[Chem. Pharm. Bull. 31(11)4130—4134(1983)]

Studies on the Chemical Synthesis of Potential Antimetabolites. XXXIV.[1] Apparent Discrepancy among Reported Proton Nuclear Magnetic Resonance Spectra of 3-Deazaadenosine Is Actually a Reflection of a Difference in Molecular Species

TOKUO ITOH,* TOYOAKI ISHIKURA, and YOSHIHISA MIZUNO

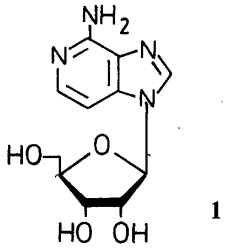
Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-ku, Sapporo 060, Japan

(Received April 4, 1983)

It was shown that the remarkable discrepancy among reported proton nuclear magnetic resonance (^{1}H NMR) spectra of 3-deazaadenosine obtained under comparable conditions is actually a reflection of a difference in molecular species, one being neutral and another being cationic, by carefully measuring the ^{1}H NMR spectra of 3-deazaadenosine and its hydrochloride as well as 3-deazaadenine derivatives, whose preparation is also described.

Keywords——3-deazaadenosine; ^{1}H NMR; molecular species; *N*-methyl-3-deazaadenine; *N*-glycosyl-3-deazaadenine

From the biomedical and physiological points of view, 3-deazaadenosine (**1**) is one of the most intriguing analogues among a large number of modified adenosines[2,3] as regards possible inhibitory activity against *S*-adenosylhomocysteinase or oncogenic transformation caused by several viruses.



1

Chart 1

Chemical synthesis of **1** has been achieved by several groups *via* closely related but definitely different approaches.[4-6] However, as already pointed out,[7] the reported proton nuclear magnetic resonance (^{1}H NMR) spectra obtained under comparable conditions (regarding concentration, temperature and so on) of putatively the same nucleoside (**1**) exhibited quite different patterns, which could be classified into two categories (hereafter referred to as type I and type II, see Table I).[4-6,8] As shown, the discrepancy exists, above all, in the chemical shifts due to H-3, H-8, 6-NH_2, and the anomeric proton.

In view of the possible importance of **1** in biomedical and physiological studies, we felt it urgent to solve this problem. This paper resolves the reported discrepancies and also deals with some related subjects.

Synthesis of 3-Deazaadenosine Derivatives

9-β-D-Arabinofuranosyl-9*H*-3-deazaadenine (**3**) was derived from 6-chloro-9-β-D-ribofuranosyl-9*H*-3-deazapurine (**2**) according to Sugawara *et al.*[9] 7-β-D-Ribofuranosyl-7*H*-

TABLE I. Reported ^{1}H NMR Chemical Shifts and Coupling Constants of **1** in Dimethylsulfoxide-d_6

	Internal standard	H-2	H-3	H-8	6-NH_2	H-1′	H-2′	H-3′	H-4′	H-5′
Type I										
Kitano *et al.*[8]	TMS	7.79 d ($J_{2,3}$=7.1 Hz)	7.46 d	8.72 s	8.59 s	5.98 d ($J_{1',2'}$=5.9 Hz)	4.33 t	4.19 t	4.07 q	3.67 m 3.71 m
May *et al.*[5a]	DSS	7.83 dd ($J_{2,3}$=3.5 Hz)	7.47 d	8.77 s	8.63 s	5.99 d ($J_{1',2'}$=3.0 Hz)	Not given			
Type II										
Montgomery *et al.*[6]	TMS	7.7 d ($J_{2,3}$=6.0 Hz)	6.9 d	8.3 s	6.2 s	5.8 d ($J_{1',2'}$=6.1 Hz)	4.3 m	4.1 m	4.0 m	3.6 m

TMS=tetramethylsilane; DSS=sodium 2,2-dimethyl-2-silapentane-5-sulfonate.

3-deazaadenine (**5**) was prepared from the corresponding 6-chloro-nucleoside (**4**)[10] by the hydrazine–Raney nickel procedure.

