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3-Methylinosine

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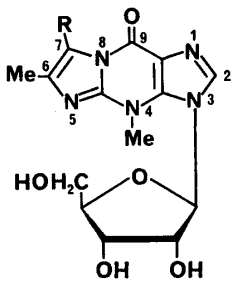
3-Methylinosine (**2a**) has been prepared in 28% yield by heating 5-(methylamino)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**5c**) with a mixture of ethyl orthoformate and acetic anhydride, followed by ammonolysis. Compound **2a** gave the stable 1,2-dihydro derivative **6** in 77% yield on catalytic hydrogenation over Pd–C. The pyrimidine moiety of **2a** has been shown to undergo ring cleavage under alkaline conditions at a rate three times faster than that of 3,9-dimethylhypoxanthine (**3a**). The glycosidic bond of **2a** is unusually susceptible to acidic hydrolysis and the rate was shown to be faster than that of inosine by a factor of 10^4.

Keywords——3-methylinosine; cyclocondensation; purine hydrogenation; purine ring cleavage; glycosidic bond cleavage; formamide hydrolysis; deamination; substituent effect

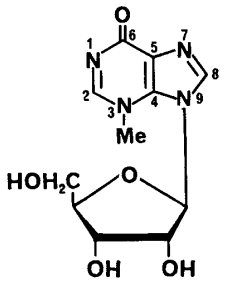
Although no natural occurrence of 3-methyl-9-β-D-ribofuranosylpurines has been reported, 3-methylinosine (**2a**) can be seen as a partial structure in **1a**, **b**, the most probable structures[1-3] for wyosine[1] from torula yeast phenylalanine transfer ribonucleic acid ($tRNA^{Phe}$) and wybutosine[2] from yeast $tRNA^{Phe}$. In connection with the unusual susceptibility of the glycosidic bonds of wyosine[1] and wybutosine[2b,c] to acidic hydrolysis, we have reported the syntheses and hydrolysis of 3-β-D-ribofuranosylwye (**1a**)[3e] and various 3-alkyl-9-β-D-ribofuranosylpurines.[3c,e,4] This paper presents a detailed account of the first synthesis and chemical properties of **2a**.[5]



1

a : R=H

b : R=CH_2CH_2–C(H)(NHCOOCH$_3$)(COOCH$_3$)



2a

3

a : $R^1=R^2=CH_3$

Chart 1

Since methylation of ionsine has been reported to take place at the 1-,[6] 7-,[6] and O^6-position,[6b] we have developed a method for the synthesis of 3,9-dialkylhypoxanthines (**3**)[7] by cyclization of 1-alkyl-5-(alkylamino)imidazole-4-carboxamides as a model for the synthesis of **2a**. We have also established the synthesis of the requisite carboxyamides **5**.[5,8] According to

