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Purines. XVIII.*,[1] Kinetic Studies of the Dimroth Rearrangement of 1-Alkoxy-9-methyladenines and 1-Benzyloxyadenosine: Effect of 1-Benzyloxy and 9-β-D-Ribofuranosyl Groups on the Rates of the Ring-opening and the Reclosure[2]

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Rates of the Dimroth rearrangements of 1-benzyloxyadenosine (Id) and 1-alkoxy-9-methyladenines (Ia—c), which give the rearranged products (IIIa—d) and the deformylated compounds (IVa—d) competitively through the isolable monocyclic intermediates (IIa—d), have been measured at different pH's and ionic strength 1.0 at 40°. It has been shown that all reactions obey good pseudo-first-order kinetics. The ring-opening (I→II) follows the rate law given by $k^{(0)}_{II,\,obsd}[I]_{total}=k^{(0)}_{II,\,ionic}[I\cdot H^+][OH^-]+k^{(0)}_{II,\,neut}[I][OH^-]$ where $k^{(0)}_{II,\,obsd}$ is the observed limiting rate constant for zero buffer concentration; $[I\cdot H^+]$ is the concentration of the protonated base; [I], the concentration of the free base; $[I]_{total}$, the sum of $[I\cdot H^+]$ and [I]. Comparison of the individual second-order rate constants thus obtained (Table III) has revealed that attack of hydroxide ion on the protonated species is faster than on the neutral species by a factor of 560—1200 and that both reaction modes are promoted by the β-D-ribofuranosyl substituent at the 9-position. Both the ring-closure (II→III) and the deformylation (II→IV) in 0.1 M buffer solutions are enhanced by the β-D-ribofuranosyl group at the 1-position of II, whereas the benzyl group on the O-atom of the amidoxime moiety of II exerts the stabilizing effect on II. The unusually low pK_a's of 9.9 to 10.6 at 40° observed for II have been assigned to the acidic ones of the formamido group of II. Probable mechanisms for the ring-closure and the deformylation of II are also described.

1-Alkoxyadenine derivatives (type I) have been known to undergo Dimroth rearrangement[4] in aqueous solution, giving N-alkoxyadenines (type III) through isolable monocyclic intermediates (type II) which afford deformylated products (type IV) competitively (Chart 1).[5] Our previous finding revealed that at pH 7.60 and above 1,9-dimethyladenine rearranges more rapidly than 1-methoxy-9-methyladenine (Ia), although the latter undergoes ring-opening *ca.* 30 times as fast as the former.[6] The acceleration of the ring-opening step (Ia→IIa) and the retardation of the recyclization step (IIa→IIIa) observed for Ia could be attributed directly to the electron-withdrawing nature of the attached methoxyl group. We also found that replacement of the 1-methyl group of the protonated 1,9-dimethyladenine by the less electron-donating benzyl group or substitution of the β-D-ribofuranosyl group for the 9-methyl group multiplied the rate of the rearrangement by a factor of five.[1] In view

* Dedicated to the memory of Prof. Eiji Ochiai.

1) Paper XVII in this series, T. Fujii, T. Itaya, and T. Saito, *Chem. Pharm. Bull.* (Tokyo), **23**, 54 (1975).

2) Presented in part at the 1st Symposium on Nucleic Acid Chemistry, Osaka, October, 1973.

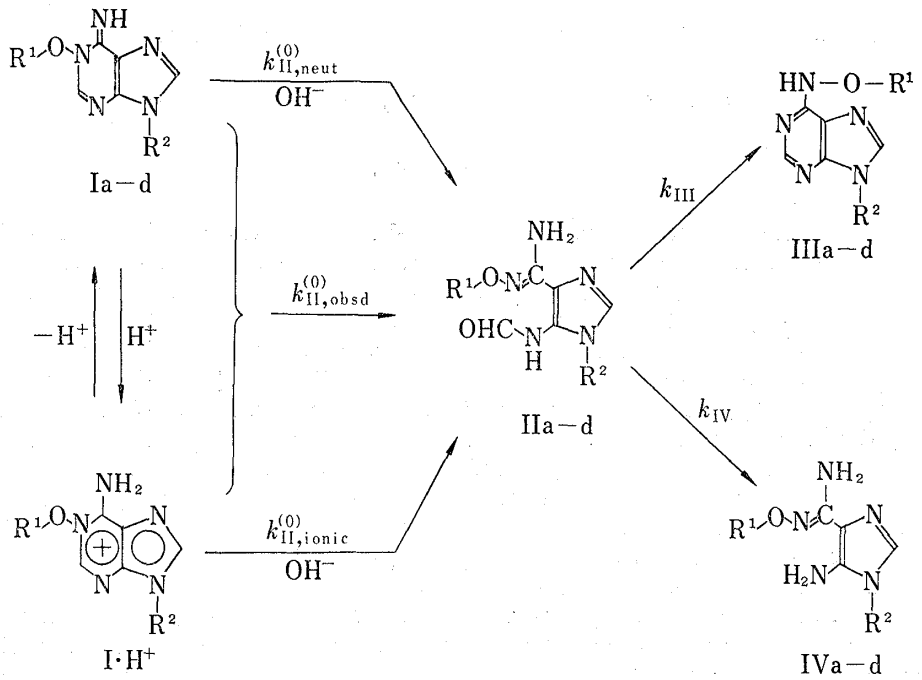
3) Location: *13-1 Takara-machi, Kanazawa 920, Japan.*

