The Action of Alkali on Inosine. The Formation of 5-Amino-1-β-D-ribofuranosyl-4-imidazolecarboxamide and 9-β-D-Ribopyranosyl Hypoxanthine

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On treating inosine with aqueous dilute alkali hydroxide (pH 10) over 100 °C, three types of reactions have been found to occur in competition with one another: (1) the hydrolysis of the glycosidic bond to give hypoxanthine and D-ribose, (2) the opening of the pyrimidine ring at C-2 to give 5-amino-1-β-D-ribofuranosyl-4-imidazolecarboxamide, and (3) the isomerization of the ribose moiety to give mainly 9-β-D-ribopyranosyl hypoxanthine. The last two are newly-found reactions, and the products were isolated through column chromatography using Dowex 50W. The products are obtainable in yields of 30 and 10% respectively under optimum conditions. Hypoxanthine was cleaved in an alkaline solution to give 5-amino-4-imidazolecarboxamide, and 5′-inosinic acid was supposed to give 5-amino-1-β-D-ribofuranosyl-4-imidazolecarboxamide-5′-monophosphate. The ring-opening reaction at C-2 was not observed for adenosine or guanosine.

In the course of an investigation of the stability of inosine to acid and alkali, 5-amino-1-β-D-ribofuranosyl-4-imidazolecarboxamide (AICA-riboside) and 9-β-D-ribopyranosyl hypoxanthine were obtained when inosine was heated over 100 °C for several hours with aqueous dilute alkali hydroxide (pH 10). The reactions are considered to be useful for preparing those compounds conveniently from inosine.

It is well known that the acid treatment of inosine causes a hydrolysis at the *N*-glycosyl bond to give hypoxanthine and D-ribose. Such a hydrolysis is regarded as an acid-base catalyzed reaction; therefore, the same reaction was expected to take place when inosine was treated with alkali. This expectation was right. However, under alkaline conditions, especially in a dilute alkaline solution (pH 10), two other interesting reactions have been found to take place besides hydrolysis.

One of these reactions is a ring-opening reaction at C-2 in the pyrimidine of the purine ring to give AICA-riboside. The cleavage has been found to occur predominantly at C-2 rather than at the glycosyl bond at pH 10. Shaw[1] and Montgomery *et al.*[2] have reported that the N-1 substitution of inosine makes it susceptible to a nucleophilic attack of a hydroxide ion at C-2 in alkali, while they considered that inosine itself is extremely stable to alkali.[3] Therefore, what the present author found is somewhat contradictory to their considerations. A brief account of the present work has already appeared elsewhere.[4]

It was once considered, without actual isolation and identification, that purine ribonucleosides undergo an attack by the hydroxide ion at C-8, resulting in the opening of the imidazole ring without the hydrolysis of the *N*-glycosyl bond. For instance, 5-amino-4-ribosylaminopyrimidine is considered to be formed from 9-β-D-ribofuranosylpurine,[5] and also a reaction product, from 7-[β-(*N*-methyl-*N*-4-formylphenylamino) ethyl] guanosine.[6] It is considered that whether the ring-opening reaction takes place at C-2 or at C-8 depends upon the nature of the purine ribosides.

Another remarkable reaction in an alkaline solution would be an isomerization in the ribose moiety to give mainly 9-β-D-ribopyranosyl hypoxanthine. Although an isomerization of the riboside has been shown for aniline riboside[7] in alcohol, no such isomerization has yet been reported in the literature for usual ribonucleosides. This is probably because the *N*-glycosyl bond is labile in the usual nucleosides, so that this bond is cleaved before the isomerization takes place. On the other hand, for an abnormal ribonucleoside, such as pseudouridine, which involves the C-C glycosyl bond in the molecule, the isomerization has been found in acid and alkali.[8] This is probably because the C-glycosyl bond is stable. 9-β-D-Ribopyranosyl hypoxanthine has been obtained by Baddiley *et al.*[9] by the deamination of the 9-β-D-ribopyranosyl adenine which had been synthesized from 4,6-diaminopyrimidine and D-ribose.