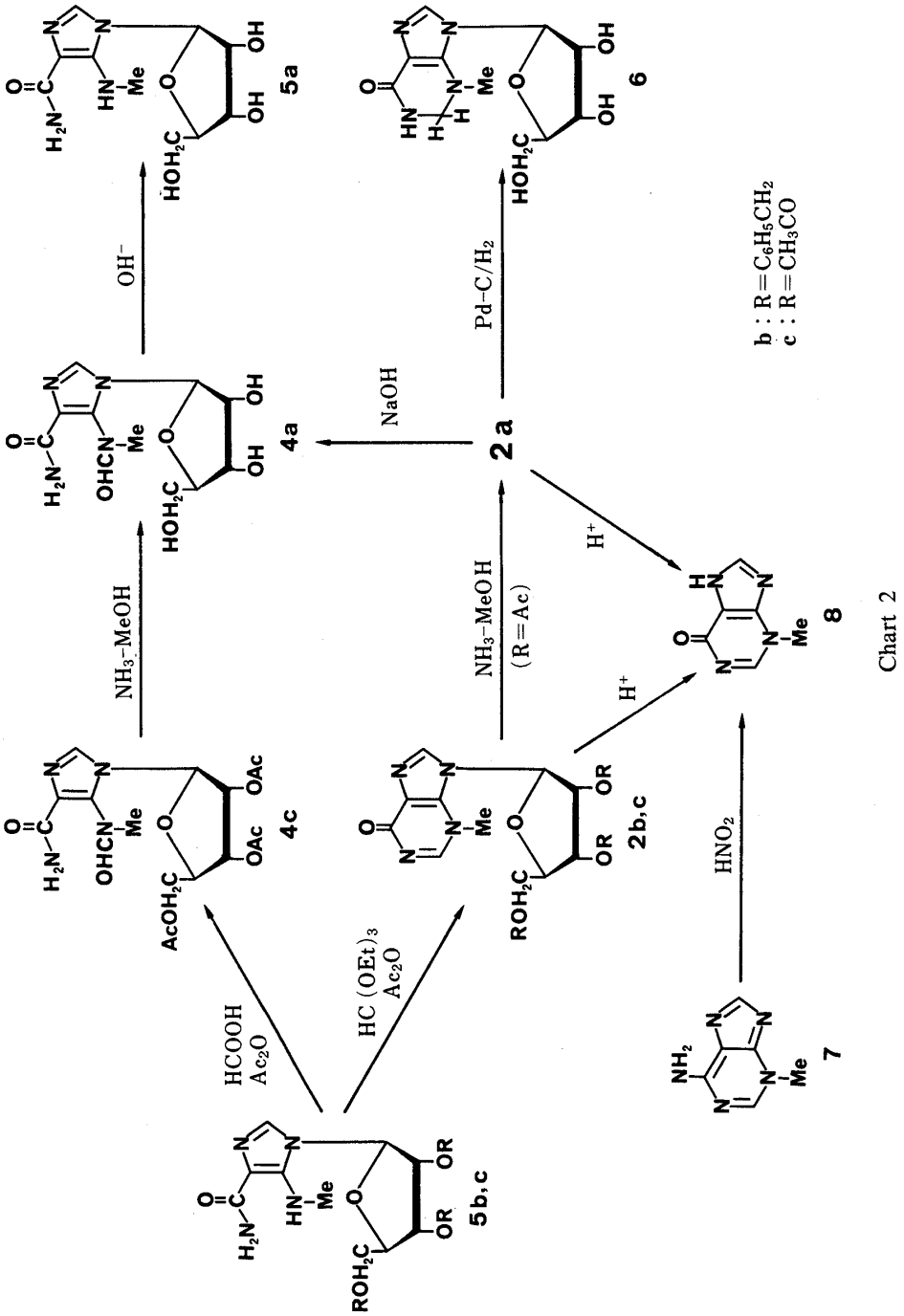


Chart 2

the procedure used for the synthesis of **3**, 5-(methylamino)-1-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**5b**)[5,8a] was heated in a mixture of ethyl orthoformate and acetic anhydride to furnish 2′,3′,5′-tri-*O*-benzyl-3-methylinosine (**2b**) in 41% yield. Treatment of **2b** with hot AcOH gave 3-methylhypoxanthine (**8**), which was identical with a specimen obtained by deamination of 3-methyladenine (**7**),[9] confirming the correctness of the structure **2b**. However, debenzylation of **2b** by catalytic hydrogenolysis over Pd–C proceeded only sluggishly, failing to provide **2a**.

2′,3′,5′-Tri-*O*-acetyl-3-methylinosine (**2c**) was then prepared in 38% yield by similar treatment of 5-(methylamino)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**5c**).[3b,5,8a] 5-(*N*-Methylformamido)-1-β-D-ribofuranosylimidazole-4-carboxamide (**4a**) was obtained as a by-product in this reaction after treatment of the rest of the products with aq. NH_3, in agreement with the results obtained in the synthesis of **3**.[7] Removal of the acetyl groups from **2c** was successfully achieved by treatment with NH_3–MeOH to give **2a** as the monohydrate in 73% yield. The structure of **2a** is supported by the close resemblance of the ultraviolet (UV) spectrum to that of **3**.[7] Further support for the structure rests on the chemical transformations described below.

As in the case of 3,9-dimethylhypoxanthine (**3a**),[7] **2a** is unstable under basic conditions: **2a** underwent complete ring-opening at the pyrimidine moiety in 0.01 N aq. NaOH at room temperature in 1 h to provide **4a** as a sole product. The structure of **4a** was confirmed by comparison with a sample derived from **5c** by formylation followed by ammonolysis. It was found that the ring-opening of **2a** proceeded at a rate three times faster than that of **3a** at pH 10.82 and 25 °C (Fig. 1). A similar accelerating effect of the 9-β-D-ribofuranosyl group on the cleavage of the pyrimidine moiety has been reported in the hydrolysis of 1-alkyladenosines,[10] 1-benzyloxyadenosine,[11] and 3-methyladenosine.[12] It is noteworthy that 3-methyladenosine has been reported to undergo reversible ring-opening under basic conditions[12,13] and the rate of the ring-opening is estimated to be faster than that of the irreversible ring-opening of **2a**. A similar relationship holds between the ring-opening of 3,9-dimethyladenine[12] and that of **3a**. It has been shown that base-catalyzed hydrolysis of **4a** is also promoted by the β-D-ribofuranosyl substitutent: 1-alkyl-5-(*N*-methylformamido)-imidazole-4-carboxamides are practically stable in 0.1 N aq. NaOH at room temperature as judged from the UV spectra,[7b] whereas **4a** changes into 5-(methylamino)-1-β-D-ribofuranosylimidazole-4-carboxamide (**5a**) under similar conditions.