4) *a*) D.J. Brown, "Mechanisms of Molecular Migrations," Vol. 1, ed. by B.S. Thyagarajan, Interscience Publishers, New York, 1968, pp. 209—245; *b*) J.H. Lister, "Fused Pyrimidines. Part II. Purines," ed. by D.J. Brown, Wiley-Interscience, New York, 1971, pp. 313—315.

5) *a*) T. Fujii, T. Itaya, C.C. Wu, and S. Yamada, *Chem. Ind.* (London), **1966**, 1967; *b*) T. Fujii, T. Itaya, C.C. Wu, and F. Tanaka, *Tetrahedron*, **27**, 2415 (1971); *c*) T. Fujii, T. Itaya, and S. Moro, *Chem. Pharm. Bull.* (Tokyo), **20**, 1818 (1972); *d*) T. Fujii, C.C. Wu, T. Itaya, S. Moro, and T. Saito, *ibid.*, **21**, 1676 (1973).

6) T. Itaya, F. Tanaka, and T. Fujii, *Tetrahedron*, **28**, 535 (1972).

of synthetic utility of the compounds II,[7] III,[8,9] and IV[10] and potential applicability of the rearrangement of I to chemical modification of nucleic acids, it is important to know more about the substituent effect on each step of the reaction sequence sketched in Chart 1. The present paper describes the results of kinetic studies on the ring-opening of 1-alkoxy-9-methyladenines (Ia—c) and 1-benzyloxyadenosine (Id) and the ring-closure and hydrolysis of the monocyclic intermediates (IIa—d).



a : $R^1=R^2=CH_3$
b : $R^1=C_2H_5$; $R^2=CH_3$
c : $R^1=C_6H_5CH_2$; $R^2=CH_3$
d : $R^1=C_6H_5CH_2$; $R^2=\beta$-D-ribofuranosyl

Chart 1

Experimental

The nuclear magnetic resonance (NMR) spectra were determined in hexadeuterated dimethyl sulfoxide with a JEOL-JNM-C-60H spectrometer using tetramethylsilane as an internal standard. See also ref. 1 for details of instrumentation and measurement. We are indebted to Mr. Y. Itatani and Miss S. Toyoshima at Kanazawa University for microanalyses and NMR spectral data.

Materials——Of analytically pure samples used, the following compounds were prepared according to the reported procedure: the perchlorates of 1-methoxy- (Ia),[11] 1-ethoxy-9-methyladenine (Ib),[6] and 1-benzyloxyadenosine (Id);[12] N′-methoxy-1-methyl- (IIa),[5b] N′-ethoxy-1-methyl- (IIb),[5b] and N′-benzyloxy-1-β-D-ribofuranosyl-5-formamidoimidazole-4-carboxamidine (IId);[5d] N-methoxy- (IIIa) and N-ethoxy-9-methyladenine (IIIb);[5b] N-benzyloxyadenosine (IIId);[5d] N′-methoxy- (IVa) and N′-ethoxy-1-methyl-5-aminoimidazole-4-carboxamidine (IVb).[5b] Other compounds were obtained in the manner described below.

1-Benzyloxy-9-methyladenine Perchlorate (Ic·$HClO_4$)——A mixture of 9-methyladenine 1-oxide[12,13] (4.13 g, 25 mmoles), benzyl bromide (21.6 g, 126 mmoles), and N,N-dimethylacetamide (50 ml) was stirred

7) T. Fujii, T. Itaya, K. Mohri, and T. Saito, *J. Chem. Soc., Chem. Comm.*, **1973**, 917.
8) T. Fujii, F. Tanaka, K. Mohri, T. Itaya, and T. Saito, *Tetrahedron Letters*, **1973**, 4873.
9) T. Ueda, M. Imazawa, K. Miura, R. Iwata, and K. Odajima, *Tetrahedron Letters*, **1971**, 2507.
10) *a*) J.A. Montgomery and H.J. Thomas, *Chem. Commun.*, **1969**, 458; *b*) *Idem*, *J. Med. Chem.*, **15**, 182 (1972); *c*) R.B. Meyer, Jr., D.A. Shuman, R.K. Robins, J.P. Miller, and L. N. Simon, *ibid.*, **16**, 1319 (1973).
11) T. Fujii and T. Itaya, *Tetrahedron*, **27**, 351 (1971).
12) T. Fujii, C.C. Wu, and T. Itaya, *Chem. Pharm. Bull.* (Tokyo), **19**, 1368 (1971).
13) T. Fujii, T. Itaya, and S. Moro, *Chem. Pharm. Bull.* (Tokyo), **20**, 958 (1972).