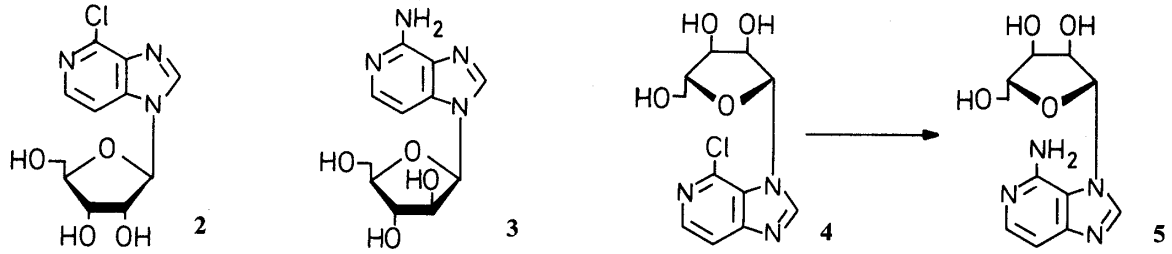


Chart 2

9-Methyl-9*H*- (**9**) and 7-methyl-7*H*-3-deazaadenine (**10**) were synthesized as shown in Chart 3. Thus, methylation of 6-chloro-3-deazapurine (**6**) with dimethyl sulfate in the presence of potassium carbonate gave rise to an isomeric mixture of **7** and **8**, which were

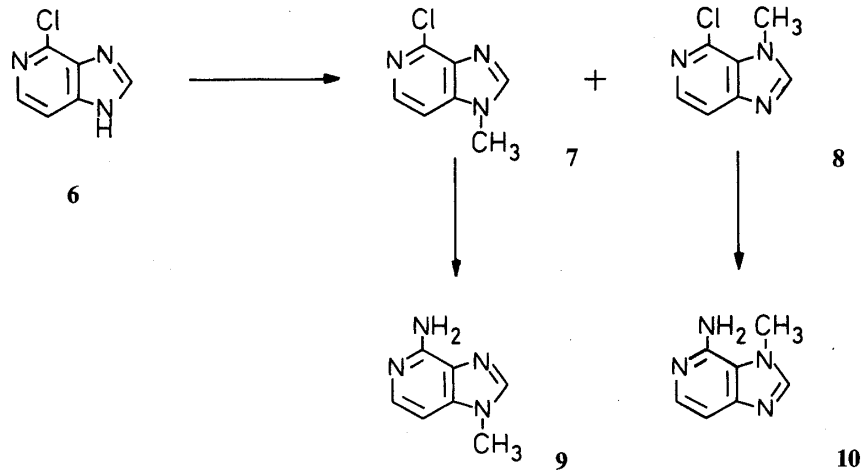


Chart 3

separated by silica gel chromatography. The sites of methylation were elucidated by the comparison of their ultraviolet (UV) spectra with those of 9-β-D-ribofuranosyl-9*H* (**2**) and 7-β-D-ribofuranosyl-7*H* (**4**) derivatives. Conversion of **7** and **8** to the corresponding 6-amino

compounds (**9** and **10**) was achieved by treatment with hydrazine, followed by Raney nickel reduction.

Results and Discussion

Clearly the discrepancy in ^{1}H NMR spectra between samples of **1** prepared by different approaches does not arise from positional isomerism, because the UV absorption spectra of the samples are superimposable on each other and are almost the same as that of **9**. In addition, they are definitely different from those of 7-substituted 3-deazaadenines (**5** and **10**), whose absorption maxima both appear at longer wavelength than do those of **1**.

During the course of tackling this perplexing problem, we repeated the synthesis of **1** (putative 3-deazaadenosine) according to Mizuno *et al.*[4] It turned out, however, that the nucleoside (hereafter referred to as **1a**) which was obtained after recrystallization from isopropanol–water is the hydrochloride of **1** rather than **1** itself, whose structural determination was based on its combustion values. In addition, the ^{1}H NMR spectrum was found to be virtually identical the reported type I spectra (see Table II). This observation prompted us to prepare an authentic sample of **1** to determine its ^{1}H NMR spectrum; **1a** was treated with Dowex 1 × 2 (OH^-) to give a sample (referred to as **1b**), whose combustion values were compatible with those calculated for the free nucleoside. The sample **1b** showed a ^{1}H NMR spectrum which was identical with reported type II spectra.