Compound **2a** provided 1,2-dihydro-3-methylinosine (**6**) in 77% yield on hydrogenation over Pd–C, analogously with the reaction of **3a**.[7b] The 1,2-dihydro structure of **6** is supported by its nuclear magnetic resonance (NMR) spectrum in Me_2SO-d_6: the $N_{(3)}$-methyl protons resonate at higher field than those of **2a** by 1.24 ppm and the signal due to the anomeric proton appears in the range where the signals of the corresponding protons of the imidazole nucleosides **4a** and **5a**[5,8a] lie. The structure is further supported by the UV spectral similarity to 1,2-dihydro-3,9-dimethylhypoxanthine.[7b] Unlike **2a**, **6** was found to be fairly stable under both acidic and basic conditions.

When a solution of **2a** in dilute aq. HCl was kept at 40 °C, cleavage of the glycosidic bond was completed within 24 h at pH 3 and **8** was obtained as a single UV-absorbing product. The progress of the reaction at pH 2.01 and 25 °C was followed by high-performance liquid chromatography (HPLC) and the pseudo-first-order rate constant (k_{obs}) was determined to be 5.7×10^{-2} min^{-1} (half-life, $t_{1/2}$ 12 min). For the hydrolysis in 0.1 N aq. HCl (pH 1.1) at 25 °C, k_{obs} 8.7×10^{-1} min^{-1} ($t_{1/2}$ 48 s) was likewise obtained (Fig. 2). Suzuki has reported the rates for the hydrolysis of the glycosidic bond of inosine at pH 1.1—1.4 and 50—70 °C,[14] and k_{obs} 1.4×10^{-5} min^{-1} may be calculated for the reaction at 25 °C from the Arrhenius equation. Even under more acidic conditions (in 1.02 N aq. HCl), a relatively small value of k_{obs} (8.6×10^{-7} s^{-1}, *i.e.* 5.2×10^{-5} min^{-1}) was reported for the hydrolysis of inosine at 25 °C.[15]

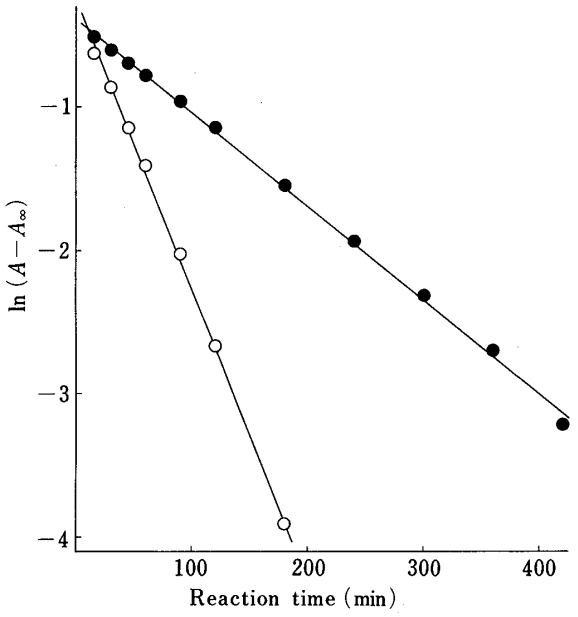


Fig. 1. First-Order Plot for Ring-Opening of **2a** (○) and **3a** (●) at 25 °C in 0.1 M Phosphate Buffer at pH 10.82 and Ionic Strength 1.0

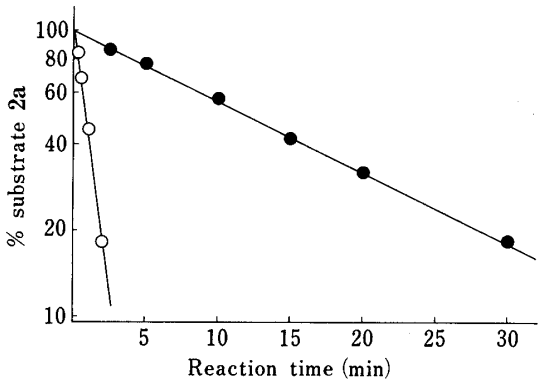


Fig. 2. First-Order Plot for Glycosidic Bond Cleavage of **2a** in 0.1 N aq. HCl (○) and in 0.1 M aq. HCl–KCl (pH 2.01) (●) at 25 °C

