^{15}N nuclear magnetic resonance spectra of nucleoside compounds. Reflection of modifications at N1 and N^6 of adenosine derivatives

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^{15}N nuclear magnetic resonance spectra of adenosine derivatives were taken at the natural abundance level, and the reflection of structural modifications on the spectra was considered on the basis of the electronic behaviour of the molecules.

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Opérant au niveau de l'abondance naturelle, on a déterminé les spectres en résonance magnétique nucléaire du ^{15}N de dérivés de l'adénosine; on a observé des variations dans les spectres qui résultaient de modifications de structure et on les a considérées en fonction du comportement électronique des molécules.

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Introduction

Several papers have been published regarding the ^{15}N nmr spectra of nucleosides (1). We would like to present here the ^{15}N nmr spectra of some adenosine derivatives to cast light on the spectral changes caused by structural modifications at N1 and N^6 of adenosine.

Results and discussion

Morris (2) has presented a method of taking ^{15}N nmr spectra at the natural abundance level, using INEPT (insensitive nuclei enhanced by polarization transfer). To assign the signals of nitrogen unambiguously, Bax, Niu, and Live (3) have adopted the selective INEPT, including the use of refocussed pulse sequence and broad band proton decoupling. If a compound has several nitrogen atoms whose $^nJ_{N,H}$ values are similar, as in the case of polypeptides (3), the selective INEPT would be effective because a τ value can be chosen unequivocally based on a J value. A preliminary study has suggested that the nitrogen atoms in adenosine have $^2J_{N,H}$ values ranging widely (8–15 Hz), and setting a τ value based on a $^2J_{N,H}$ value seems to be difficult. Deviation from the optimum τ values ($\tau = 1/4J$) causes, if the refocussing period is included in the sequence, the decrease of the S/N ratio (4). Thus, we adopted the basic INEPT pulse sequence,[2] and τ values were chosen experimentally to reach analyzable spectra (Table 1). The assignment of nitrogen resonance signals in adenine compounds at the natural abundance level was accomplished as follows: selective irradiation[3] at the H-2 resonance resulted in the appearance of two antiphase doublets assignable to N1 and N3 (8). In the same manner the signals of N7 and N9 were observed by irradiating the H-8 signal. Distinction between N1 and N3 signals was accomplished by the method of Städeli, Bigler, and Philipsborn (5), which included the combination of INEPT with selective ^1H decoupling. Because of the coupling (<2 Hz) between N1 and one (two) hydrogen(s) at N^6, the N1 signal was broader than the N3 signal, being sharpened by weak irradiation[4] at the N^6-H resonance during the time of acquisition.

The position of protonation on adenosine was determined to be N1, based on the fact that addition of an acid causes upfield shifts of the N1 signal without affecting the other nitrogen signals (6). From a different viewpoint this experiment seems to illustrate the localization of positive charge without perturbing the π-electron sextet, which would have caused upfield shifts of other N atoms.

To extend the above reasoning, the ^{15}N nmr spectra of compounds having various functional groups at N1 of adenosine ($\gtrless N\rightarrow O$, $\gtrless N^+—OR$, $\gtrless N^+—R$, $\succ N—R$) were inspected. Unexpectedly, adenosine **1** and adenosine *N*-oxide **15** were found to have almost the same ^{15}N chemical shifts, giving no information about the position of oxygen. Wasylishen and Schaefer (7) reported that protonation of azines was accompanied by the decrease of $^2J_{N,H}$ and, in fact, a small value of $^2J_{N1,H\text{-}2}$ (4.9 Hz) in **15** compared to **1** (16 Hz) was observed. Other N1-modified derivatives of adenosine showed a $^2J_{N1,H\text{-}2}$ value of 8.8 Hz, compared to a value of 16 Hz in N^6-substituted adenosine derivatives.

The introduction of positive charge at N1 seemed not to affect the π-electron sextet, as was shown by the ^{15}N chemical shifts of **11**, **12**, and **13**.

