The Isomerization of Purine Nucleosides in a Dilute Alkaline Solution

Yoshihisa SUZUKI and Setsuko YATABE

Central Research Laboratories, Ajinomoto Co., Inc., Suzuki-cho, Kawasaki 210

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A further study of the isomerization of inosine (**1a**) in a dilute alkaline solution has revealed the formation of α-pyranosyl isomer (**4a**) and its β-isomer (**3a**). *Adenosine (**1b**) was isomerized at 130 °C for 12 hr at pH 10,* giving α-furanosyl (**2b**) (2% yield), β-pyranosyl (**3b**) (11%), and the α-pyranosyl isomer (**4b**) (0.8%), respectively; 65% of the **1b** also remained. 9-β-D-Ribopyranosyl-6-mercaptopurine (**3c**) was prepared through this reaction from the corresponding β-furanoside (**1c**). Arabinosyl or xylosyl purine nucleosides were also isomerized, whereas neither 2′-deoxy nor 2′-substituted nucleoside showed any clear evidence of isomerization. In conclusion, the purine base and the 2′-hydroxyl anion are essential to the alkaline isomerization of nucleosides. By means of NMR, both **4a** and **4b** were found to have a 1C conformation, while **3a** and **3b** have a C1 conformation.

The isomerization of inosine (**1a**) in a dilute alkaline solution (pH 10), giving 9-β-D-ribopyranosyl hypoxanthine (**3a**), has been reported in the preceding paper.[1] This paper will describe the isolation of 9-α-D-ribopyranosyl hypoxanthine (**4a**) from the same mixture as that described in the preceding paper, and will further describe the isomerization of adenosine (**1b**) and other purine nucleosides under the standard conditions at 130 °C for 6 hr at pH 10 (Chart 1).

The action of alkali on some purine nucleosides was examined by Jones *et al.*[2] in 1 M NaOH at 100 °C for 1 hr; they found that **1a** was extremely stable under their conditions and that **1b** gave two degraded compounds (adenine and 4,5,6-triaminopyrimidine) and one deaminated nucleoside, **1a**. Garret and Mehta[3] examined the solvolysis of adenine nucleosides in 0.05—1.0 M NaOH at 60—80 °C and obtained similar products. Neither of these groups, however, found any isomerization. On the basis of what we found, it does not occur under their conditions (pH values over 13 and temperatures below 100 °C); a more diluted alkali and a temperature higher than 100 °C are essential for isomerization.

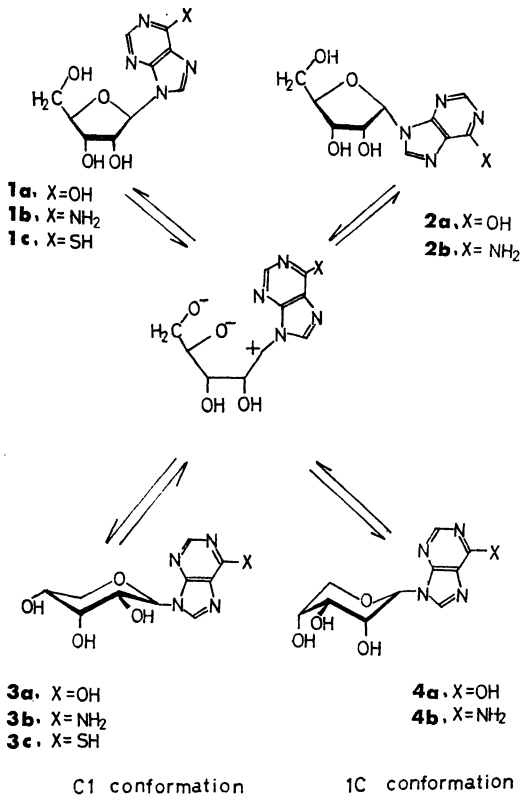

Chart 1.

The action of alkali on pyrimidine nucleosides was examined by Jones *et al.*[4] under the same conditions as with purine nucleosides; they found that thymidine is quite stable, that some amounts of urea and ammonia are formed from uridine, and that a considerable degree of deamination takes place for cytidine. These pyrimidine nucleosides did not show any clear evidence of isomerization even under the conditions where the isomerization of purine nucleosides takes place. On the other hand, an isomerization of an abnormal pyrimidine nucleoside, pseudouridine, was found in acid and in alkali by Chambers *et al.*[5]

Although both α- and β-pyranosyl anomers of pseudouridine were illustrated in the C1 form by Thomasz *et al.*,[6] the α-pyranosyl anomers of adenosine (**4b**) and **4a** were found to be in the 1C conformation, and their β-pyranosyl anomers, to be in the C1 form.