Comparison of these data supports the conclusion that introduction of a methyl group at the 3-position of inosine accelerates the acidic hydrolysis of the glycosidic bond by a factor of 10^4. An analogous accelerating effect of the 3-methyl group has been reported in the hydrolysis of the glycosidic bonds of all the known 3-methyl-9-β-D-ribofuranosylpurines.[3b,c,e,4a,b,13] The rates of hydrolysis of these nucleosides in 0.1 N aq. HCl at 25 °C are in the order 3-methylguanosine[3e] > **2a** > 3-methylxanthosine[4a] > 3-methyladenosine[13] > 3-methylisoguanosine.[4b] We have reported the rates for the hydrolysis of the glycosidic bond of **1a** at 25 °C (k_{obs} 4.4×10^{-1} min^{-1} for the reaction in 0.1 N aq. HCl and 3.7×10^{-2} min^{-1} for the reaction at pH 2.1).[3e] These are of the same order of magnitude as those of **2a**. It seems likely that the partial structure **2a** in **1** is responsible for the unusual susceptibility of the glycosidic bond of **1** to acidic hydrolysis.

Experimental

General Notes——All melting points are corrected. UV spectra were measured with a Hitachi 323 spectrometer using solutions in 95% aq. EtOH, 0.1 N aq. HCl (pH 1), 0.005 M phosphate buffer (pH 7), and 0.1 N aq. NaOH (pH 13). NMR spectra were recorded on a JEOL JNM-PS-100 or a JNM-FX-100 spectrometer at 25 °C with Me_4Si as an internal standard. Optical rotations were measured with a JASCO DIP-SL or a DIP-181 polarimeter. Spectrophotometric determinations were carried out with a Hitachi Model 181 spectrometer. The liquid chromatographic system was a Waters Model 204 ALC which included a 6000A pump, a U6K injector, and a Model 440 absorbance detector operating at 254 nm. pH's were measured with a Hitachi-Horiba F-5 pH meter. Elemental analyses were performed by Mr. Y. Itatani and his associates at Kanazawa University. The following abbreviations are used: br = broad, d = doublet, dd = doublet of doublets, m = multiplet, q = quartet, s = singlet, sh = shoulder.

2′,3′,5′-Tri-*O*-acetyl-3-methylinosine (2c)——5-(Methylamino)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**5c**)[3b,5,8a] (7.00 g, 17.6 mmol) was heated under reflux for 1 h in a mixture of ethyl orthoformate (70 ml) and acetic anhydride (26 ml). The resulting solution was concentrated *in vacuo* to leave a brown viscous oil. This was purified by column chromatography on silica gel (70 g), with $CHCl_3$–EtOH (4 : 1, v/v) as an eluent. The eluate containing **2c** was concentrated *in vacuo* to give **2c** (2.71 g, 38% yield) as a colorless caramel, which crystallized on treatment with Me_2CHOH, mp 161—162 °C. Recrystallization from Me_2CHOH gave an analytical sample as colorless needles, mp 161.5—162.5 °C. UV $\lambda_{max}^{95\%\ EtOH}$ 255 nm (ε 12100); $\lambda_{max}^{H_2O}$ (pH 1) unstable; $\lambda_{max}^{H_2O}$ (pH 7) 259 (13000); $\lambda_{max}^{H_2O}$ (pH 13) unstable. ^{1}H-NMR ($CDCl_3$) δ: 2.01, 2.15, and 2.19 (3H each, s, $3CH_3CO$'s), 4.08 (3H, s,

NCH_3), 4.28 (2H, br, $C_{(5')}$-H_2), 4.47 (1H, m, $C_{(4')}$-H), 5.44 (1H, dd, $J=5$ and 5 Hz, $C_{(3')}$-H), 5.91 (1H, dd, $J=5$ and 5 Hz, $C_{(2')}$-H), 6.32 (1H, d, $J=5$ Hz, $C_{(1')}$-H), 7.77 and 7.86 (1H each, s, purine protons). $[\alpha]_D^{25}$ $-24.9°$ ($c=0.614$, H_2O). *Anal.* Calcd for $C_{17}H_{20}N_4O_8$: C, 50.00; H, 4.94; N, 13.72. Found: C, 49.78; H, 4.88; N, 13.94.

The eluate which contained less polar substances than **2c** was collected, and removal of the solvent by evaporation left a brown viscous oil. A solution of this material in 28% aq. NH_3 (60 ml) was allowed to stand at room temperature for 3 h and then concentrated *in vacuo* to leave a brown oil. Crystallization from EtOH gave **4a** (711 mg, 13% yield) as colorles needles, mp 177—181 °C (dec.), identical with an analytical sample described below.