Accordingly, the apparent discrepancy among the reported ^{1}H NMR spectra of 3-deazaadenosine prepared by different routes is actually a reflection of a difference in molecular species. To substantiate this conclusion, mixed ^{1}H NMR spectra of **1a** and **1b** were determined. As shown in Table II, the chemical shifts in question (H-3, H-8, 6-NH_2, and H-1′) tend to converge to the corresponding values of the pure species as the composition ratio

Table II. ^{1}H NMR Spectra of **1a**, **1b**, and Mixed Samples Thereof in Dimethylsulfoxide-d_6

	Ratio of **1a**/**1b**	H-2	H-3	H-8	6-NH_2	H-1′	H-2′	H-3′	H-4′	H-5′
1a	(10/0)	7.74 d	7.41 d	8.67 s	8.57 s	5.91 d	4.27 q	4.11 q	4.01 q	3.64 m
		($J_{2,3}$ = 7.1 Hz)				($J_{1',2'}$ = 6.2 Hz)				
	(8/2)	7.72 d	7.32 d	8.59 s	8.02 s	5.89 d	4.28 q	4.12 q	4.01 q	3.64 m
	(4/6)	7.67 d	7.09 d	8.43 s	7.12 s	5.80 d	4.29 q	4.09 q	3.96 q	3.62 m
1b	(0/10)	7.64 d	6.89 d	8.27 s	6.15 s	5.73 d	4.30 q	4.07 q	3.93 q	3.61 m
		($J_{2,3}$ = 5.9 Hz)				($J_{1',2'}$ = 6.3 Hz)				

Table III. ^{1}H NMR Spectra of **3** and **9** and Their Hydrochlorides in Dimethylsulfoxide-d_6

		H-2	H-3	H-8	6-NH_2	H-1′	H-2′	H-3′	H-4′	H-5′	CH_3
3	Free	7.62 d	6.80 d	8.16 s	6.08 s	6.10 d	4.13 q	4.06 q	3.73 m	3.61 m	
		($J_{2,3}$ = 5.9 Hz)				($J_{1',2'}$ = 4.9 Hz)					
	HCl salt	7.72 d	7.27 d	8.61 s	8.41 s	6.31 d	4.25 q	4.10 q	3.82 m	3.71 m	
		($J_{2,3}$ = 7.0 Hz)				($J_{1',2'}$ = 4.9 Hz)					
9	Free	7.68 d	6.77 d	8.00 s	6.07 s						3.75 s
		($J_{2,3}$ = 5.8 Hz)									
	HCl salt	7.75 d	7.25 d	8.41 s	8.44 s						3.89 s
		($J_{2,3}$ = 7.1 Hz)									

approaches zero.

Similar situations were noted with the spectra of 9-β-D-arabinofuranosyl-9*H*- (**3**) and 9-methyl-9*H*-3-deazaadenine (**9**).

In summary, the ^{1}H NMR spectra reported by Kitano *et al.*[8] and May *et al.*[5a] are those of 3-deazaadenosine hydrochloride, and it is likely that data obtained with "3-deazaadenosine" prepared by Mizuno *et al.*[4] and May *et al.*[5a] are those of the hydrochloride rather than the free nucleoside.

Experimental

Melting points were determined on a Yamato melting point apparatus, type MP-1, and are uncorrected. UV absorption spectra were taken on a Hitachi 323 recording spectrometer. ^{1}H NMR spectra were taken on a JEOL FX-200 spectrometer using 3% (w/v) solution at ordinary probe temperature (*ca.* 25 °C): signals are designated as s (singlet), d (doublet), t (triplet or pseudo-triplet), q (quartet or pseudo-quartet), or m (multiplet). Mass spectra (MS) were taken on a JEOL JMS D-300 mass spectrometer.

3-Deazaadenosine Hydrochloride (1a)——Compound **1a** was prepared from **2** according to Mizuno *et al.*[4] A crude sample was crystallized from isopropanol–water. UV $\lambda_{max}^{H_2O}$ nm: (pH 1) 261.5; (pH 11) 266. *Anal.* Calcd for $C_{11}H_{14}N_4O_4 \cdot HCl$: C, 43.65; H, 5.00; Cl, 11.79; N, 18.51. Found: C, 43.52; H, 5.03; Cl, 11.49; N, 18.51.

3-Deazaadenosine (1b)——Compound **1a**, prepared from **2** (196 mg), was dissolved in water (3 ml) and chromatographed on a Dowex 1×2 (OH^-) column with 40% methanol as an eluent. Concentration of the main fraction gave a crude sample of **1b**, which was recrystallized from water to give needles, mp 224—226 °C. UV $\lambda_{max}^{H_2O}$ nm: (pH 1) 261.5; (pH 11) 266. *Anal.* Calcd for $C_{11}H_{14}N_4O_4$: C, 49.62; H, 5.30; N, 21.04. Found: C, 49.61; H, 5.33; N, 20.94.