These alkaline isomerizations will be useful for the synthesis of various analogs of nucleic acid components.

Results and Discussion

Identification of 4a. Under the standard conditions, **1a** was treated in a KOH aqueous solution. The reaction mixture was fractionated by use of a column of Diaion SK1B-S, as is illustrated in Fig. 1. The I-1 and I-2 fractions are assignable to **3a** and **1a** respectively on the basis of our previous study.[1]

The I-4 fraction was found by means of tlc to be a mixture of hypoxanthine and 5-amino-1-β-D-ribofuranosyl-4-imidazolecarboxamide (AICA-riboside); this is consistent with our previous results. The new fraction, I-3, which was not clearly detected in the previous experiments,[1] was found to be a mixture of two other components: hypoxanthine and another compound which is similar to **1a** in its behavior in tlc and UV spectroscopy. The former is suspected to be a degraded product of the latter in the isolation procedure. The latter reacts with the orcinol reagent; therefore, its ribose moiety is intact. This compound was separated from hypoxanthine by means of the acetylation of the ribose moiety, followed by the extraction of the acetylated compound with dichlormethane and then deacetylation.

Table 1. The analytical data on the four isomers of 9-ribosyl adenine

Compound	R_f[a]	Mp[b] (°C)	UV maximum 0.1 M HCl	UV maximum H_2O	UV maximum 0.1 M NaOH	$[\alpha]_D$ in H_2O[c]	NMR[d] H-1′	$J_{1'2'}$, Hz
A-1	0.37	142—3 278—82[e]	257	259.5	260	−61.7 (*c*, 0.4)	5.79 d	0—1
A-2	0.32	249	257.5	260	260	−37 (*c*, 0.4)	5.74 d	8—9
A-3	0.46	130—1 225—6[e]	259	260	260	+21.5 (*c*, 1.1)	6.3 d	5.2
A-4	0.46	234—5	257.5	260	260	−59 (*c*, 0.2)	5.9 d	6.1
4b	—	—	—	—	—	— —	—	—
3b	—	254[9] 250—4[12]	258.5[12]	—	260	−37 (*c*, 0.6)[9] −32.4 (*c*, 0.28)[12]	5.72 d[12]	9.0[12]
2b	—	201[11]	257[10]	259[10]	260[10]	+24 (*c*, 0.65)[11]	6.38 d[10]	4.8[10]
1b	—	233—4[10]	257[10]	259[10]	—	−60.4 (*c*, 1.0)[10,11]	6.02 d[10]	5.9[10]

a) Tlc in solvent system *n*-BuOH–AcOH–H_2O (4 : 1 : 1). b) For the compound crystallized from water and uncorrected. c) At room temperature. d) DMSO-d_6. e) For the compound crystallized from ethanol and uncorrected.

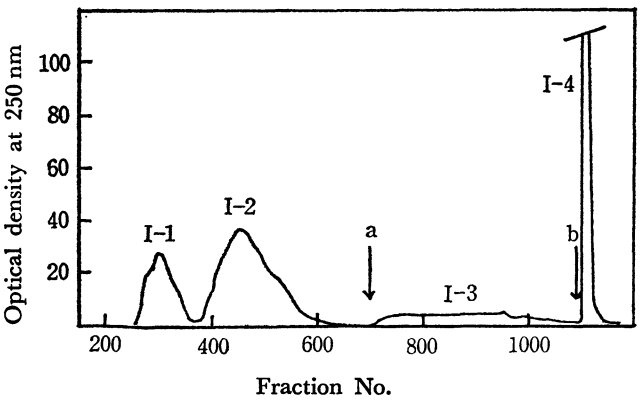

Fig. 1. The elution of UV-absorbing compounds contained in alkaline-treated inosine from the Diaion SK1B-S column with water followed 0.1 M NH_4Cl(a) and 0.1 M NH_4OH(b).
I-1 : 9-β-D-Ribopyranosyl hypoxanthine (**3a**).
I-2 : Inosine (**1a**).
I-3 : 9-α-D-Ribopyranosyl hypoxanthine (**4a**) and hypoxanthine.
I-4 : AICA-riboside and hypoxanthine.