3-Methylinosine (2a)——Compound **2c** (1.84 g, 4.51 mmol) was dissolved in a saturated solution (80 ml) of NH_3 in MeOH and the solution was kept at 0 °C overnight. It was concentrated *in vacuo* and treatment of the resulting caramel with MeOH (30 ml) gave **2a** (982 mg, 73% yield) as the monohydrate, mp 172—173 °C (dec.). Recrystallization from MeOH gave an analytical sample as colorless needles, mp 172—173 °C (dec.). UV $\lambda_{max}^{95\%\,EtOH}$ 256 nm (ε 11700); $\lambda_{max}^{H_2O}$ (pH 1) unstable; $\lambda_{max}^{H_2O}$ (pH 7) 259 (13100); $\lambda_{max}^{H_2O}$ (pH 13) unstable. ^{1}H-NMR (Me_2SO-d_6) δ: 3.38 (2H, br, H_2O), 3.64 (2H, br, $C_{(5')}$-H_2), 3.85—4.24 (2H, m, $C_{(4')}$- and $C_{(3')}$-H), 4.00 (3H, s, CH_3), 4.42 (1H, m, $C_{(2')}$-H), 5.13 (1H, br, $C_{(5')}$-OH), 5.30 (1H, d, $J=7$ Hz, $C_{(3')}$-OH), 5.74 (1H, d, $J=7$ Hz, $C_{(2')}$-OH), 6.07 (1H, d, $J=5$ Hz, $C_{(1')}$-H), 8.12 and 8.30 (1H each, s, purine protons). $[\alpha]_D^{26}$ $-44.1\pm0.1°$ ($c=0.368$, H_2O). *Anal.* Calcd for $C_{11}H_{14}N_4O_5\cdot H_2O$: C, 44.00; H, 5.37; N, 18.66. Found: C, 44.24; H, 5.24; N, 18.93.

2',3',5'-Tri-*O*-benzyl-3-methylinosine (2b)——5-(Methylamino)-1-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)-imidazole-4-carboxamide (**5b**)[5,8a] (970 mg, 1.79 mmol) was refluxed with a mixture of ethyl orthoformate and acetic anhydride (8:3, v/v) (40 ml) for 30 min. The mixture was then concentrated *in vacuo* to leave a partly crystallized residue. This was treated with a mixture of C_6H_6 (5 ml) and hexane (5 ml), and the resulting solid was filtered off, dried over P_2O_5 at 2 mmHg and 50 °C for 2 h and then at 75 °C for 2 h to give **2b** (340 mg), mp 127—129 °C (resolidified and melted again at 150—151 °C). The mother liquor was concentrated *in vacuo* to leave a yellow oil. This was chromatographed on silica gel (5 g) using $CHCl_3$–EtOH (10:1, v/v) as an eluant to afford a second crop of **2b** (69 mg, total yield 41%). Recrystallization from EtOH followed by drying over P_2O_5 at 2 mmHg and 50 °C for 1 h and then at 75 °C for 5.5 h gave an analytical sample as colorless needles, mp 150—151 °C (sintered at *ca.* 120 °C). UV $\lambda_{max}^{95\%\,EtOH}$ 259 nm (ε 11800). ^{1}H-NMR ($CDCl_3$) δ: 3.53 and 3.69 (1H each, ABX type q, $J_{AB}=11$ Hz, $J_{AX}=J_{BX}=2.5$ Hz, $C_{(5')}$-H_2), 3.86 (3H, s, CH_3), 4.21 (1H, br, $C_{(4')}$-H), 4.53 (8H, m, $3C_6H_5CH_2$'s, $C_{(2')}$-H, and $C_{(3')}$-H), 6.10 (1H, d, $J=6$ Hz, $C_{(1')}$-H), 7.25 (15H, m, $3C_6H_5$'s), 7.70 and 7.80 (1H each, s, purine protons). $[\alpha]_D^{25}$ $-74.1\pm0.3°$ ($c=0.442$, MeOH). *Anal.* Calcd for $C_{32}H_{32}N_4O_5$: C, 69.55; H, 5.84; N, 10.14. Found: C, 69.46; H, 5.70; N, 9.98.