Results and Discussion

The three reactions found in the action of alkali on inosine are summarized in Fig. 1. The results of the paper chromatography of the reaction mixture are shown in Table 1. The spots were revealed by the color development of diazotizable amine and by UV absorption. The two spots on the paper chromatogram, named X_1 and Y in Table 1, were coincident with

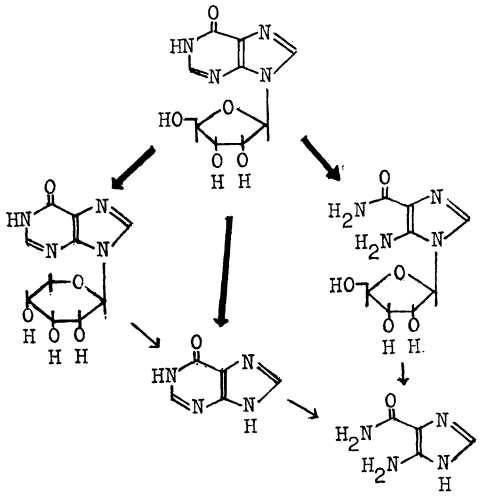

Fig. 1

Table 1. Reaction products of alkaline treated inosine[a]

Component	R_f[b]	UV-absorption	Color development
K	0.96	−[c]	−
X_2	0.83	+	+ (orange)
Hypoxanthine	0.74	++	−
X_1	0.64	++	+ (purple-red)
R	0.55	−	± (purple-red)
Inosine	0.52	++	−
Y	0.43	++	−

a) Initial pH 9.6 at 120 °C for 16 hr. b) On the paper chromatogram in solvent A (thrice development). c) Fluorescence was observed.

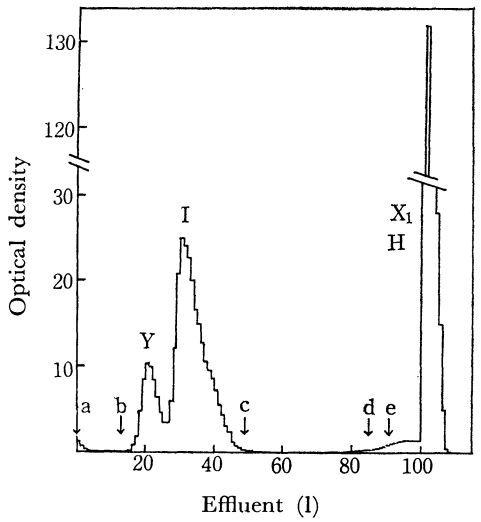

Fig. 2. Fractionation of alkaline treated reaction mixture of inosine by Dowex 50 W.
Y: 9-β-D-Ribopyranosyl hypoxanthine, I: Inosine, X_1: AICA-riboside, H: Hypoxanthine, (a) water, (b) 0.004 M NH_4Cl, (c) 0.01 M NH_4OH, (d) 0.05 M NH_4OH, (e) 0.1 M NH_4OH.

those of AICA-riboside and 9-β-D-ribopyranosyl hypoxanthine respectively. They were later identified by isolation through column chromatography using Dowex 50 W (Fig. 2).

The analytical data, the UV spectrum and the melting point of X_1 agreed with those reported for AICA-riboside by Greenberg and Spilman.[10] The X-ray diffraction data agreed with those of authentic AICA-riboside (Table 2).

On the other hand, the melting point (253 °C, decomp., uncorrected) of Y agreed with that (259—260 °C decomp., only available datum) reported for 9-β-D-ribopyranosyl hypoxanthine by Baddiley.[9] Elementary analysis data supported the assumption that Y was 9-β-D-ribopyranosyl hypoxanthine. The acid hydrolysis of Y gave two components, hypoxanthine and D-ribose. The fact that the UV spectrum of Y is similar to that of inosine indicated that the glycosyl bond of Y was attached at N-9. Therefore, Y may be considered to be 9-ribosyl hypoxanthine. By examining the periodate oxidation, the ring structure of Y

Table 2. X-ray diffraction data

9-β-D-Ribopyranosyl hypoxanthine				AICA-riboside	
α-Form		β-Form			
d(Å)	I/I_1[a]	d(Å)	I/I_1[a]	d(Å)	I/I_1[a]
10.78	60	17.00	100	10.74	5
6.86	80	5.52	60	7.44	40
5.34	100	5.25	20	5.40	40
4.58	60	5.16	30	5.13	20
3.60	20	4.73	80	4.77	30
3.56	40	4.62	15	4.70	70
3.48	10	4.51	15	4.62	20
3.40	5	4.20	5	4.53	20
3.34	10	3.73	5	4.44	20
3.30	5	3.52	5	4.08	95
3.15	20	3.18	20	3.71	30
		3.06	25	3.56	30
				3.55	35
				3.35	100

a) Peak heights scaled that the most intense line is given a value of 100.