The compound now in question has been found to be **4a** in the following way: On the thin-layer plate this compound gave a spot between those of **1a** and **3a**. On paper chromatography (PC), the acid hydrolysate gave one UV-absorbing spot (the R_f value agreed with that of authentic hypoxanthine), and also one positive spot with a periodate reagent (the R_f value agreed with that of authentic D-ribose). The UV spectrum was similar to that of **1a**, and after acid hydrolysis it came into accord with that of authentic hypoxanthine. These facts suggested that the compound was 9-α-D-ribosyl hypoxanthine. The optical rotation in water was −50°. Martinez *et al.*[7] reported the amounts of the optical rotation of four isomers of arabinosyl adenine. By comparison with their data, this compound was assumed to be α-pyranoside rather than α-furanoside because the latter is expected to have an opposite sign or at least a levorotatory value smaller than the −50° actually observed. The measurements of the NMR spectra supported this assumption. When the compound is acetylated, the signals in CD_3OD display the acetylation shifts for H-2′, 3′, and 4′. This fact means that three hydroxyl groups are attached, at C-2′, 3′, and 4′ respectively, and that the ribose ring has a pyranosyl structure. Signals appeared at 4.01 δ (J_{AB}=14 Hz, J_{AX}=2 Hz) and 4.28 δ (J_{AB}=14 Hz, J_{BX}=2 Hz), indicating that two protons of H-5′ and one proton of H-4′ are in the *gauche* relation around the C-4′—C-5′ bond. Thus, the pyranose ring was determined to have the 1C conformation. The small value of $J_{1'2'}$ (0—1 Hz) also explains well that the compound is the α-pyranoside in the 1C conformation.[8]

Thus, α-pyranoside of inosine has been found in the reaction mixture; therefore, the concomitant formation of α-furanoside is considered to be probable. However, this was not satisfactorily detected by the column chromatography. Presumably the amount is too small to be detected.

Identification of Four Isomers of Adenosine. **1b** was treated at 130 °C for 12 hr in a KOH aqueous solution whose pH was kept at 9—10. The reaction mixture gave three major spots on the thin-layer plate, as has been reported before.[1] They were suspected to be adenine, unreacted **1b**, and the β-pyranosyl isomer (**3b**) on the basis of the known results in the case of inosine. The reaction products were fractionated by a column of Dowex-1 with an eluting reagent of 25% methanol (Fig. 2). Four fractions were obtained, but adenine was not eluted from the column. Each of the four compounds from the four fractions were confirmed to be adenine nucleosides through some analytical tests such as orcinol reaction, UV spectrometry, and acid hydrolysis. The results of the individual identifications are summarized in Table 1 in comparison with the known data in the literature. In spite of a lack of available data on **4b** in the literature, the optical rotation datum of A-1 made it reasonable to identify it as **4b**, in comparison with the data on 9-D-arabinopyranosyl adenine.[1,7]

The conformations of the pyranosyl anomers (**3b** and **4b**) were determined on the basis of the chemical

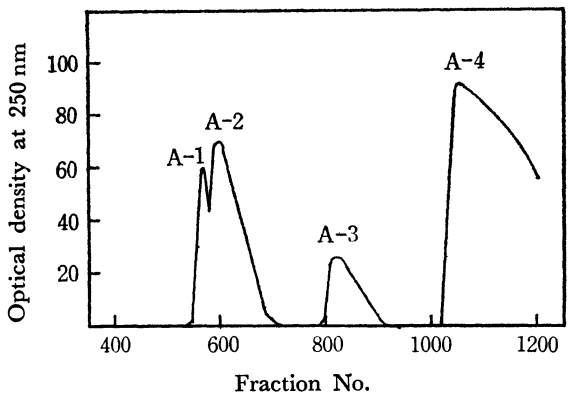

Fig. 2. The elution of UV-absorbing compounds contained in alkaline-treated adenosine from theD owex-1 column with 25% methanol.
A-1 : 9-α-D-Ribopyranosyl adenine (**4b**).
A-2 : 9-β-D-Ribopyranosyl adenine (**3b**).
A-3 : 9-α-D-Ribofuranosyl adenine (**2b**).
A-4 : 9-β-D-Ribofuranosyl adenine (adenosine, **1b**).

shifts in the NMR spectra in DMSO-d_6. An NMR signal of **3b** attributable to the anomeric proton appears at 5.74 δ, and the coupling constant, $J_{1'2'}$, is found to be 8—9 Hz. Since such a large coupling constant could only be expected for an axial-axial interaction between the protons on C-1′ and C-2′, **3b** was unambigously identified as β-ribopyranoside in the Cl conformation as was determined by Pan *et al.*[12] On the other hand, the 1C conformation of **4b** was corroborated on the basis of the signals of two protons for H-5′ after acetylation. The H-5′ and H-4′ protons appear as ABX-type signals at 4.04 δ (J_{AB}= 14 Hz, J_{AX}=2 Hz) and 4.38 δ (J_{AB}=14 Hz, J_{BX}= 2 Hz).