5-(*N*-Methylformamido)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (4c)——A mixture of acetic anhydride (6 ml) and formic acid (3 ml) was heated at 50 °C for 15 min then cooled. Compound **5c**[3b,5,8a] (490 mg, 1.23 mmol) was dissolved in the mixture, and the solution was kept at room temperature for 1 h. It was concentrated *in vacuo* to leave an oil, which was crystallized from EtOH (7 ml). The resulting precipitate was filtered off, washed with EtOH (4 ml), and dried to give **4c** (434 mg, 83% yield), mp 171—175 °C. Recrystallization from EtOH gave an analytical sample as colorless needles, mp 175.5—176.5 °C. UV $\lambda_{max}^{95\%\,EtOH}$ 230 nm (sh) (ε 9400); $\lambda_{max}^{H_2O}$ (pH 1) 231 (sh) (9100); $\lambda_{max}^{H_2O}$ (pH 7) 231 (sh) (9200); $\lambda_{max}^{H_2O}$ (pH 13) 233 (sh) unstable. ^{1}H-NMR (Me_2SO-d_6) δ: 2.04 (6H, s, $2CH_3CO$'s), 2.10 (3H, s, CH_3CO), 3.01 and 3.23 ($3\times3/4$H and $3\times1/4$H, s, NCH_3), 4.31 (3H, br, $C_{(4')}$-H and $C_{(5')}$-H_2), 5.35 (1H, dd, $J=5$ and 6 Hz, $C_{(3')}$-H), 5.56 (1H, dd, $J=6$ and 6 Hz, $C_{(2')}$-H), 5.71 (1H, d, $J=6$ Hz, $C_{(1')}$-H), 7.28 and 7.48 (1H each, br, NH_2), 8.04 and 8.11 (a total of 7/4H, s, a combination of $C_{(2)}$-H and 3/4CHO), 8.28 (1/4H, s, 1/4CHO).[16] $[\alpha]_D^{27}$ $-24.5\pm0.4°$ ($c=0.601$, MeOH). *Anal.* Calcd for $C_{17}H_{22}N_4O_9$: C, 47.88; H, 5.20; N, 13.14. Found: C, 47.80; H, 5.19; N, 12.85.

5-(*N*-Methylformamido)-1-β-D-ribofuranosylimidazole-4-carboxamide (4a)——i) Compound **4c** (150 mg, 0.352 mmol) was dissolved in a saturated solution (50 ml) of NH_3 in MeOH and the solution was kept at 0 °C for 16 h. Removal of the solvent by evaporation left a colorless oil, which was crystallized from EtOH (2 ml) to give **4a** (93 mg, 88% yield), mp 176—180 °C (dec.). Recrystallization from EtOH gave an analytical sample as colorless needles, mp 183—184 °C (dec.). UV $\lambda_{max}^{95\%\,EtOH}$ 232 (sh) (ε 9200); $\lambda_{max}^{H_2O}$ (pH 1) 232 (sh) (8400); $\lambda_{max}^{H_2O}$ (pH 7) 232 (sh) (8800); $\lambda_{max}^{H_2O}$ (pH 13) 233 (sh) unstable. ^{1}H-NMR (Me_2SO-d_6) δ: 3.02 and 3.24 ($3\times3/4$H and $3\times1/4$H, s, CH_3), 3.55 (2H, br, $C_{(5')}$-H_2), 3.80—4.40 (3H, m, $C_{(4')}$-, $C_{(3')}$-, and $C_{(2')}$-H), 5.00 (1H, br, $C_{(5')}$-OH), 5.20 (1H, br, $C_{(3')}$-OH), 5.25 (1H, d, $J=6$ Hz, $C_{(1')}$-H), 5.44 (1H, d, $J=6$ Hz, $C_{(2')}$-OH), 7.20 and 7.37 (1H each, br, NH_2), 7.98 and 8.02 (a total of 7/4H, s, a combination of $C_{(2)}$-H and 3/4CHO), 8.20 (1/4H, s, 1/4CHO).[16] $[\alpha]_D^{27}$ $-46.5\pm0.3°$ ($c=0.505$, H_2O). *Anal.* Calcd for $C_{11}H_{16}N_4O_6$: C, 44.00; H, 5.37; N, 18.66. Found: C, 44.10; H, 5.52; N, 18.46.

ii) A solution of **2a**·H_2O (60 mg, 0.2 mmol) in 0.01 N aq. NaOH (5 ml) was kept at room temperature for 1 h and then neutralized with 0.1 N aq. HCl. It was concentrated *in vacuo* to leave a colorless caramel, which was crystallized from H_2O to give **4a** (14 mg, 23% yield) as colorless needles, mp 182—184 °C (dec.), identical with the analytical sample described above.

The stability of **4a** under basic conditions was studied. Although **4a** was found to be stable in 0.1 M phosphate buffer (pH 10.82, ionic strength 1.0), at 25 °C for 5 h, the UV spectrum of **4a** in 0.1 N aq. NaOH changed at room temperature to become superimposable on that of **5a**, showing an isosbestic point at 246 nm. When **4a** (30 mg, 0.1 mmol) was dissolved in 0.1 N aq. NaOH (10 ml), it disappeared in about 10 h. The solution was neutralized with 1 N aq. HCl and concentrated *in vacuo*. The residue was adsorbed on silica gel (0.3 g) and placed on top of a silica gel

(0.3 g) column. Elution with EtOAc–EtOH (4 : 1, v/v) gave **5a** (27 mg, 100% yield), mp 177—179 °C, identical with an authentic sample.[8]