Table 3. Optical rotations of some ribonucleosides

Nucleosides	$[\alpha]_D^{(°)}$ in water
Adenosine	−65.5[b], −60.4[c], −60.7[d]
9-β-D-Ribopyranosyl adenine	−38[e,f], −37[g,h], −30[d]
9-α-D-Ribofuranosyl adenine	+24[c]
Inosine	−49.2[a]
Y	−26.5

a) The Merck Index. b) J. Davoll and B. A. Lowry, *J. Amer. Chem. Soc.*, **73**, 1650 (1951). c) R. S. Wright, G. M. Tener, and H. G. Khorana, *ibid.*, **80**, 2004 (1958). d) Y. Furukawa and M. Honjo, *Chem. Pharm. Bull.*, **16**, 1076 (1968). e) Ref. 7. f) M. Viscontini and S. Huwyler, *Helv. Chim. Acta*, **43**, 782 (1960). g) J. Davoll and B. A. Lowry, *J. Amer. Chem. Soc.*, **74**, 1563 (1952). h) K. Onodera, S. Hirano, N. Kashimura, F. Masuda, T. Yajima, and N. Miyazaki, *J. Org. Chem.*, **31**, 1291 (1966).

was assumed to have a pyranosyl form; the slow velocity on acid hydrolysis as compared with inosine supported this assumption. (Ribopyranosyl adenine has been known to be hydrolyzed by acid slower than ribofuranosyl adenine.[11]) The optical rotation data are shown in Table 3, along with some available data on adenosine derivatives. As β-pyranosyl adenine has about one-half the value of β-furanosyl adenine, Y also showed about one-half the value of inosine (β-furanoside). Thus, it is very likely that Y is 9-β-D-ribopyranosyl hypoxanthine. The X-ray diffraction patterns of Y were obtained in two forms (Table 2). One of the forms seems to be unstable (named the α-form) and to be changed into another stable form (named the β-form) during storage. The crystals of the α-form were obtained on the first experimental run; afterward they were never obtainable in several succeeding experimental runs. These facts suggest that there is a large energy difference between the two crystal forms.

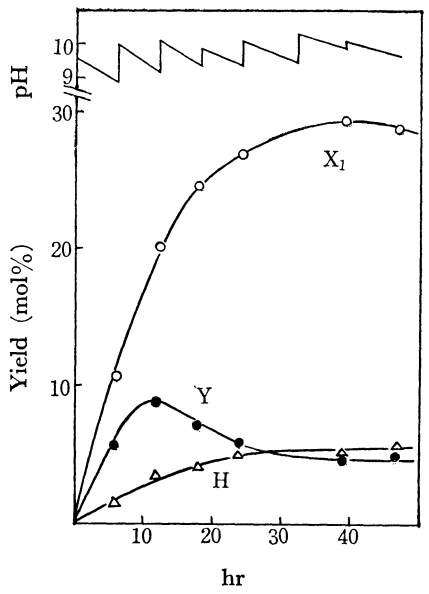

Fig. 3. Dependence of the yield of three major products on the reaction period in alkaline treatment of inosine at 130 °C (pH between 10 and 9).
X_1(○): AICA-riboside, Y(●): 9-β-D-Ribopyranosyl hypoxanthine H(△): Hypoxanthine

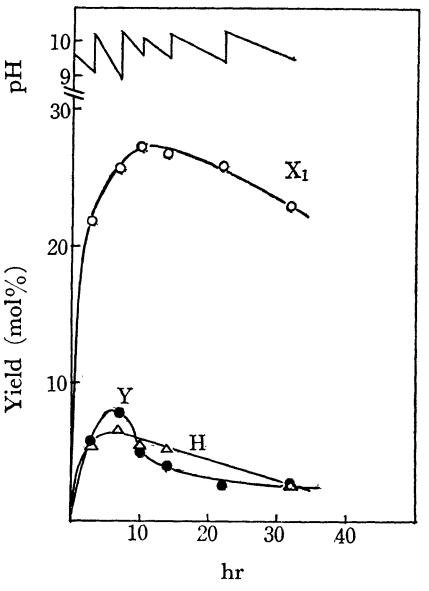

Fig. 4. Dependence of the yield of three major products on the reaction period in alkaline treatment of inosine at 150 °C (pH between 10 and 9).
X_1(○): AICA-riboside, Y(●): 9-β-D-Ribopyranosyl hypoxanthine, H(△): Hypoxanthine