at room temperature for 20 hr. Benzene (50 ml) was added to the mixture and the precipitates that separated were collected by filtration and washed with ether (50 ml). The solid was dissolved in H_2O (100 ml) and 15% (w/v) aq. NH_4ClO_4 (30 ml) was added to the solution. After cooling in an ice-water bath, the precipitates that separated were filtered off, washed successively with a small volume of H_2O and ethanol, and dried to give colorless pillars (7.82 g, 88%), mp 193—194° (decomp.). Recrystallization from 50% (v/v) aq. ethanol yielded colorless pillars, mp 195—196° (decomp.); pK_a (see Table I); UV $\lambda_{max}^{95\%\ EtOH}$ 261 nm (ε 12300); $\lambda_{max}^{H_2O}$ (pH 1) 261 (12200); $\lambda_{max}^{H_2O}$ (pH 7) 261 (12200); $\lambda_{max}^{H_2O}$ (pH 13) 258 (12600), 264 (sh) (11600); NMR τ: 6.16 (3H, s, CH_3), 4.59 (2H, s, $C_6H_5C\underline{H}_2$), 2.50 (5H, m, C_6H_5), 1.54 and 1.17 (1H each, s, purine protons), 0.0 (1H, b, NH). *Anal.* Calcd. for $C_{13}H_{14}O_5N_5Cl$: C, 43.89; H, 3.97; N, 19.69. Found: C, 43.75; H, 4.04; N, 19.60.

N′-Benzyloxy-1-methyl-5-formamidoimidazole-4-carboxamidine (IIc)——A mixture of the perchlorate (Ic·$HClO_4$) (3.56 g, 10 mmoles) and 0.5 M carbonate buffer (pH 9.5, 200 ml) was stirred at 41° for 4.5 hr. After cooling, the precipitates that separated were filtered off, washed with a small amount of H_2O, and dried to afford a colorless solid (2.22 g, 81%), mp 148—151°. Recrystallization from 50% (v/v) aq. ethanol gave colorless prisms, mp 156—157°; pK_a (Table I); UV $\lambda_{shoulder}^{95\%\ EtOH}$ 223 nm (ε 15100), 256 (7500); $\lambda_{max}^{H_2O}$ (pH 1) 254 (8100); $\lambda_{shoulder}^{H_2O}$ (pH 7) 250 (7200); $\lambda_{max}^{H_2O}$ (pH 13) 259 (12100); IR $\nu_{max}^{CHCl_3}$ (0.005 M solution) cm^{-1}: 3505 (NH_2), 3390 (NH_2, CONH), 1705 (CONHAr); NMR τ: 6.58 and 6.54 (s each, CH_3's due to *cis–trans* isomerism[5a,b,d,14,15] of IIc), 5.09 and 5.03 (s each, $C_6H_5C\underline{H}_2$'s due to *cis–trans* isomerism of IIc), 4.30 (2H, slightly dull, NH_2), 2.67 (5H, s, C_6H_5), 2.40 (1H, s, $C_{(2)}$–H), 1.89 (d, J=11 Hz, HCON, *trans*-IIc), 1.80 (s, HCON, *cis*-IIc), 0.70 (d, J=11 Hz, CONH, *trans*-IIc), 0.46 (CONH, *cis*-IIc). *Anal.* Calcd. for $C_{13}H_{15}O_2N_5$: C, 57.13; H, 5.53; N, 25.63. Found: C, 56.92; H, 5.52; N, 25.70.

N-Benzyloxy-9-methyladenine (IIIc)——A suspension of Ic·$HClO_4$ (178 mg, 0.5 mmole) in 0.5 M phosphate buffer (pH 6.5, 10 ml) was refluxed for 2 hr. After cooling, the precipitates that formed were separated by filtration, washed successively with H_2O (10 ml) and ethanol (3 ml), and dried to furnish a colorless solid (84 mg, 66%), mp 231—233° (decomp.). Recrystallization from ethanol gave colorless plates, mp 232—234° (decomp.); UV $\lambda_{max}^{95\%\ EtOH}$ 270 nm (ε 14900); $\lambda_{max}^{H_2O}$ (pH 1) 273 (14200); $\lambda_{max}^{H_2O}$ (pH 7) 270 (16500); $\lambda_{max}^{H_2O}$ (pH 13) 286 (12600). *Anal.* Calcd. for $C_{13}H_{13}ON_5$: C, 61.16; H, 5.13; N, 27.44. Found: C, 61.30; H, 5.19; N, 27.51.