7-β-D-Ribofuranosyl-7*H*-3-deazaadenine (5)——A hydrazine hydrate (5 ml) solution of **4** (18 mg) was heated at 100 °C for 1 h under a nitrogen atmosphere. Concentration of the reaction mixture *in vacuo* gave the 6-hydrazino derivative [$\lambda_{max}^{H_2O}$ nm: (pH 7) 272], which was dissolved in water (5 ml). Raney nickel (*ca.* 20 mg) was added to the solution and the mixture was refluxed for 1 h under a nitrogen atmosphere. The catalyst was filtered off and washed with water (2 ml). Evaporation of the water gave a crude sample of **5**, which was crystallized from water (0.5 ml) containing a drop of concentrated NH_4OH to give **5** (10 mg), mp 236—237 °C. UV $\lambda_{max}^{H_2O}$ nm: (pH 1) 243, 294; (pH 7), 245, 290; (pH 11) 245, 289. MS *m/z*: 266 (M^+), 135 (B+2H), 134 (B+H). ^{1}H NMR (DMSO-d_6) δ: 8.40 (1H, s, H-8), 7.69 (1H, d, J=5.9 Hz, H-2), 6.90 (1H, d, J=5.9 Hz, H-3), 5.94 (2H, br s, 6-NH_2), 5.89 (1H, d, J=6.8 Hz, H-1′), 5.52 and 5.28 (each 1H, each d, OH), 5.16 (1H, t, 5′-OH), 4.26 (1H, q, H-2′), 4.09 (1H, q, H-3′), 3.97 (1H, q, H-4′), 3.62 (2H, m, H-5′). *Anal.* Calcd for $C_{11}H_{14}N_4O_4$: C, 49.62; H, 5.30; N, 21.04. Found: C, 49.50; H, 5.32; N, 20.88.

6-Chloro-9-methyl-9*H*-deazapurine (7) and 6-Chloro-7-methyl-7*H*-3-deazapurine (8)——To a suspension of **6** (1.06 g, 6.95 mmol) in *N,N*-dimethylformamide (DMF) (30 ml) was added 1 N $NaOCH_3$ (7 ml, 7 mmol) and dimethyl sulfate (0.88 g, 7 mmol), and the mixture was stirred at 50 °C for 4 h. Evaporation of the solvent *in vacuo* gave a white residue, which was partitioned between chloroform (30 ml) and water (10 ml). The aqueous solution was extracted with two 15-ml portions of chloroform. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to give a mixture of methylated products (0.70 g), which were chromatographed on a silica gel column (column size, 1.2 cm × 20 cm) with chloroform as an eluent. The column was washed with 140 ml of the eluent, then **8** was eluted out. Concentration of the fraction (*ca.* 150 ml) containing **8** left crystals (324 mg, 27.4%), mp 150—151 °C. UV $\lambda_{max}^{H_2O}$ nm: (pH 1) 243, 250 sh, 273, 279, 287 sh; (pH 7 and 11) 238 sh, 244, 250 sh, 279.5, 287 sh. MS *m/z*: 167/169 (M^+), 132 (M−Cl). ^{1}H NMR ($CDCl_3$) δ: 8.20 (1H, d, J=5.9 Hz, H-2), 7.96 (1H, s, H-8), 7.64 (1H, d, J=5.9 Hz, H-3), 4.18 (3H, s, CH_3). *Anal.* Calcd for $C_7H_6ClN_3$: C, 50.16; H, 3.61; Cl, 21.16; N, 25.06. Found: C, 50.15; H, 3.57; Cl, 21.25; N, 25.11.

From the subsequent fraction (*ca.* 200 ml), 288 mg (24.7%) of **7** was obtained after evaporation, mp 129 °C. UV $\lambda_{max}^{H_2O}$ nm: (pH 1) 259, 267 sh, 273.5 sh; (pH 7 and 11) 258, 267 sh, 273.5. MS *m/z*: 167/169 (M^+), 132 (M−Cl). ^{1}H NMR ($CDCl_3$) δ: 8.25 (1H, d, J=5.9 Hz, H-2), 7.98 (1H, s, H-8), 7.32 (1H, d, J=5.9 Hz, H-3), 3.90 (3H, s, CH_3). *Anal.* Calcd for $C_7H_6ClN_3$: C, 50.16; H, 3.61; Cl, 21.16; N, 25.06. Found: C, 50.05; H, 3.70; Cl, 20.96; N, 24.88.