In the alkaline treatment of inosine, the dependence of the yield of the three major UV-absorbing products on the reaction time is shown in Figs. 3 and 4. When the pH's of the solutions were kept between 9 and 10, the maximum yield of AICA-riboside reached 28—30% and that of 9-β-D-ribopyranosyl hypoxanthine, 8—9% (that of hypoxanthine was 6—7%). The relative amount of the yield was found to be independent of the temperature in the 130 °C—150 °C range. The reaction time for the maximum yield, however, depends on the temperature. The higher the temperature, the shorter was the reaction period needed to give the maximum yield of both compounds. The amounts of the three major products (including hypoxanthine) all decreased when too long a reaction period was employed. This fact suggested that a further degradation occurs into simple compounds which have no more UV-absorption.

Among the minor reaction products of the alkaline treatment of inosine, X_2 in Table 1 is considered to be 5-amino-4-imidazolecarboxamide (AICA) on the basis of its UV spectrum, the characteristic orange color caused by the NBDF reagent,* and the R_f value on the paper chromatogram (compared with the authentic standard of AICA). Undoubtedly AICA is obtained by a base-catalyzed hydrolysis at the glycosyl bond of AICA-riboside. AICA may be also obtainable by a base-catalyzed ring-opening reaction at C-2 in the pyrimidine ring of hypoxanthine (Fig. 1). In an acid solution AICA has been obtained from hypoxanthine both in the presence of zinc[12] and in its absence.[13] In this report the AICA formation from hypoxanthine in 0.4 M NaOH at 150 °C is shown (Table 4) in a yield of 30%, corresponding with the reaction period of 4 hr. Under these conditions, the original hypoxanthine remained 52%, though 99% of the hypoxanthine has been reported by Albert and Brown[14] to remain unreacted in 10 M NaOH at 100 °C for 1 hr.

A ring-opening reaction at C-2 in the pyrimidine section of the purine ring was also postulated for 5'-inosinic acid to give 5-amino-1-β-D-ribofuranosyl-4-imidazolecarboxamide-5'-monophosphate (AICA-ribotide) on the basis of a characteristic color reaction found by the NBDF reagent and a comparison of its R_f value with that of authentic AICA-ribotide (Table 5). It should be pointed out here that, for the purpose

Table 4. Reaction products of alkaline treated hypoxanthine[a]

Component	R_f[b]	UV-absorption	Color development
AICA[c]	0.71	+	+ (orange)
Hypoxanthine	0.50	++	−

a) Initial pH 11.8 at 150 °C for 4 hr. b) On the paper chromatogram in Solvent B. c) 5-Amino-4-imidazolecarboxamide.

Table 5. Reaction products from disodium 5'-inosinic acid[a]

Component	R_f[b]	UV-absorption	Color development
Hypoxanthine	0.44	+	−
X_1 (AICA-riboside)	0.33	+	+
Inosine	0.23	++	−
5'-Inosinic acid+X_0[c]	0.07	++	±
AICA-ribotide standard	0.07	++	+

a) Initial pH 7.8 at 120 °C for 8 hr. b) On the paper chromatogram in Solvent A. c) Suspected to be AICA-ribotide.

* See Experimental Section.

of obtaining AICA-ribotide through the ring-opening at C-2, Shaw[1c] made the N-1 substitution of isopropylidene-inosine-5′-di(*p*-nitrophenyl)phosphate and left out the direct cleavage of inosinic acid. Also, Magrath and Brown[15] reported a ring-opening reaction at C-8 in the imidazole section of the purine ring on 9-β-D-ribofuranosylpurine-5′-phosphate in dilute aqueous alkali.