N′-Benzyloxy-1-methyl-5-aminoimidazole-4-carboxamidine (IVc)——A mixture of 1-benzyloxy-9-methyladenine hydrobromide hemihydrate (Ic·HBr·1/2H_2O)[12] (863 mg, 2.5 mmoles) and NaOH (0.75 g) in H_2O (15 ml) was refluxed for 15 min. After cooling, the mixture was neutralized with 20% aq. HCl and filtered. The filter cake was purified by silica gel (50 g) column chromatography using ethyl acetate–ethanol (6: 1, v/v) as eluent. The UV-absorbing fractions that appeared first were combined and removal of the solvent gave a slightly brown solid (501 mg, 82%), mp 129—135°. Recrystallization from ethyl acetate yielded colorless prisms, mp 138—140°; UV $\lambda_{max}^{95\%\ EtOH}$ 265 nm (ε 12100); $\lambda_{max}^{H_2O}$ (pH 1) 281 (9200); $\lambda_{max}^{H_2O}$ (pH 7) 264 (10000); $\lambda_{max}^{H_2O}$ (pH 13) 264 (10000); NMR τ: 6.76 (3H, s, CH_3), 5.23 (2H, s, $C_6H_5C\underline{H}_2$), 4.85 (2H, dull, NH_2), 4.61 (2H, slightly dull, NH_2), 3.05 (1H, s, $C_{(2)}$–H), 2.78 (5H, s, C_6H_5). *Anal.* Calcd. for $C_{12}H_{15}ON_5$: C, 58.76; H, 6.16; N, 28.56. Found: C, 58.51; H, 6.23; N, 28.41.

The second UV-absorbing eluate was collected and evaporated to dryness, giving a slightly brown solid (71 mg, 11%), mp 224—226° (decomp.). Recrystallization from ethanol gave colorless plates, mp 232—234° (decomp.), which were identified with the sample of IIIc described above by comparison of IR spectrum and by mixed melting-point test.

The picrate of IVc was prepared from IVc (50 mg) by dissolving it in ethanol (1 ml) and adding a saturated solution (1 ml) of picric acid in ethanol. The precipitates that separated were filtered off and washed with a little ethanol to give a yellow solid (97 mg, 96%, as a hemiethanolate), mp 200—201° (decomp.). Recrystallization from ethanol and drying over P_2O_5 at 2 mmHg and room temperature for 24 hr afforded yellow needles; mp 201—202° (decomp.). *Anal.* Calcd. for $C_{18}H_{18}O_8N_8 \cdot 1/2C_2H_5OH$: C, 45.87; H, 4.26; N, 22.52. Found: C, 45.81; H, 4.17; N, 22.66.

N′-Benzyloxy-1-β-D-ribofuranosyl-5-aminoimidazole-4-carboxamidine Picrate (IVd·picrate)——A mixture of 1-benzyloxyadenosine perchlorate monohydrate (Id·$HClO_4 \cdot H_2O$)[12] (984 mg, 2 mmoles) and NaOH (0.60 g) in H_2O (10 ml) was refluxed for 15 min. After cooling, the solution was neutralized with 10% aq. HCl. To the solution was added 1 N NaOH (2 ml) and the total volume was adjusted to 20 ml with H_2O. The aqueous solution was extracted quickly with ethyl acetate (20 ml) and the organic layer was dried over $MgSO_4$ and evaporated to dryness. The resulting residual caramel was purified by silica gel (50 g) column chromatography by using ethyl acetate–ethanol (10: 1, v/v) as eluent. Thin-layer chromatographically pure fractions were combined and removal of the solvent gave a colorless glass. This was dissolved in warm ethanol (2 ml) and a saturated solution (10 ml) of picric acid in ethanol was added. The precipitates that separated were filtered off, washed with ethanol (1 ml), and dried to give yellow needles (240 mg, 20%), mp 142—145° (decomp.). Recrystallization from ethanol gave yellow needles, mp 154—

14) T. Fujii, T. Sato, and T. Itaya, *Chem. Pharm. Bull.* (Tokyo), **19**, 1731 (1971).
15) T. Fujii, F. Tanaka, K. Mohri, and T. Itaya, *Chem. Pharm. Bull.* (Tokyo), **22**, 2211 (1974).

156° (decomp.) (lit.[10b] mp 146—150°). *Anal.* Calcd. for $C_{22}H_{24}O_{12}N_8$: C, 44.60; H, 4.08; N, 18.91. Found: C, 44.58; H, 4.18; N, 18.73.

Kinetic Studies——Buffer solutions used for kinetic runs were 0.05, 0.10, and 0.15 M NaH_2PO_4—Na_2HPO_4 (pH 6.20 at 40°); 0.05, 0.10, 0.15, and 0.30 M NaH_2PO_4—Na_2HPO_4 (pH 7.00 and 7.80 at 40°); 0.05, 0.10, 0.15, and 0.30 M $NaHCO_3$—Na_2CO_3 (pH 9.00 and 10.00 at 40°); 0.05, 0.10, and 0.15 M Na_2HPO_4—Na_3PO_4 (pH 11.00 and 11.40 at 40°); and were brought to ionic strength 1.0 with KCl. All the reactions were followed with at least six determinations and the pH was found never to vary more than ±0.02 unit.

