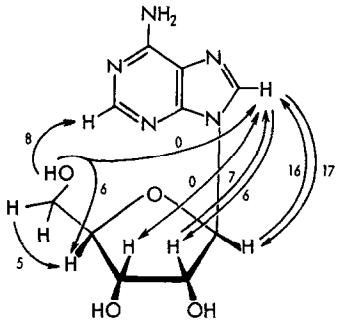
Multiplicities:
s : singlet,
d : doublet,
t : triplet,
q : quartet,
m : multiplet,
and
br: broad signal

[a] Obtained by computer analysis using the LAOCN3 program.
[b] Obtained by the first-order analysis.
[c] Not determinable owing to exchanging of OH signals.
[d] Not exactly determinable owing to line broadening of 5'-H signals: errors are probably ±0.5 Hz or less.

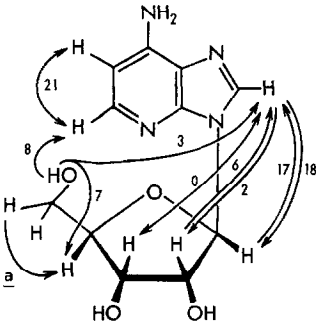
60% of the 2'-endo form (5), and that conformations in the 4'-C — 5'-C bonds are preferentially gauche-gauche (4,5). The signals due to 2'-H of I and II appear at lower fields by ca. +0.35 ppm than does the signal due to the corresponding proton in III. The signals arising from 8-H of the former two appear at higher fields by ca. -0.4 ppm than does the signal due to the corresponding signal of the latter. These results may be explained by the assumptions that I and II adopt similar conformations about the N-glycosyl linkage, whereas III assumes a conformation different from those of I and II, that 2'-H of I and II is situated in the deshielding region of the 3-N lone-pairs of the base, and that 8-H of III is deshielded by the close proximity to the furanose ring moiety.

In order to confirm the above assumptions, NOE interactions were measured for most pairs of proton signals in these nucleosides. The results pertinent to the present discussion are shown in Fig. 1. As has been well known (10), much caution should be exercised in interpretation of NOE results in rotating (13) and inverting (14) systems. In the present cases also, NOE results must reflect time-averaged structures on the NOE time-scale (2,3,10). The observations of f_8(5'-OH) in II and f_3(2') in III (see Fig. 1), though in slight extents, evidence the rapid equilibria of their conformations, although f_2(5'-OH) in II, and f_3(1') and $f_{1'}$(3) in III observed in considerably large extents, seem to prove fixed conformations.

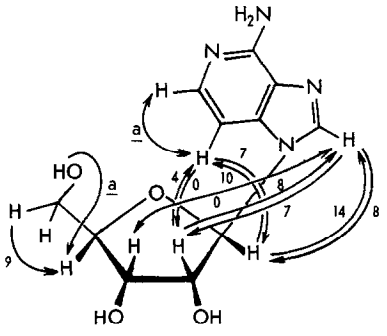
The most important interactions f_8(1') in the present compounds decreased in the following order: 17% in II, 16% in I, and 14% in III (see Fig. 1). This order may be taken as an index of distance r between the anomeric proton and 8-H as the first approximation because their furanose-ring moieties have been considered to be similar to each other as described above. However, the treatment by Eq.(A) (10) may give somewhat more quantitative estimations for relative distances between 8-, 1'-, and 2'-H, because f_8(1') and f_8(2') were the only large enhancements of 8-H;



Adenosine (I)



1-Deazaadenosine (II)



3-Deazaadenosine (III)

[a] Not determinable owing to close signal positions or exchanging.

Fig. 1. Structures and NOE Enhancement Values (±2%) in DMSO: i-H → j-H indicates that an NOE, $f_j(i)$, was observed on the j-H signal when the i-H frequency was saturated.

$$r_{is}/r_{it} \approx [\, f_i(t)/\, f_i(s)\,]^{1/6} \qquad (A)$$

Thus the ratios $r_{8,2'}/r_{8,1'}$ in I, II, and III were estimated to be about 1.15, 1.19, and 1.10, respectively. Moreover, the proportion of time spent in the syn-conformation P_{syn} is approximately given by Eq.(B) according to Son et al. (3);

$$P_{syn} = 1 - P_{anti} \approx 3f_8(1')/[3f_8(1') - f_8(2') - f_8(3')] \qquad (B)$$

Then, we can obtain P_{syn} values for I, II, and III to be about 0.87, 0.89, and 0.84, respectively, neglecting the weak f_8(5'-OH) observed in II. These results strongly suggest that II predominatly adopts syn-conformation similar to that of I (or more syn than I), whereas III has a greater anti component than I has, or the rotation about the N-glycosyl bond in III occurs more freely.

Spin-lattice relaxation times T_1 were also determined for all protons of the nucleosides, as also listed in Table I. The T_1 values for 8-H and all furanose protons are virtually similar to each other from compound to compound, except that for 1'-H in III. The smaller T_1 value for 1'-H in III (0.39 sec) than those in I and II (0.55 and 0.58 sec, respectively) can be interpreted as showing that another relaxation mechanism, probably through 2-H, becomes effective in III, the result which is in harmony with those described above. Detailed analyses of the results with T_1 measurements as well as those with NOE interactions between furanose-ring protons will be reported in future.

In conclusion, it is found that both syn- and anti-conformations exist in rapid equilibria for these nucleosides, and that the decrease in proportion of syn to anti is ordered as II > I > III, the former two, II as well as I, predominatly adopting the N-glycosyl conformation in the syn region. We would like to suggest that the formation of a hydrogen-bond between the 5'-hydroxyl group and the 3-N atom plays an important role in stabilizing the syn-conformations of I and II.

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