Guanosine and adenosine were subjected to alkaline treatment under the same conditions as those used for inosine. By the NBDF reagent, however, no compounds with the ring opened at C-2 were detected on the chromatogram. The amino group at the 2 position of guanosine probably interferes with the approach of the hydroxide ion and prevents its attack at C-2. In adenosine, on the other hand, the amino group at the 6 position may increase the electron density at C-2, and this would prevent a nucleophilic attack at C-2; the attack may take place, however, at C-8.[3] However, it is known that the alkaline treatment of adenosine causes some deamination, thus giving inosine,[3] so that it may be expected that a little AICA-riboside can be obtained, through inosine. It may need longer reaction period than that in the present study. The characteristic spots corresponding with β-pyranoside in the case of inosine were detected in both nucleosides with UV light on the chromatogram. The mechanism of the alkaline isomerization of these usual ribonucleosides including inosine is expected to occur through an intermediate of the oxide anion, whose negative charge is located on one of the oxygen atoms in the ribose moiety. Such an intermediate, *i. e.*, a mono oxide anion in which an electron is shared by the ring oxygen and the 5′-oxygen in the ribose moiety, is suspected to induce the formation of β-pyranoside. However, if the case of pseudouridine, examined by Chambers *et al.*[8a] is taken into consideration, the concomitant formation of the α-anomer can be expected in the present experiments. The α-anomer was not isolated here, probably because too little of it was formed to be perceived in this brief period of detection. In this respect, detailed experimental results and a discussion will be given in a later paper.

Experimental

Material and Equipment. Inosine, hypoxanthine, disodium 5′-inosinic acid, adenosine, and guanosine crystals, all indicated free from any impurities by paper chromatography, and the authentic standard samples of AICA, AICA-riboside, and AICA-ribotide were all prepared by the Ajinomoto Co., Inc. The ultraviolet absorbance was measured with a Hitachi 139 spectrophotometer, and the pH's of the solutions were measured at room temperature with a Toa Dempa pH meter HM-5A. Automatic Polarimeter Bendix-NPL 143-A was used for the measurement of the optical rotation. The X-ray powder diffraction patterns were obtained by means of a Rigaku Denki X-ray diffractometer.

Paper Chromatography. One-dimensional ascending paper chromatography was carried out on Toyo-Roshi No. 51A, the following two solvent systems being used: Solvent A, *n*-butanol-acetic acid-water (4 : 1 : 1); Solvent B, distilled-*i*-butylic acid-*n*-butanol-28% ammonia aq.-water (36 : 30 : 3 : 31). The papers were developed thrice if necessary for the complete separation of the components on the chromatogram. The components of the reaction mixture were detected on the paper by two different methods: (a) absorption in ultraviolet light was used to detect the ultraviolet absorbing compounds, (b) color development with NBDF reagent was used to detect the diazotizable amines. The NBDF reagent was prepared by an addition of 100 mg of *p*-nitrobenzenediazonium fluoroborate to 100 ml of 2 M acetic acid.

Alkaline Treatment on Inosine. Inosine (0.6 g) was dissolved in 20 ml of 0.1 M NaOH and heated in a sealed stainless steel tube at 120 °C on an oil bath for 16 hr. The pH of the solution was varied from 9.6 to 9.1 during the reaction period. The reaction mixture was paperchromatographed in Solvent A three times (Table 1).

Isolation of Y (9-β-D-ribopyranosyl hypoxanthine) and X_1 (AICA-riboside) in the Crystalline Form. Potassium hydroxide (4.1g) and inosine (20 g) were dissolved in 45 ml of water, and the mixture was heated in a sealed tube at 130 °C for 6 hr. The pH of the solution was varied from 10.1 to 9.5 during the reaction period. A granule of potassium hydroxide was added to correct the pH of the solution to 10.0. Then, the reaction was continued for 6 hr more. The reaction mixture was acidified (pH 2) by 10 ml of conc. HCl and applied to a column (95 cm×3.7 cm) of Dowex 50W X-8 (H^+-form, 50—100 mesh, 1 l). The column was developed with 13 l of water and 36 l of 0.004 M NH_4Cl, 36 l of 0.01 M NH_4OH, 6 l of 0.05 M NH_4OH, and 23 l of 0.1 M NH_4OH in turn. The fraction was collected every 1 l. The optical densities of the fractions at 250 nm were measured. The first and the second UV-absorbing peaks appeared in the fractions with NH_4Cl. The first peak contained Y, and the second, inosine. The third peak appeared in the fractions with 0.1 M NH_4OH; it was found to be a mixture of a large amount of X_1 and a little hypoxanthine. The fractions between No. 17 and No. 25 (9 l) in the first peak were neutralized with ammonia and concentrated *in vacuo* until 20 ml. When the concentrated solution was cooled, crystallization occurred. The crystals were washed with cold 50% aqueous methanol and weighed (1.5 g). Recrystallization from water gave 0.7 g (dry) of plate-like crystals (Y). The fractions between No. 101 and No. 107 (7 l) in the third peak were concentrated *in vacuo* until 90 ml. The concentrated solution was neutralized (pH 7) with a drop of dil. HCl and cooled for precipitation. After the removal of the precipitated fine powder of hypoxanthine, the filtrate was concentrated *in vacuo* until 5 ml and then cooled again. The precipitated powder crystals were triturated with water and the insoluble parts (hypoxanthine) removed by filtration. The filtrate was evaporated in a desiccator; thus some large cubic crystals and fine powder crystals different in appearance from each other were obtained. On filtration with a fiber net of 50 mesh, the fine powder crystals were passed out through the net with the mother liquor. The large cubic crystals remaining on the net were collected and recrystallized from water. The filtration with a fiber net was repeated again; subsequent recrystallization gave 1.4 g of large cubic crystals (X_1) which were free from any contaminant on the paper chromatogram.