Since the ring-opening step (I→II) proceeded rapidly enough relative to the subsequent steps (III←II→IV), it was possible to treat it approximately as a separate one from the other. The perchlorates (Ia—d) were individually dissolved in the buffer solutions (0.10 to 0.30 M and pH 7.00 to 11.40) at a concentration of *ca.* 6×10^{-4} M. Aliquots (*ca.* 3 ml) of the solutions were sealed in ampoules and kept at 40±0.05°. At intervals the ampoules were removed, cooled, and broken and the contents were diluted with 0.17 M citrate buffer (pH 5.80 at 20°, adjusted to ionic strength 1.0 with KCl) by a factor of 10 to quench the reaction. Concentrations of the components of the diluted solutions were determined spectrophotometrically at 260 nm in the manner described before.[6]

For the determination of the rates of the cyclization of II to III and the deformylation of II to IV, experiments starting with 6.5×10^{-4} to 8.5×10^{-4} M solutions of II in various 0.1 M buffers at pH 6.20 to 11.40 were carried out as described above. After cooling, the reaction mixtures were diluted tenfold with 0.1 M tartaric acid—0.01 N HCl (for the reactions of IIa, b) or 0.2 M H_3PO_4 (for the reactions of IIc, d). In the case of IIa, similar kinetic runs were performed in 1 N NaOH and in 0.05 and 0.15 M buffers at different pH's. Analysis of the resulting three-component system was performed by measuring optical densities at the isoabsorptive point of II and IV and at that of III and IV. The isoabsorptive points and the molecular extinction coefficients at them utilized for the analysis were determined by measurement of ultraviolet (UV) spectra of pure samples of the three components in mixed solvents prepared by diluting the requisite buffers by a factor of 10 with 0.1 M tartaric acid—0.01 N HCl (for IIa, b, IIIa, b, and IVa, b) or 0.2 M H_3PO_4 (for IIc, d, IIIc, d, and IVc, d). Fig. 1 and 2 illustrate the UV spectra of IIb, IIIb, and IVb in 0.1 M tartaric acid—0.01 N HCl and those of IId, IIId, and IVd in 0.2 M H_3PO_4, which are essentially identical with those in the mixed solvents mentioned above. Since IVd failed in crystallization in the form of either the free base or the salt with a transparent anion, the UV spectrum of IVd was obtained by subtracting molecular extinction coefficients of picric acid from those of IVd·picrate at the wavelength involved.[16]

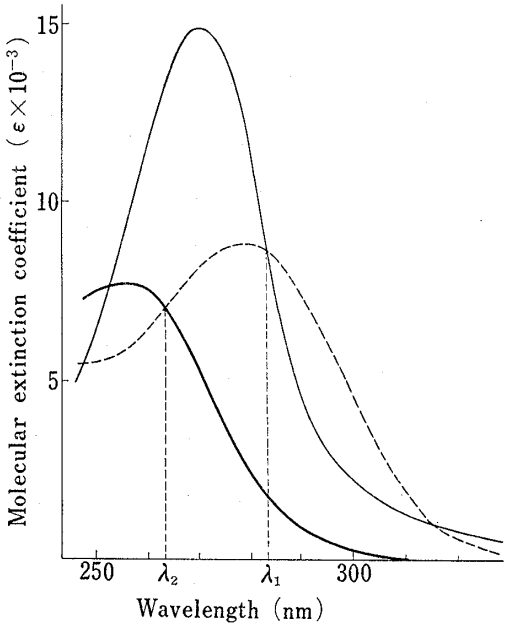


Fig. 1. UV Spectra of the Ethoxy Derivatives (IIb, IIIb, IVb) in 0.1 M Tartaric Acid—0.01 N HCl (pH 1.8) at 20°

——: IIb, ——: IIIb, ·····: IVb

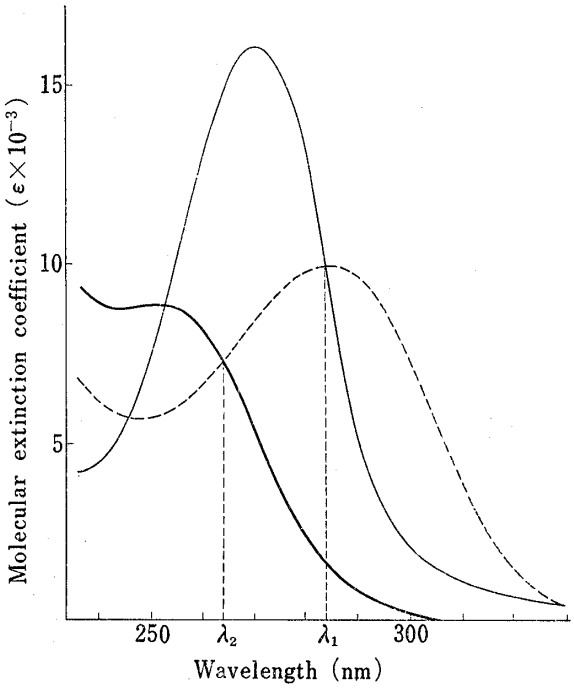


Fig. 2. UV Spectra of the Nucleosides (IId, IIId, IVd) in 0.2 M H_3PO_4 (pH 1.4) at 20°

——: IId, ——: IIId, ·····: IVd

16) This treatment may be justified by the analogy that the same process for the spectra of IVc·picrate and of picric acid in 0.2 M H_3PO_4 produced a spectrum identical with that of the free base (IVc) itself in the range of 235 to 360 nm.