1,2-Dihydro-3-methylinosine (6)——Compound **2a**·H_2O (300 mg, 1 mmol) was hydrogenated over 10% Pd–C (300 mg) in H_2O (20 ml) at room temperature for 3 h. The catalyst was filtered off and washed with H_2O (40 ml). The filtrate and washings were combined and concentrated *in vacuo* to leave a partly crystallized caramel. This was crystallized by treatment with cold EtOH–Me_2CHOH (1 : 1, v/v) (6 ml), and the resulting precipitate was collected by filtration, washed with the mixed solvent (3 ml), and dried to give **6** (218 mg, 77% yield), mp 181—190 °C (dec.). Recrystallization from EtOH gave an analytical sample as colorless prisms, mp 189—190 °C (dec.). UV $\lambda_{max}^{95\%\ EtOH}$ 265 nm (ε 5000); $\lambda_{max}^{H_2O}$ (pH 1) 261 (4900); $\lambda_{max}^{H_2O}$ (pH 7) 267 (4800); $\lambda_{max}^{H_2O}$ (pH 13) 268 (4900). ^{1}H-NMR (Me_2SO-d_6) δ: 2.76 (3H, s, CH_3), 3.58 (2H, br, $C_{(5')}$-H_2), 3.91 (1H, m, $C_{(4')}$-H), 4.06 (1H, m, $C_{(3')}$-H), 4.38 (3H, br, $C_{(2)}$-H_2 and $C_{(2')}$-H), 4.99 (1H, br, $C_{(5')}$-OH), 5.17 (1H, br, $C_{(3')}$-OH), 5.39 (1H, d, J=6 Hz, $C_{(1')}$-H), 5.47 (1H, br, $C_{(2')}$-OH), 7.25 (1H, br, NH), 7.81 (1H, s, $C_{(8)}$-H). $[\alpha]_D^{27}$ −34.9° (c=0.404, H_2O). *Anal.* Calcd for $C_{11}H_{16}N_4O_5$: C, 46.47; H, 5.67; N, 19.71. Found: C, 46.37; H, 5.77; N, 19.97.

The stability of **6** under acidic conditions was investigated. No UV spectral change was observed for a solution of **6** in 0.1 N aq. HCl at 25 °C for 10 h. Even in 1 N aq. HCl, **6** was shown to be stable at room temperature by paper chromatography. A solution of **6** (50 mg) in 1 N aq. HCl (5 ml) was allowed to stand at room temperature for 6 h and then passed through a column of Amberlite IRA-410 (HCO_3^-) (3 ml). The column was eluted with H_2O (40 ml). The combined eluate was concentrated *in vacuo* and the resulting caramel was treated with EtOH to recover **6** (47 mg, 94%). When a solution of **6** in 1 N aq. HCl was refluxed for 1 h, many products were found by paper chromatography. Similar results were obtained with a solution in 0.1 N aq. HCl.

3-Methylhypoxanthine (8)——i) A solution of **2a**·H_2O (50.0 mg, 0.167 mmol) in H_2O (20 ml) was adjusted to pH 3.0 with 0.01 N aq. HCl, kept at 40 °C for 24 h, and then passed through a column of Amberlite IRA-410 (HCO_3^-) (1 ml). The column was eluted with H_2O (15 ml). The combined eluate was concentrated *in vacuo* and the residue was crystallized from H_2O (0.5 ml) to give **8** (10.2 mg, 35% yield) as colorless needles, mp 298—302 °C, identical with an analytical sample of the sesquihydrate described below.

ii) 3-Methyladenine (**7**)[9] (1.79 g, 12 mmol) was dissolved in 0.8 N aq. HCl (450 ml) and the solution was kept at 50—55 °C. An aqueous solution of $NaNO_2$ (8.3 g in 175 ml) (35 ml) was added to the warm solution. At 1 h intervals, two 35-ml portions and a 17.5-ml portion of the $NaNO_2$ solution were added and the solution was kept at 50—55 °C for a further 3.5 h. The mixture was then concentrated *in vacuo* and the solid residue was dissolved in hot H_2O (14 ml). The solution was brought to pH 8 with 10% aq. NaOH and cooled. The resulting precipitate was filtered off, washed with cold H_2O (6 ml), and dried to give **8**·3/2H_2O (1.44 g, 68% yield) as colorless needles, mp 300—303 °C (dec.). Recrystallization from 50% v/v aq. EtOH and drying over P_2O_5 at 2 mmHg and 90 °C for 6 h gave an analytical sample of anhydrous **8**, mp 303—305 °C (dec.) [lit.[17] mp 307—309 °C (dec.)]. UV $\lambda_{max}^{95\%\ EtOH}$ 267 nm (ε 13200); $\lambda_{max}^{H_2O}$ (pH 1) 254 (11700); $\lambda_{max}^{H_2O}$ (pH 7) 265 (14600); $\lambda_{max}^{H_2O}$ (pH 13) 265 (11300). ^{1}H-NMR (Me_2SO-d_6) δ: 3.79 (3H, s, CH_3), 8.15 and 8.25 (1H each, s, purine protons), 13.44 (1H, br, NH). *Anal.* Calcd for $C_6H_6N_4O$: C, 48.00; H, 4.03; N, 37.32. Found: C, 47.95; H, 3.97; N, 37.37. This sample was exposed to air until constant weight was reached to give an analytical sample of **8** as the sesquihydrate. *Anal.* Calcd for $C_6H_6N_4O$·3/2H_2O: C, 40.68; H, 5.12; N, 31.63. Found: C, 40.70; H, 4.87; N, 31.59.