Isolation of ***1a*** *from Alkaline-treated* ***3a***. The isomerization of **3a** to give **1a** was confirmed. The reaction mixture which was obtained by heating **3a** at 150 °C for 6 hr at pH 10 was subjected to column chromatography on a Dowex-50W column. Subsequent elution with 0.001 M NH_4Cl gave two UV-absorbing fractions; the first one is considered to be the unreacted starting material, and the second, **1a**. The crystals obtained from the latter fraction were identified as **1a** on the basis of the R_f value on the tlc and the X-ray diffraction pattern of its anhydrous α-crystal form.[13]

*Preparation of 9-β-D-Ribopyranosyl-6-mercaptopurine (***3c***).* **3c** was obtained through the isomerization of **1c**. The products were fractionated by column chromatography on a Dowex-50W column (Fig. 3). The two UV-absorbing fractions (M-1 and M-2) were obtained upon elution with water. The first was assigned to **3c**, and the second, to the original **1c**, on the basis of an analogy with the fractionation of alkaline-treated inosine. The mobilities of these two fractions on tlc supported these assignments. It is empirically established that the β-pyranosyl isomer shows a little less mobility than that of the corresponding β-furanosyl one on tlc and PC in the *n*-butanol–acetic acid–water (4 : 1 : 1) solvent system. From the M-1 fraction, needle crystals were obtained. The UV spectrum of

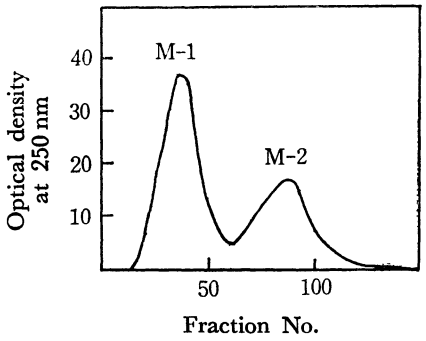

Fig. 3. The elution of UV-absorbing compounds contained in alkaline-treated 9-β-D-ribofuranosyl-6-mercaptopurine from the Dowex-50 W column with water.
M-1 : 9-β-D-ribopyranosyl-6-mercaptopurine (**3c**).
M-2 : 9-β-D-ribofuranosyl-6-mercaptopurine (**1c**).

this compound was found to be similar to that of **1c**. The results of elementary analysis and mass spectrometry after trimethylsililation were consistent with what would be expected for **3c**. An NMR signal of **3c** attributable to the anomeric proton appears at 5.62 δ, with a large coupling constant, $J_{1'2'}$, of 9 Hz; this can be explained unambigously by the fact that **3c** is β-pyranoside in the Cl conformation.

Alkaline Treatment of Various Nucleosides. Tables 2 and 3 summarize the reaction products of various nucleosides with alkali. All the reaction mixtures of adenosine, guanosine, and xantosine gave three major UV-absorbing spots on the chromatograms corresponding to the base, β-pyranoside, and unreacted β-furanoside. Usually too few of the α-isomers of these nucleosides were produced for their spots to be given clearly on the chromatograms.

1a and **1c** exhibit more complicated reactions. They are cloven at C-2 of the purine ring, giving 5-amino-4-imidazolecarboxamide (AICA) derivatives as has been reported in the previous paper. The AICA derivatives are detectable by means of the NBDF-reagent,[1] giving a purple-red color on the chromatogram. Isomerization was observed in competition with these degrading reactions. The substitution also took place in the case of **1c**, giving **1a**.

9-β-D-Arabinofuranosyl adenine and 9-β-D-xylofuranosyl hypoxanthine are also considered to isomerize in alkali in a way similar to that of purine ribonucleosides on the basis of the presence of a characteristic UV-absorbing spot located a little lower than that of the original furanoside on the chromatogram. A change in the sugar moiety from ribose to arabinose or from ribose to xylose of the purine nucleoside does not exert any significant effect on the isomerization.

However, the substitution of the sugar 2′-hydroxyl group serves to prevent the isomerization. 2′-*O*-Methyladenosine gave only adenine and no other UV-absorbing products under the same conditions as when adenosine gives its β-pyranoside. 2′-Deoxyinosine is hydrolyzed completely and gives a large amount of hypoxanthine and a small amount of a NBDF-reagent-positive compound. The latter is considered to be AICA-2′-deoxy-riboside from the fact that λ_{max} in 0.1

Table 2. The reaction products of alkaline treated purine nucleosides[a]