Spectrophotometric Determination of Acid Dissociation Constants of Ia—d and IIa—d (Table I)—— The pK_a's at 40° and ionic strength 1.0 were determined spectrophotometrically at the wavelength indicated in Table I in a manner similar to that described previously.[1] The pH regions covered by individual buffer systems at 40° were 2.0—3.0, H_3PO_4—NaH_2PO_4; 3.2—4.2, formic acid—sodium formate; 4.4—4.6, acetic acid—sodium acetate; 5.5—8.5, NaH_2PO_4—Na_2HPO_4; 8.8—10.7, $NaHCO_3$—Na_2CO_3; 10.9—11.3, Na_2HPO_4—Na_3PO_4. Absorbances of pure species of the anions of IIa—c were determined in 0.5 N NaOH and absorbance of the anion of IId was obtained in 0.1 N NaOH. The NaOH solutions had been brought to ionic strength 1.0 with KCl. Similarly, the absorbances of the neutral molecules of IIa—d were determined in 0.1 M phosphate buffer at pH 7.0 and those of the protonated species, in 0.1 M H_3PO_4 (for IIa—c) or 0.1 N HCl (for IId). Table I assembles the pK_a's of compounds I and II.

Table I. Acid Dissociation Constants of I and II

Compound	pK_a at 40° and ionic strength 1.0			
	Basic	Analytical wavelength (nm)	Acidic	Analytical wavelength (nm)
Ia·$HClO_4$	8.48±0.06[a]	300	—	—
Ib·$HClO_4$	8.50±0.04	300	—	—
Ic·$HClO_4$	8.30±0.06	300	—	—
Id·$HClO_4$	7.90±0.05	300	—	—
IIa	3.78±0.08[c]	255	10.36±0.04[b]	258
IIb	3.91±0.11[c]	255	10.42±0.06	256
IIc	3.67±0.09[c]	254	10.60±0.06	260
IId	2.94±0.10[c]	252	9.93±0.04	252

a) at ionic strength 0.5, pK_a 8.44±0.04[6]
b) pK_a 10.77±0.07, determined titrimetrically at 0.01 M and 26°
c) The considerably large error may be attributed to the difficulty of determining the absorbance of the protonated species of II, which stems from instability of II·H^+ at low pH and/or coexistence of a doubly protonated species.

Results

It may be seen from Table I that the benzyl and the β-D-ribofuranosyl group affect the acid dissociation constants of compounds of type I and those of type II. The effect of the benzyl group was small and the 1-benzyloxy derivative (Ic) showed pK_a value lower than that of the 1-methoxy derivative (Ia) by *ca.* 0.2 pK_a unit. The benzyloxy derivative (IIc) also showed basic pK_a lower than that of the methoxy (IIa) or the ethoxy derivative (IIb) by *ca.* 0.1—0.2 pK_a unit, whereas IIc had acidic pK_a higher than that of IIa or IIb by *ca.* 0.2 pK_a unit. The effect of the β-D-ribofuranosyl group was found to be larger: the 1-benzyloxy nucleoside (Id) showed pK_a lower than that of the corresponding 9-methyl derivative (Ic) by *ca.* 0.4 pK_a unit; the imidazole nucleoside (IId) had basic and acidic pK_a's lower than those of the 1-methyl analog (IIc) by *ca.* 0.7 pK_a unit, respectively.

Figure 3 shows typical first-order plots for the ring-opening of Id in six different 0.1 M buffers at pH 7.00 to 11.40 at 40° and ionic strength 1.0. The reaction obeyed fairly good pseudo-first-order kinetics at all pH's through at least two half-lives. A similar treatment of data obtained from the reaction in 0.15 and 0.30 M buffer solutions showed that the rate constant was approximately proportional to the buffer concentration at constant pH, but the catalytic coefficients of the buffer components were small. Extrapolation of the plot of the rate constants *vs.* buffer concentration to zero buffer concentration gave the limiting rate constant, $k^{(0)}_{II,obsd}$, at the pH involved. Kinetic runs of the other substrates (Ia—c) were also handled in a similar manner.