We previously reported that zinc bromide coordinates at N1 and N7 to the same extent, as is known by upfield shifts of the ^{15}N signals (9). The present study revealed that both $^2J_{N1,H\text{-}2}$ and $^2J_{N7,H\text{-}8}$ were not altered by coordination of the zinc cation until a salt concentration of 0.5 equivalents,[5] in spite of large upfield shifts of the N1 and N7 signals (N1, $\Delta\delta$ 24; N7, $\Delta\delta$ 18).

The fact that the resonance signal of N7 in **6** was at high field compared to **7** ($\Delta\delta$ 5) led us to the assumption that the bulkiness of the di-*p*-methoxytrityl group at N6 in **6** forced hydrogen bonding between N^6H and N7. In the case of 7-deazaadenosine compounds **19** and **20**, the ^{13}C chemical shifts of C7 were similar (**19**, δ_{C7} 100.0; **20**, δ_{C7} 100.5).

Effects of the substituents at N^6 seemed to be explicable by Hammett's rule: electron-releasing alkyl and electron-withdrawing acyl or trityl groups exerted opposite effects on the electron densities at N^6, N1, and N3. The ^{15}N chemical shifts of N7 and N9 were not altered, showing that the nature of 10π-electron aromatics could hardly be attributed to adenine compounds. Modifications of the imidazole ring as were seen in 8-bromoadenosine and 7-deazaadenosine did not alter the ^{15}N chemical shifts of N^6, N1, and N3.

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[2]$90°_x(^1H)$–τ–$180°_x(^1H)$, (^{13}C)–τ–$90°_y(^1H)$, $90°_\phi(^{13}C)$-acquisition.

[3]$\gamma B_2/2\pi$ = 21 Hz; pulse width 11 ms.

[4]$\gamma B_2/2\pi$ = 16 Hz.

[5]At the concentration of 1.5 equivalents $ZnBr_2$, all of the $^2J_{N,H}$ values were found to decrease to the same extent (10–20%), the reason being unknown.

TABLE 1. ^{15}N chemical shifts and spin coupling constants of adenosine derivatives[a]

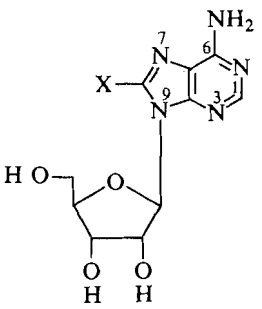
Compound	N1	N3	N7	N9	N^6
1	215.3 (15.6)[b]	202.6 (14.6)	219.5 (11.7)	150.3 (8.8)	62.5 (90.3)
2	218.6 (16.6)	201.9 (15.6)	[c]	[c]	65.4 (90.4)
3	217.6 (18.6)	204.1 (15.6)	222.6 (12.7)	151.7 (8.8)	63.3 (90.3)
4	218.4 (15.6)	203.3 (15.6)	[c]	[c]	64.6 (90.3)
5	214.1 (18.6)	201.5 (15.6)	[c]	[c]	59.5 (95.7)
6	216.4 (16.6)	205.1 (15.6)	223.0 (11.7)	151.9 (8.8)	63.1 (90.3)
7	223.8 (18.5)	207.1 (15.6)	217.9 (11.7)	152.5 (8.8)	87.9 (89.8)
8	217.4 (18.6)	204.8 (15.6)	222.6 (11.7)	150.6 (8.8)	62.9 (90.3)
9	240.9 (17.6)	225.3 (16.6)	226.3 (11.7)	150.6 (8.8)	[d]
10	250.0 (15.6)	231.3 (14.6)	221.8 (11.7)	152.0 (8.8)	[c]
11	134.1 (8.8)	206.4 (13.7)	223.6 (11.7)	159.1 (8.8)	—
12	135.7 (8.8)	205.9 (12.7)	[c]	[c]	—
13	193.2 (8.8)	207.6 (12.7)	225.8 (12.7)	160.6 (8.8)	—
14	131.0 (8.8)	186.1 (12.7)	224.5 (11.7)	155.7 (8.8)	—
15	215.9 (4.9)	200.2 (13.7)	219.7 (12.6)	153.0 (8.8)	57.6 (91.3)
16	217.5 (4.9)	200.8 (13.7)	219.5 (12.6)	153.8 (8.8)	—
17			241.9 (12.7)	168.0 (7.8)	84.3 (93.8)
18	211.5 (16.6)	204.2 (15.6)		135.2 (7.8)	—
19	211.4 (15.6)	205.5 (14.6)		135.5 (7.8)	63.5 (89.4)
20	220.8 (18.6)	207.1 (15.6)		134.7 (7.8)	88.0 (89.9)