Nucleoside	R_f[b]	Yield(%)[c]	Products
Inosine	0.35	3	Hypoxanthine
	0.31[i]	17	AICA[d]-riboside
	0.22	64	SM[e] **1a**
	0.22	0.7	α-Inosine(P)[f] **4a**
	0.19	11	Inosine(P) **3a**
Adenosine	0.56	20	Adenine
	0.46	58	SM **1b**
	0.46	2	α-Adenosine **2b**
	0.37	0.8	α-Adenosine (P) **4b**
	0.32	10	Adenosine(P) **3b**
6-MP[g]-riboside	0.51[j]	—	AITCA[h]
	0.42[j]	—	AITCA-riboside
	0.30[i]	—	AICA-riboside
	0.27[j]	—	SM **1c**
	0.22[j]	—	6-MP-riboside(P) **3c**
	0.19	—	Inosine
Guanosine	0.24	12	Guanine
	0.19	71	SM
	0.14	8	Guanosine(P)
Xantosine	0.32	2	Xanthine
	0.20	78	SM
	0.13		Xantosine(P)
Arabinosyl adenine	0.56	2	Adenine
	0.39	85	SM
	0.26	13	Arabinosyl adenine(P)
Xylosyl hypoxanthine	0.35	5	Hypoxanthine
	0.27[i]	24	AICA-xyloside
	0.20	47	SM
	0.11	24	Xylosyl hypoxanthine(P)
Inosine(P)	0.41[i]	trace	AICA
	0.34	1	Hypoxanthine
	0.32[i]	1	AICA-riboside
	0.27[i]	22	AICA-riboside(P)
	0.22	5	Inosine
	0.19	68	SM

a) Treated in aq. KOH (pH 10) at 130 °C for 6 hr except adenosine (12 hr) and guanosine (additional 4 hr at 140 °C). b) UV-absorbing spots on tlc. Solvent system, *n*-butanol–acetic acid–water (4 : 1 : 1). c) See experimental section. d) 5-Amino-4-imidazolecarboxamide. e) Starting material remained. f) Pyranoside. g) Mercaptopurine. h) 5-Amino-4-imidazolethiocarboxamide. i) Purple-red color development by NBDF reagent. j) Circle white spot appeared by NBDF reagent.

M NaOH (267.5 nm) is similar to that of AICA-riboside (268 nm) and unlike that of AICA (278.5 nm) and from the fact that the presence of deoxysugar was proved by the method of Buchanan.[14] 2′-Deoxyguanosine was more stable than 2′-deoxyinosine, and no evidence of isomerization was observed. Some guanine and a trace amount of an unknown compound were obtained. 2′,3′-Isopropylidene-inosine gave several spots (Table 3); however, none of them was considered to be responsible for the isomerization.

The isomerization was also significantly affected by a change in the base moiety from purine to pyrimidine or from purine to imidazole. Thymidine was found

Table 3. The reaction product of alkaline treated some nucleosides[a]

Nucleoside	R_f[b]	Yield(%)[c]	Products
2′-Deoxyinosine	0.40[f]	trace	2′-Deoxy-AICA-riboside
	0.36	71	Hypoxanthine
2′-Deoxyguanosine	0.31	75	SM[d]
	0.26	—	Guanine
	0.18	trace	Unknown
	0.11	trace	Unknown
2′-*O*-Methyl-adenosine	0.75	90	SM
	0.63	4	Adenine
Ip[e]-inosine	0.80[f]	30	Ip-AICA-riboside
	0.70	17	SM
	0.39[f]	trace	AICA
	0.34	1	Hypoxanthine
	0.32[f]	trace	AICA-riboside
	0.25	—	Inosine
	0.23[f]	trace	Unknown
AICA-riboside	0.40[f]	1	AICA
	0.31[f]	87	SM
Uridine	0.55	2	Uracil
	0.38	52	SM
Cytidine	0.55	—	Uracil
	0.38	—	Uridine
	0.34	—	SM
Thymidine	0.70	trace	Thymine
	0.63	84	SM

a) Treated in aq. KOH (pH 10) at 130 °C for 6 hr except uridine (12 hr), thymidine (13 hr), AICA-riboside (42 hr) and Ip-inosine (additional 11 hr at 160 °C at pH 10.5). b) UV-absorbing spots on tlc. Solvent system, *n*-butanol–acetic acid–water (4 : 1 : 1). c) See experimental section. d) Starting material remained. e) 2′,3′-isopropylidene. f) Purple-red color development was observed with NBDF reagent.

to be resistant against alkali, and even when the reaction period was prolonged up to 12 hr, no spot was observed except that of the starting material and a trace of thymine. Uridine was less resistant than thymidine; however, no UV-absorbing products could be detected except uracil. No effect was found even under more severe conditions (1 M KOH, 140 °C, 6 hr). In the case of cytidine, deamination followed by hydrolysis at the glycosyl bond was dominantly observed under the standard conditions, so that chromatography revealed three UV-abosorbing compounds corresponding to uridine, uracil, and cytidine. AICA-riboside was also resistant against alkali; even when it was reacted for 42 hr, the only reaction product was found to be AICA. The above four nucleosides showed no evidence of isomerization, even though the starting pH of the solution was changed from 10 to 8 or 12.