Table II assembles the limiting rate constants for zero buffer concentration for the ring-opening of Ia—d. It may be seen that the reaction rate increased with increasing pH of the reaction medium in every case. The 1-methoxy derivative (Ia) underwent ring-opening more

rapidly than did the 1-ethoxy derivative (Ib) at all pH's. At pH 9.00 and above Ia also underwent ring-opening more rapidly than the 1-benzyloxy derivative (Ic) although the reaction of the latter proceeded faster at pH 7.80. The 1-benzyloxy nucleoside (Id) underwent ring-opening more rapidly than the corresponding 9-methyl derivative (Ic), in agreement with the previously observed relationship between the Dimroth rearrangement rate of 1-alkyl-9-methyladenine and that of 1-alkyladenosine.[1)]

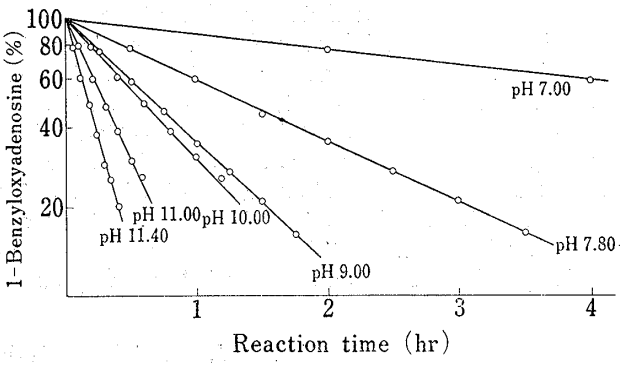


Fig. 3. First-Order Plot for the Ring-Opening of Id to IId in 0.1 M Buffer Solutions at 40° and Ionic Strength 1.0

Table II. The Limiting Rate Constants for Zero Buffer Concentration for the Ring-Opening of Ia—d to IIa—d at 40° and Ionic Strength 1.0

Substrate	Pseudo-first-order rate constant, $k^{(0)}_{II,obsd} \times 10^3$, min^{-1} pH value						
	7.00	7.50	7.80	9.00	10.00	11.00	11.40
Ia·$HClO_4$	—	1.7	2.8	14	17	26	42
Ib·$HClO_4$	—	1.5	2.7	12	16	24	30
Ic·$HClO_4$	0.7	—	3.3	12	14	20	31
Id·$HClO_4$	2.0	—	7.8	18	20	41	69

When the limiting rate constant for the ring-opening of Ia (Table II) is plotted as a function of pH, the pH—rate profile shown in Fig. 4 is obtained. If in this reaction a mechanism similar to that[1,6)] proposed for the ring-opening of Ia or the Dimroth rearrangement of 1,9-dialkyladenine is operative, the rate law will be given by

$$k^{(0)}_{II,obsd}[Ia]_{total} = k^{(0)}_{II,ionic}[Ia\cdot H^+][OH^-] + k^{(0)}_{II,neut}[Ia][OH^-] \quad (1)$$

where $[Ia]_{total}$ is the total concentration of Ia; $[Ia\cdot H^+]$, the concentration of the protonated species of Ia; [Ia], the concentration of the neutral species of Ia; $[OH^-]$, hydroxide ion concentration; $k^{(0)}_{II,ionic}$ and $k^{(0)}_{II,neut}$, rate constants for hydroxide attack on the protonated and neutral species at zero buffer concentration. A theoretical rate profile was calculated from the rate equation [Eq. (1)], adopting a pK_a of 8.48 for Ia (Table I) and a negative logarithm of ionic product of H_2O (pK_w) of 13.53 (at 40°).[17)] It corresponds to curve 1 plotted in Fig. 4 if $k^{(0)}_{II,ionic}$= 1900 and $k^{(0)}_{II,neut}$=3.4, time being in minutes. A similar treatment of the rest of the data in Table II provided the second-order rate constants listed in Table III. It may be seen that the protonated species of 1-benzyloxy-9-methyladenine (Ic) undergoes ring-opening 1.2 times as fast as the corresponding 1-methoxy derivative (Ia); the protonated species of the nucleoside (Id), 3.4 times more rapidly than that of Ic. On the other hand, the neutral species of Ia—d all undergo ring-opening only at an extremely slower rate and the bulkier alkoxy groups at the 1-position appear to have a rate-retarding effect. However, the rate-promoting effect of the ribofuranosyl group at the 9-position is still operative. These results are in harmony with those obtained in the Dimroth rearrangement of the 1-alkyladenine derivatives.[1)]

In the ring-closure of IId to IIId and the simultaneous deformylation of IId to IVd, concentration of IId can be directly followed by measuring the increase of UV absorbance at the isoabsorptive wavelength (λ_1 in Fig. 2) where molecular extinction coefficients of IIId and

17) H.S. Harned and R.A. Robinson, *Trans. Faraday Soc.*, **36**, 973 (1940).