[a]*Solvent: DMSO-d_6.*

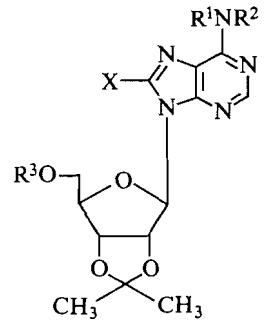
[b]The minus sign for all of the $^2J_{N,H}$ values listed here is quite likely according to literature values, although the sign assignment experiments were not carried out.

[c]No detection of signal based on the principle of INEPT.

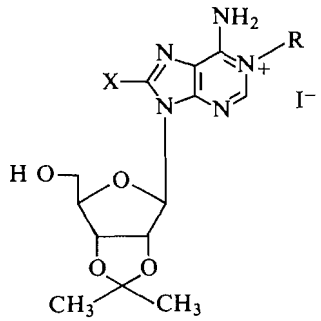
[d]The signals were not obtained.



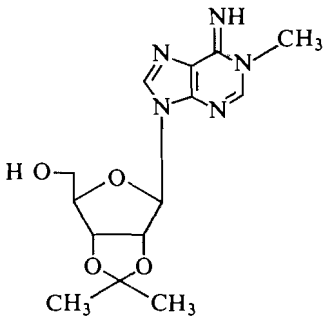
1 X = H
2 X = Br



3 X, R^1, R^2, R^3 = H
4 X = Br, R^1, R^2, R^3 = H
5 X = Br, R^1 = CH_3, R^2, R^3 = H
6 X, R^1, R^2 = H, R^3 = (p-$OCH_3C_6H_4$)$_2C_6H_5$—
7 X, R^1 = H, R^2, R^3 = (p-$OCH_3C_6H_4$)$_2C_6H_5$—
8 X, R^1, R^2 = H, R^3 = p-$CH_3C_6H_4CO$—
9 X, R^1 = H, R^2, R^3 = p-$CH_3C_6H_4CO$—
10 X = H, R^1, R^2, R^3 = p-$CH_3C_6H_4CO$—



11 X = H, R = CH_3
12 X = Br, R = CH_3
13 X = H, R = OCH_3



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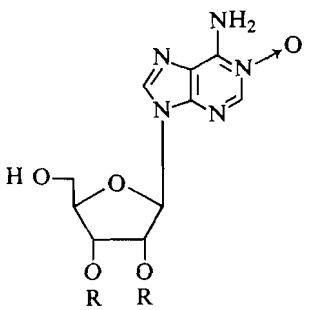
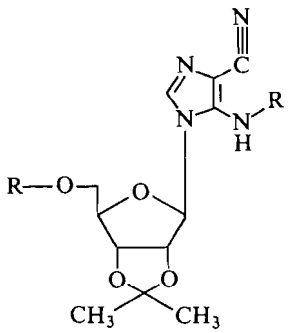
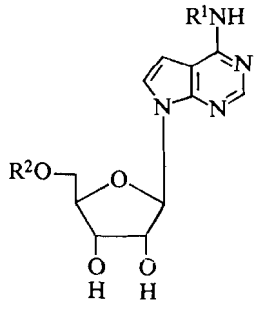