9-Methyl-9*H*-3-deazaadenine (9)——Compound **7** (46 mg) was dissolved in hydrazine hydrate (2 ml) and the mixture was heated at 100 °C for 1 h under a nitrogen atmosphere. Concentration of the reaction mixture gave a crude sample of 6-hydrazino-9-methyl-9*H*-3-deazapurine, which was dissolved in water (10 ml). Raney nickel (*ca.* 40 mg) was added to the solution and the mixture was refluxed for 1 h under a nitrogen atmosphere. After filtration of the catalyst, the reaction mixture was concentrated *in vacuo* to give a crude sample of **9** hydrochloride. *Anal.* Calcd for $C_7H_8N_4 \cdot HCl \cdot H_2O$: C, 41.47; H, 5.47; Cl, 17.50; N, 27.65. Found: C, 41.06; H, 5.63; Cl, 17.22; N, 27.79. The hydrochloride salt was dissolved in water (5 ml). After addition of two drops of concentrated NH_4OH, the solution was shaken with three 5-ml portions of chloroform. The dried ($MgSO_4$) extract was concentrated *in vacuo* to give a crude sample of **9** (15 mg, 41%), which was recrystallized from water, mp 224—228 °C. UV $\lambda_{max}^{H_2O}$ nm: (pH 1) 263—270

(flat peak); (pH 11) 266. MS *m/z*: 148 (M^+). *Anal.* Calcd for $C_7H_8N_4$: C, 56.72; H, 5.44; N, 37.72. Found: C, 56.52; H, 5.52; N, 37.82.

7-Methyl-7*H*-3-deazaadenine (10)——Compound **8** (47 mg) was converted to **10** (21 mg, 50%) as described in the preparation of **9**, mp 186—190 °C. UV $\lambda_{max}^{H_2O}$ nm: (pH 1) 260 sh, 289; (pH 11) 250, 284. MS *m/z*: 148 (M^+). ^1H NMR (DMSO-d_6) δ: 8.03 (1H, s, H-8), 7.60 (1H, d, J=5.7 Hz, H-2), 6.84 (1H, d, J=5.7 Hz, H-3), 5.90 (2H, br s, 6-NH_2), 4.00 (3H, s, CH_3). *Anal.* Calcd for $C_7H_8N_4$: C, 56.72; H, 5.44; N, 37.72. Found: C, 56.35; H, 5.50; N, 37.55.

Acknowledgement This research was supported in part by a Grant-in-Aid for Special Project Research (Grant No. 56470014) from the Ministry of Education, Science, and Culture, to whom we are indebted. We also thank the staff of the Analytical Center of Hokkaido University for valuable assistance in the analytical work.

References and Notes

1) Part XXXIII: Y. Mizuno, K. Tsuchida, and H. Tampo, *J. Org. Chem.*, "submitted."
2) R. T. Borchardt, *J. Med. Chem.*, **23**, 347 (1980).
3) J. W. Daly, *J. Med. Chem.*, **25**, 197 (1982).
4) Y. Mizuno, S. Tazawa, and K. Kageura, *Chem. Pharm. Bull.*, **16**, 2011 (1968).
5) *a*) J. A. May, Jr. and L. B. Townsend, *J. Chem. Soc., Perkin Trans. 1*, **1975**, 125; *b*) R. J. Rousseau, L. B. Townsend, and R. K. Robins, *Biochemistry*, **5**, 756 (1966).
6) J. A. Montgomery, A. T. Shortnacy, and S. D. Clayton, *J. Heterocycl. Chem.*, **14**, 195 (1977).
7) J. P. Horwitz and T. Endo, personal communication.
8) S. Kitano, Y. Mizuno, M. Ueyama, K. Tori, M. Kamisaku, and K. Ajisaka, *Biochem. Biophys. Res. Commun.*, **64**, 996 (1975).
9) T. Sugawara, T. Ishikura, T. Itoh, and Y. Mizuno, *Nucleosides & Nucleotides*, **1**, 239 (1982).
10) Y. Mizuno, T. Itoh, and K. Saito, *J. Org. Chem.*, **29**, 2611 (1964).