Reaction Mechanism. The available information concerning the alkaline isomerization of usual nucleosides is insufficient for us to determine clearly its reaction mechanism. One of the most important pieces of information is that the isomerization takes place only for 2′-hydroxypurine nucleosides. The C-2′ hydroxyl group is known to be the most acidic hydroxyl group of the three OH groups in the ribofuranoside,

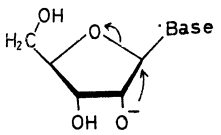

Chart 2. C-2′ Oxyanion participation of β-nucleoside for cleavage of sugar ring at C-1′.

It is supposed that the formation of the carbonium ion at C-1′ is facilitated by the anchimeric assistance[15] of an ionized hydroxyl group at C-2′, and that then the isomerization proceeds (Chart 2). The final equilibrium state of the four isomers and the rate of the reaction are not known.

Garret *et al.*[3] reported that adenosine and 2′-deoxyadenosine in an alkaline solution (pH higher than 13) have almost equal rates of hydrolysis at the glycosyl bond. Therefore, it may be supposed that the oxyanion participation affects only the cleavage of the sugar-ring bond at C-1′, while it does not affect that of the glycosyl bond.

The dissociation constant, pK_a, of the ribose moiety in nucleosides is approximately 12, and the isomerization in question is dependent on the pH of the solution. The optimum pH is between 7 and 12, about 10.[16] This pH range results from the competition among several parallel and sequential reactions, and outside this pH range these concomitant reactions occur in preference to isomerization. It has not been demonstrated whether or not the rate of isomerization would depend on the concentration of the hydroxyl ion, because parallel reactions, for example, hydrolysis, disturb the system.

It has not yet been explained why pyrimidine nucleosides do not isomerize, while purine nucleosides do.

Experimental

Materials and Equipment. The **1a**, **1b**, guanosine, and AICA-riboside used were commercial products of the Ajinomoto Co., Inc. The xantosine, uridine, cytidine, and 9-β-D-arabinofuranosyl adenine used were reagents of the Nutritional Biochem. Corp. The 2′-deoxyinosine and 2′-deoxyguanosine were obtained from Sigma Chemicals. The 2′,3′-isopropylideneinosine and 9-β-D-xylofuranosyl hypoxanthine were supplied by Dr. Akihiro Yamazaki of this department. The **3a** and **1c** were prepared according to the method described in the preceding paper[1] and according to the method of J. J. Fox *et al.*[17] respectively. The **1b** was methylated by the method of Martin *et al.*[18]; however, the separation of 2′-*O*-methyladenosine from the 3′-isomer by this method was unsatisfactory. It was, though, achieved with a method similar to that described by Gin *et al.*[19] The pHs of the solutions were measured at room temperature. The NMR spectra were recorded at 100 MHz with a Varian XL-100 spectrometer, using TMS as the internal standard. The melting points were measured by the use of a Yanagimoto MP-S2 apparatus and are uncorrected.

PC and Tlc. One-dimensional ascending PC on Toyo-Roshi No. 51A for quantitative analysis, and tlc on DC-Fertigplatten Cellulose F for qualitative analysis, were both carried out in the *n*-butanol–acetic acid–water (4 : 1 : 1) solvent system. Whichever chromatography was used, paper or thin-layer, similar R_f values were obtained for the same compounds. A paper or a plate was thrice developed if necessary to separate the components completely on the chromatogram. The components of the reaction mixture were detected on the paper or plate by two methods: (a) absorption in UV light, and (b) color development with a NBDF reagent[1] for the detection of diazotizable amines.

Conditions of Alkaline Treatment. The compound was dissolved in aq. KOH (1.5—4 g/dl), and the pH was adjusted to 10. Unless otherwise stated, a solution was heated at 130 °C for 6 hr in a sealed stainless steel tube on an oil bath. Adenosine and AICA-riboside were reacted for 12 hr and 42 hr respectively. The tube was removed from an oil bath after 2—3 hr, and the decreased pH of the solution was corrected to 10. The pH was corrected two times 2 hr and 6 hr after heating for adenosine and six times, at six-hour intervals, for AICA-riboside. Guanosine was subjected to further heating for 4 hr at 140 °C in addition to that under the standard conditions. In the cases of some nucleosides, several different conditions were also employed where the initial pH of the solution was changed. Two solutions (pH 9.6 and 11.3) of AICA-riboside were heated at 130 °C for 42 hr, and two solutions (pH 8.6 and 11.5) of cytidine, at 130 °C for 6 hr. Uridine was heated at 140 °C for 6 hr in 1 M KOH.