15 R = H
16 R, R = $(CH_3)_2C$



17 R = p-$CH_3C_6H_4CO$—



18 R^1, R^2 = H
19 R^1 = H, R^2 = $(C_6H_5)_3C$—
20 R^1, R^2 = $(C_6H_5)_3C$—

In conclusion, although recent advances in nmr techniques enable us to take ^{15}N nmr spectra at the natural abundance level, the procedure for taking the spectra is still less accessible than for ^{1}H and ^{13}C nmr measurements. However, the laboriousness of taking the spectra may be balanced by the information obtained about the electronic states of nitrogen (lone-pair existence, metal coordination, protonation, and salt formation). Thus, detailed information about the structure of nitrogen compounds is made available, as exemplified in this paper.

Experimental[6]

^{15}N nuclear magnetic resonance measurements

The ^{15}N nmr spectra were recorded on a JEOL FX-100 spectrometer at 10.10 MHz; τ value, 25 ms or 2.8 ms; proton pulse width, 240 μs or 11 ms (for selective INEPT); repetition time, 1.8 s; 15 000–125 000 transients; sample concentration, 0.6–0.1 mol in DMSO-d_6; ^{15}N chemical shifts were measured relative to external standard of aqueous $^{15}NH_4NO_3$.

N^6,5′-O-Bis(4,4′-dimethoxytrityl)-2′,3′-O-isopropylideneadenosine (7)

The isolation of 5′-*O*-(4,4′-dimethoxytrityl)-2′,3′-*O*-isopropylideneadenosine from the reaction mixture of **3** (1.04 g, 5 mmol) and 4,4′-dimethoxytrityl chloride (1.8 g, 5.3 mmol) in pyridine (50 mL) as the main product (yield 1.6 g, 53%) has been reported (9). The presence of two minor products was observed on tlc and the less polar one was isolated by silica gel chromatography (4:1 C_6H_6–EtOAc), yield 370 mg (8%), mp 104–108°C (ligroin); ^{1}H nmr data (100 MHz, $CDCl_3$) δ: 3.72 and 3.76 (2s, 6H, $2CH_3O$). *Anal.* calcd. for $C_{55}H_{53}N_5O_8$: C 72.43, H 5.86, N 7.68; found: C 72.78, H 5.94, N 7.38.

8-Bromo-9-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)-1-methyladeninium iodide (12)

A solution of **4** (386 mg, 1 mmol) and CH_3I (1 mL) in DMF (20 mL) was left standing at room temperature for 5 days. The solvent was removed by evaporation to obtain a solid, which was dissolved in hot PrOH. A powder decomposing in the range 290–300°C separated when this solution was left in a refrigerator, yield 405 mg (77%); ^{1}H nmr data (100 MHz, DMSO-d_6) δ: 1.40 and 1.62 (2s, 6H, two isopropylidene methyls), 3.84 (s, 3H, N1-methyl), 6.14 (d, 1H, H-1′, J = 3 Hz), 8.16 and 8.68 (2s, 2H, H-8 and H-2). *Anal.* calcd. for $C_{14}H_{19}N_5O_4BrI$: C 31.83, H 3.63, N 13.26, I 24.03; found: C 32.08, H 3.68, N 13.44, I 23.64.

8-Bromo-2′,3′-O-isopropylidene-N^6-methyladenosine (5)

A solution of **7** (528 mg, 1 mmol) in 0.1 *M* NaOH (20 mL) was left standing at room temperature overnight to give a precipitate, which was crystallized from toluene, yield 300 mg (75%), mp 175–177°C; ^{1}H nmr data (100 MHz, $CDCl_3$) δ: 1.43 and 1.72 (2s, 6H, two isopropylidene methyls), 3.40 (d, 3H, $HNCH_3$, J = 5 Hz), 6.10 (d, 1H, H-1′, J = 4 Hz), 8.35 (s, 1H, H-2). *Anal.* calcd. for $C_{14}H_{18}N_5O_4Br$: C 42.15, H 4.52, N 17.46; found: C 42.10, H 4.45, N 17.46.