Characterization of AICA-riboside. The large cubic crystals (X_1) were analyzed as follows. (a) Paper chromatography. The R_f agreed with the authentic standard in Solvent A; 0.64 (thrice developed). (b) UV spectrum. Agreed with the data of Greenberg and Spilman in 0.1 M HCl and in 0.1 M NaOH. (c) Melting point. Found; 213 °C (decomp.) uncorrected. (d) X-ray diffraction data (Cu K_α). Identical with the authentic standard under a diffraction angle of 30 degrees (2θ).

Characterization of 9-β-D-Ribopyranosyl Hypoxanthine, The

plate-like crystals (Y) were analyzed as follows. (a) Paper chromatography. The R_f was a little lower than that of inosine in Solvent A [0.43 (thrice-developed)] and the same as that of inosine in Solvent B [0.55 (twice-developed)]. (b) UV spectrum. The same behavior as inosine in 0.1 M HCl, H_2O, and 0.1 M NaOH. (c) Elementary analysis. Agreed with inosine or 9-β-D-ribopyranosyl hypoxanthine. Found: C, 44.49; H, 4.57; N, 21.15%. Calcd for $C_{10}H_{22}O_5N_4$: C, 44.78; H, 4.51; N, 20.89%. (d) Components of acid hydrolysate. In 1 ml of water 10 mg of crystals were dissolved, and then 1 ml of conc. HCl was added. The mixture was heated at 80 °C for 1 hr and chromatographed in Solvent A. Hypoxanthine (R_f 0.29) was revealed by UV light and D-ribose (R_f 0.25), by periodate oxidation, by Ohkuma.[16] (e) Periodate oxidation. The Fleury-Lange[17] method was employed for a 1% solution of crystals. The consumptive mole number of periodate was 1.92, which could be regarded as nearly 2. (f) Velocity of acid hydrolysis. In 1 ml of 0.1 M HCl, 10 mg of crystals were dissolved, and then the mixture was heated at 85 °C for 1 hr. Inosine was also used for control. Paper chromatogram in Solvent A showed two UV-absorbing spots, hypoxanthine (R_f 0.40) and the original compounds (R_f 0.22). The spots were cut off and eluted with 0.1 M HCl, and the absorbance of the eluate was measured at 250 nm. The absorption ratio of hypoxanthine to the original compounds was 12 : 88 for these plate crystals and 90 : 10 for ionsine. (g) Optical rotation. Found: $[\alpha]_D^{25}$ −26.5° (*c*, 1 water). (h) Melting point. 252—254 °C (decomp.) uncorrected. (i) X-ray diffraction data (Cu K_α). The measurements were carried out under a diffraction angle of 30 degrees (2θ).