Determination of the Yields of the Products. Samples of the reaction mixtures were made up to appropriate volumes with water and submitted to PC. The spots of the samples and available authentic references, which were developed on the same paper with the samples, were cut off, together with their appropriate blanks, and eluted in 0.1 M HCl. The optical density of the eluent at the wavelength of the maximum absorption of each component was measured. The percentage of the remaining original material and the products, which were obviously identified as known compounds by means of the R_f values and UV spectra, were calculated from the optical density of the spots of the samples, which were compared with that of each authentic standard reference. The percentages of the products were converted into those of the original compound by the use of the equivalent mole ratio. The yields in Tables 2 and 3 were obtained by this procedure except in the cases of **4a**, **2b**, and **4b**. The yields of these three compounds were roughly calculated from the optical density of the individual fraction, and the molar extinction coefficients were assumed to be 12,000. The recovery yields of **1a** and **1b** were corrected by the subtraction of the above yields of **4a** and **2b** respectively because they are not separable on PC.

*Isolation of **4a**.* **1a** (5 g) was dissolved in 125 ml of 0.2 M KOH and heated at 130 °C for 2 hr. A few drops of 1M KOH were added to correct the pH of the solution to 10.0. After the reaction had been continued for another 4 hr, the mixture was made acid (pH 2) with conc. HCl and applied to a column (28 cm×3 cm) of Diaion SK1B-S (H-form, 50—100 mesh). The column was eluted with 7 l of water. Each 20-ml fraction was collected. The first and second UV-absorbing peaks were obtained within 7 l. When an eluting reagent was changed to 3.9 l of 0.1 M NH_4Cl, a third peak was obtained. It was composed of two components on tlc, the one was hypoxanthine (R_f 0.35), and the other, a compound closely resembling inosine (R_f 0.22). A fourth, obtained by the use of an eluting reagent of 0.1 M ammonia, was a mixture of hypoxanthine and AICA-riboside (R_f 0.31). The fractions between No. 718 and No. 1,080 in the third peak were collected, neutralized with 1 M NaOH, and concentrated *in vacuo*. The resultant solution was applied to a column (30 cm×0.7 cm) of granular activated carbon (Tsurumi-Coal GVA-80) in order to eliminate

inorganic salts passing through the column. The column was then eluted with a solvent mixture (1 : 1) of ethanol and 20% aq. ammonia. The eluate was concentrated *in vacuo* to dryness. The residue was dissolved in a small portion of pyridine and added to the same volume of acetic anhydride for acetylation. After refluxing for 1 hr, the solution was allowed to stand for 30 min at room temperature. To the solution some portion of ethanol was added and then it was evaporated *in vacuo*. The addition of ethanol to the solution and the evaporation of the resultant mixture were repeated several times, and finally it was evaporated to dryness. The residue was extracted with CH_2Cl_2, and the extract was evaporated to dryness, giving an acetylated compound. This compound was dissolved into ammonia-saturated methanol for deacetylation. The solution was then allowed to stand overnight and concentrated *in vacuo* to dryness. The resultant solid was extracted with $CHCl_3$ in order to remove the acetamide. The remaining solid was dried for analysis. (a) R_f on tlc. (thrice-developed) 0.39, for control inosine 0.43 and β-pyranoside 0.33. (b) UV_{max}; 249.2 nm in 0.1 M HCl, 248.2 nm in H_2O, and 253.9 nm in 0.1 M NaOH, for control, inosine corresponds to 249.9 nm, 249.2 nm and 254.5 nm respectively. (c) Components of acid hydrolysate: The compound was refluxed for 1.5 hr in 4 M H_2SO_4 followed by neutralization with $Ba(OH)_2$ and filtration of the resultant $BaSO_4$. UV_{max}; the values of 249.5 nm, in 0.1 M HCl and 264.5 nm in 0.1 M NaOH agreed with those of hypoxanthine. PC; an UV-absorbing spot, R_f 0.35 (hypoxanthine). A positive spot with a periodate reagent, R_f 0.33 (D-ribose). (d) Optical rotation. $[\alpha]_D^{20}$ −50° (c, 0.2, water). (e) NMR. (DMSO-d_6) δ 8.09 (H-2), 8.32 (H-8), 5.75 (H-1′, d, $J_{1'2'}$=0—1 Hz), 3.7—4.0 (broad, m); the acetylated compound (CD_3OD), δ 4.01 (J_{AB}=14 Hz, J_{AX}=2 Hz) and 4.28 (J_{AB}=14 Hz, J_{BX}=2 Hz) for two protons of H-5′.