2′,3′-O-Isopropylidene-1-methyladenosine (14)

This compound has already been synthesized (10) and the following is our method of preparation. The adeninium iodide **11** (449 mg, 1 mmol) was dissolved in 0.3 *M* NaOH (40 mL) and the product was immediately extracted with $CHCl_3$. The solvent was removed by evaporation and the residue was crystallized from toluene, yield 370 mg (87%), mp 180–181°C; ^{1}H nmr data (100 MHz, $CDCl_3$) δ: 1.42 and 1.68 (2s, 6H, two isopropylidene methyls), 3.60 (s, 3H, $=N—CH_3$), 5.81 (d, 1H, H-1′, J = 3 Hz), 7.72 and 7.80 (2s, 2H, H-8 and H-2). *Anal.* calcd. for $C_{14}H_{19}N_5O_4$: C 52.33, H 5.92, N 21.80; found: C 52.27, H 5.94, N 21.73.

5′-O-Trityltubercidin (19) and N^6,5′-O-ditrityltubercidin (20)

To a solution of **18** (2.66 g, 10 mmol) in pyridine (50 mL) was added trityl chloride (5.57 g, 20 mmol) portionwise. After leaving it at room temperature for 2 days, the solution was poured into an ice-cooled solution of $NaHCO_3$. The products were extracted with $CHCl_3$ and separated on a silica gel column. Compound **20** was eluted with EtOAc and obtained as a glass from EtOAc–ligroin, yield 1.63 g (22%); uv λ_{max} (MeOH): 283 nm (ε 17 600); ^{1}H nmr data (100 MHz, DMSO-d_6) δ: 3.2 (m, 2H, H-5′), 6.10 (d, 1H, H-1′, J = 4 Hz), 7.76 (s, 1H, H-2), 8.1 (s, 1H, NH, quenched with D_2O); ^{13}C nmr data (25 MHz, DMSO-d_6) δ: 87.3 (C1′), 73.7 (C2′), 70.3 (C3′), 82.4 (C4′), 64.1 (C5′), 149.9 (C2 and C4), 104.9 (C5), 155.4 (C6), 100.5 (C7), 121.6 (C8), 86.1 (Ph_3C—O), 70.3 (Ph_3C—N), 143.6 (phenyl C1 of Ph_3C—O), 145.4 (phenyl C1 of Ph_3C—N). *Anal.* calcd. for $C_{49}H_{42}N_4O_4$: C 78.37, H 5.64, N 7.46; found: C 78.15, H 5.42, N 7.22.

Compound **19** was eluted with 9:1 EtOAc–MeOH. The crystals precipitated during concentration of the eluate were washed with benzene, yield 2.9 g (57%), mp 210–212°C ($CHCl_3$–benzene); uv λ_{max} (MeOH): 270 nm (ε 13 500); ^{1}H nmr data (100 MHz, DMSO-d_6) δ: 3.2 (m, 2H, H-5′), 6.14 (d, 1H, H-1′, J = 4 Hz), 8.10 (s, 1H, H-2), 7.05 (s, 2H, NH_2, quenched with D_2O); ^{13}C nmr data (25 MHz, DMSO-d_6) δ: 87.3 (C1′), 73.7 (C2′), 70.4 (C3′), 82.2 (C4′), 64.0 (C5′), 151.8 (C2), 150.3 (C4), 102.8 (C5), 157.5 (C6), 100.0 (C7), 121.3 (C8), 86.1 (Ph_3C—O), 143.6 (phenyl C1 of Ph_3C—O). *Anal.* calcd. for $C_{30}H_{27}N_4O_4$: C 70.99, H 5.36, N 11.04; found: C 70.69, H 5.22, N 10.89.

[6]For the preparation of **13**, **15**, and **16** see refs. 11, 13, and 14, respectively. The present authors have reported the synthesis of **6**, **8**, **9**, **10**, and **17** (9, 12).

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