iii) A solution of **2b** (55 mg, 0.1 mmol) in AcOH (1 ml) was heated under reflux for 30 min and then concentrated *in vacuo*. The residue was partitioned between H_2O (5 ml) and C_6H_6 (5 ml). The aqueous layer was washed with C_6H_6 (2 × 5 ml) and concentrated to give a colorless solid, which was dried over P_2O_5 at 2 mmHg and room temperature for 20 h to give **8**·3/2H_2O (16 mg, 89% yield).

Rate Studies of the Glycosidic Bond Cleavage of 2a——Compound **2a** was found to be quantitatively converted into **8** under acidic conditions. The progress of the reaction was followed by HPLC since no suitable wavelength for spectrophotometric analysis was found owing to the close resemblance of the UV spectra of the substrate and the product. The two compounds were well resolved on a μBondapak C_{18} column using MeOH–H_2O (1 : 9, v/v) as an eluent at a flow rate of 0.8 ml/min. Concentrations of **2a** in the reaction mixtures were calculated by using a calibration line which had been constructed by plotting the ratio of known concentrations of **2a** and **8** against the ratio of peak heights of the two components.

A solution of **2a**·H_2O (12.0 mg) in 0.1 M HCl–KCl buffer (pH 2.01 at 25 °C) (20 ml) was kept at 25 °C (accurate to ±0.05 °C). At intervals, aliquots (*ca.* 1 ml) of the solution were withdrawn and mixed with 0.05 M phosphate buffer of pH 7 (5 ml) with as little delay as possible. The resulting solutions (15-μl portions) were analyzed by HPLC. Good pseudo-first-order kinetics [k_{obs} 5.7×10^{-2} min^{-1} ($t_{1/2}$ 12 min)] was obtained (Fig. 2).

Since the reaction of **2a** in 0.1 N aq. HCl proceeds too rapidly to allow measurement by the method described above, the solution in "0.1 N aq. HCl" was approximately prepared as follows. Compound **2a**·H_2O (39.6 mg) was made up to 100 ml with H_2O. An aliquot (4.5 ml) of this solution was transferred to a small vial with a cap. An open small vessel containing 1 N aq. HCl (0.5 ml) was floated on the solution in the vial. The apparatus was kept at 25 ± 0.05 °C, and the reaction was initiated by mixing the two separate solutions with vigorous shaking. After a set time the reaction was quenched by quick addition of 0.5 M aq. Na_2HPO_4 (2 ml). A portion (15 μl) of the resulting solution was analyzed by HPLC. This operation was repeated for various reaction times and the concentrations of **2a**

were determined to give k_{obs} 8.7×10^{-1} min^{-1} ($t_{1/2}$ 48 s) (Fig. 2).

Rates of Ring Cleavage of 2a and 3a——As a solution of **2a** in 0.1 M phosphate buffer (pH 10.82 at 25 °C, ionic strength 1.0) gave **4a** as a sole product, the reaction was followed by UV spectroscopy. Compound **2a** (11.015 mg) was dissolved in the phosphate buffer and the total volume was made up to 50 ml. The solution was kept at 25 ± 0.05 °C. At 15—60 min intervals, 2-ml aliquots were withdrawn and diluted with 0.05 M phosphate buffer of pH 7 to 20 ml to quench the reaction. The optical densities of the diluted solutions were determined at 260 nm. The change in optical density was completed within 6 h and the absorbance finally reached that of an equimolar solution of **4a**. Good pseudo-first-order kinetics with k_{obs} 2.0×10^{-2} min^{-1} ($t_{1/2}$ 34 min) was obtained (Fig. 1).

The ring-opening of **3a** under the same conditions was also found to obey pseudo-first-order kinetics and k_{obs} 6.5×10^{-3} min^{-1} ($t_{1/2}$ 106 min) was obtained by similar analysis at 261 nm (Fig. 1).

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