Four Isomers of Adenosine. **1b** (5 g) was dissolved in 120 ml of 0.005 M KOH at 60 °C and heated in a sealed tube at 130 °C for 2 hr. The pH of the solution varied from 10.0 to 9.4 during the reaction period. A few drops of 1 M KOH were added to correct the pH to 10.0, and then the reaction was continued for another 4 hr. The pH of the solution decreased again to 8.9. After the pH adjustment, the reaction was continued for 6 hr more. When the reaction mixture was submitted to tlc, three major UV-absorbing spots were obtained (R_f 0.56, 0.46, 0.32). Then the reaction mixture was neutralized with conc. HCl and submitted to a column (36 cm×3 cm) of Dowex-1, X-4, (OH-form, 200—400 mesh). The column was eluted with 25%(w/v) methanol. Each 4-ml fraction was collected. Four UV-absorbing fractions were obtained. The first fraction (from No. 551 to No. 580), the second (from No. 581 to No. 700), the third (from No. 795 to No. 910), and the fourth (from No. 1,011 to No. 1,200) were severally concentrated to dryness *in vacuo*, and the residues were recrystallized from water. Two of them (A-1 and A-3) were finally crystallized from ethanol. The results of the analysis are summarized in Table 1. Pieces of the four compounds were individually hydrolyzed in 6 M HCl at 80 °C for 1 hr. The four hydrolysates all showed, on PC, one UV-absorbing spot (R_f 0.56) (adenine) and one positive spot (R_f 0.33) with a periodate reagent (D-ribose). NMR: Compound A-1 (DMSO-d_6), δ 8.18(H-2), 8.39(H-8), 7.26(NH_2-6), 5.79(H-1′, d, J_{AB}= 14 Hz); an acetylated compound ($CDCl_3$), δ 4.04 (J_{AB}= 14 Hz, J_{AX}=2 Hz) and 4.38(J_{AB}=14 Hz, J_{BX}=2 Hz) for two protons of H-5′.

Isomerization of ***3a***. **3a** (10 g) was dissolved in 250 ml of 0.15 M KOH to give a solution with a pH of 10. When the solution was heated at 150 °C for 3 hr, the decreased pH (8.4) of the solution was corrected to 10 with 13 ml of 1 M KOH. After further heating for 3 hr, the reaction mixture was acidified to pH 4 with 1 M HCl and submitted to a column (53 cm×3.1 cm) of Dowex 50W X-4 (H-form, 100—200 mesh). The column was washed with 2 l of water, and then eluted with 0.001 M NH_4Cl. Each 20-ml fraction was collected. The first UV-absorbing fraction was obtained from No. 260 to No. 370, and the second, from No. 450 to No. 590. The latter fraction was concentrated *in vacuo* until 3 ml and cooled for crystallization overnight. Subsequent recrystallization from water gave 50 mg (dried in a desiccator) of needle crystals. PC: R_f 0.23. UV_{max}: 250 nm in 0.1 M HCl, 249 nm in H_2O, 254 nm in 0.1 M NaOH. The X-ray (Cu$K\alpha$) diffraction pattern: α-form of inosine.[12]

Preparation of ***3c***. To 50 ml of 0.01 M KOH, **1c** (1 g) was added and the pH was adjusted to 10.2 with a few drops of 1 M KOH. The solution was heated in a sealed tube at 130 °C for 6 hr. The reaction mixture was neutralized with 1 M HCl, concentrated *in vacuo* to 10 ml, and submitted to a column (40 cm×1.3 cm) of Dowex-50W (H-form, 50—100 mesh). The column was eluted with 1.6 l of water. Each 10-ml fraction was collected. The first and second UV-absorbing peaks appeared from No. 20 to No. 60 and from No. 61 to No. 110 respectively. The first fractions were concentrated *in vacuo* until 2 ml and then allowed to stand in an ice box overnight for crystallization. The precipitated crystals were filtered and recrystallized from water. Needle crystals were thus obtained (47 mg). Found: C, 41.90; H, 4.32; N, 19.56%; Calcd for $C_{10}H_{12}N_4O_4S \cdot 0.2\,H_2O$: C, 42.25; H, 4.23; N, 19.72%. Mp 232—233 °C. UV_{max}; 317.5 nm at pH 7. Mass: M^+ 500 (trimethylsililated). NMR (DMSO-d_6): δ 5.62 (H-1′, d, $J_{1',2'}$=9.0 Hz), 1.14 (SH-6).

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