Dependence of the Yields of the Three Major Reaction Products on the Reaction Period on the Alkaline Treatment of Inosine. Potassium hydroxide (7.5 g) and inosine (40 g) were dissolved in 90 ml of water to obtain a clear alkaline solution (pH 9.6). After the solution had been heated in a sealed tube at 130 °C or 150 °C for an appropriate period of several hours, it was cooled quickly and sampled (1 ml). The pH's of the solutions were usually decreased to some extent. A granule of potassium hydroxide was added to correct the pH to about 10, and the solution was again heated for several hours; these treatments were repeated many times over. Aliquots of 1 ml samples were diluted to 25 ml, and 10 μl of the diluted solution was spotted on the paper for chromatography in Solvent A (thrice-developed). Ten microliters of a 1% solution of three compounds, hypoxanthine, AICA-riboside, and inosine (used in place of 9-β-D-ribopyranosyl hypoxanthine), were also spotted on the same paper as the standard. The UV-absorbing spots on the chromatogram corresponding to the three compounds were cut off, together with their appropriate blanks, and eluted with 0.1 M HCl, and the absorbances of the eluates were measured at 267 nm for AICA-riboside and at 250 nm for the hypoxanthine derivatives. The concentration of each component in the reaction mixture was calculated from the absorbance compared with that of the standard references. The yields of the three compounds in mol% were computed from the known concentration of the initial solution.

Identification of X_2 as AICA. The R_f of the X_2 spot on the chromatogram shown in Table 1 was identical with that of the authentic reference sample of AICA. The spot was cut off and eluted with water, and the spectra of the eluate were measured in 0.1 M HCl (λ_{max} 267 nm and 240 nm; λ_{min} 250 nm and 220 nm) and in 0.1 M NaOH (λ_{max} 278 nm and λ_{min} 240 nm). An orange color development which was characteristic of AICA was found on the paper with the NBDF reagent.

Formation of AICA from Alkaline-treated Hypoxanthine. Hypoxanthine (0.8 g) was dissolved in 20 ml of 0.4 M NaOH and heated in a sealed tube at 150 °C for 4 hr. The pH of the solution varied from 11.8 to 10.6 during the reaction period. The reaction mixture showed two major UV-absorbing spots on the paper; one was estimated to be AICA, and the other, unreacted hypoxanthine. A clear separation of the two spots was achieved in Solvent B (AICA, R_f 0.71; hypoxanthine, R_f 0.50); three developments were required in Solvent A (AICA, R_f 0.83; hypoxanthine, R_f 0.74). The R_f value, a characteristic orange color development with the NBDF reagent, and the behavior of the UV spectra in acid and alkali were all in accordance with the authentic reference sample of AICA and also with the X_2 described before. The spots on the paper were cut off, together with their appropriate blanks, and eluted with 0.1 M HCl, and the absorbances of the eluates were measured at 267 nm for AICA and at 250 nm for hypoxanthine. The yield of AICA was determined to be 30%, and that of the unreacted hypoxanthine, 52%.

Formation of AICA-ribotide from 5′-Inosinic Acid. Disodium 5′-inosinic acid crystals (0.28 g) were dissolved in 20 ml of water and heated in a sealed tube at 120 °C for 8 hr. The pH value of the solution was varied from 7.8 to 7.2 during the reaction period. The chromatography with UV light of the resultant solution in Solvent A revealed the formation of as much inosine (R_f 0.23) as of unreacted 5′-inosinic acid (R_f 0.07), along with other minor reaction products. On the spot corresponding with 5′-inosinic acid, a pale purple-red color development was found with the NBDF reagent. The R_f value of the authentic AICA-ribotide was superimposed on 5′-inosinic acid in Solvent A.

Alkaline Treatment on Adenosine and Guanosine. Adenosine (0.1 g) was added to 20 ml of 0.008 M NaOH and heated in a sealed tube at 130 °C for 3 hr. The reaction mixture showed three UV-absorbing spots on the chromatogram in Solvent A: R_f 0.6 Adenine; R_f 0.47 Unreacted adenosine; R_f 0.34 presumably pyranoside. Nothing of the colored spot with the NBDF reagent was detected. Guanosine (0.5 g) was added to 20 ml of 0.038 M NaOH, and the mixture was heated in a sealed tube at 130 °C for 8 hr. The pH of the solution was varied from 11.0 to 9.8. The reaction mixture showed five UV-absorbing spots on the chromatogram in Solvent A (thrice developed): R_f 0.7 Guanine; R_f 0.54 Unreacted Guanosine; R_f 0.43 Presumably pyranoside; R_f 0.34 Unknown; R_f 0.27 Unknown. Nothing of the spot with the NBDF reagent was detected.

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