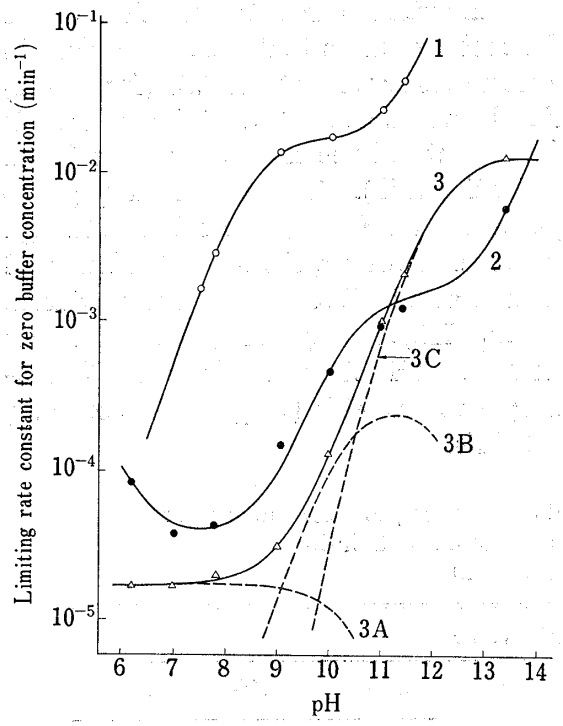
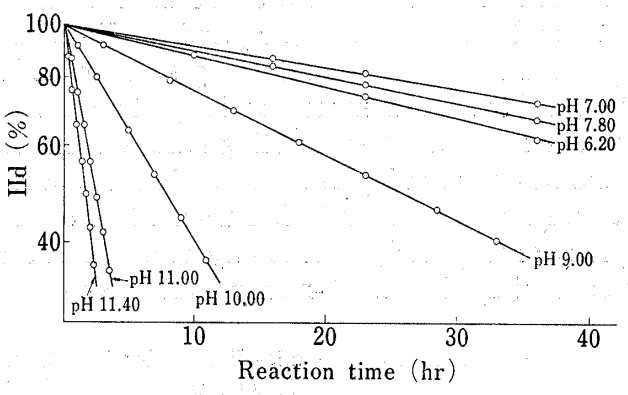


Fig. 4. pH—Rate Profiles for the Ring-Opening of Ia (○), Ring-Closure of IIa (●), and Deformylation of IIa (△)



F g. 5. First-Order-Plot for the Simultaneous Reactions of IId (IIId←IId→IVd) in 0.1 M Buffer Solution at 40° and Ionic Strength 1.0

TABLE III. Effect of Substituents on the Ring-Opening of the Protonated and Neutral Species of 1,9-Disubstituted Adenines at 40° and Ionic Strength 1.0

Substrate	Second-order rate constants, liters mole^{-1} min^{-1}	
	$k^{(0)}_{\mathrm{II,ionic}}$	$k^{(0)}_{\mathrm{II,neut}}$
1-Methoxy-9-methyladenine (Ia)	1900	3.4
1-Ethoxy-9-methyladenine (Ib)	1700	2.3
1-Benzyloxy-9-methyladenine (Ic)	2300	2.3
1-Benzyloxyadenosine (Id)	7900	6.8

IVd are equal. Figure 5 shows semilogarithmic plots of mole fractions of IId against the reaction time for the reactions of IId in 0.1 M buffer solutions at different pH's at 40° and ionic strength 1.0. Although IIId was not stable enough under the reaction conditions, fairly good first-order plots were obtained in all cases until IId decreased to 40% of the initial concentration. The rate constant for the disappearance of IId, which is the sum of the rate constants for the formation of IIId and for that of IVd, was thus obtained. On the other hand, for the two simultaneous first-order reactions, there is a relationship:[18]

$$f_{\mathrm{IIId}}(k_{\mathrm{III}}+k_{\mathrm{IV}}) = k_{\mathrm{III}}\{1-\exp[-(k_{\mathrm{III}}+k_{\mathrm{IV}})t]\} \qquad (2)$$

where f_{IIId} is the mole fraction of IIId; k_{III} and k_{IV}, rate constants for the ring-closure and the deformylation; t, the reaction time. Measurement of UV absorbance at the isoabsorptive wavelength of IId and IVd (λ_2 in Fig. 2) can determine f_{IIId} in the reaction mixture of the three components. Knowing f_{IIId} and $k_{\mathrm{III}}+k_{\mathrm{IV}}$ as described above, we can get the value of k_{III} as a slope of the straight line which is given by plotting the value of f_{IIId} $(k_{\mathrm{III}}+k_{\mathrm{IV}})$ as a function of $1-\exp[-(k_{\mathrm{III}}+k_{\mathrm{IV}})t]$. The rate constant, k_{IV}, was then obtained by subtracting the value

18) K.J. Laidler, "Chemical Kinetics," 2nd ed., McGraw-Hill Book Co., New York, 1965, p. 10.

of k_{III} from that of $k_{III}+k_{IV}$. A similar treatment of data obtained from the reactions of the other compounds (IIa—c) gave the pseudo-first-order rate constants that are summarized in Table IV. It may be seen that the rate of the ring-closure is slowest at pH 7.80 (for IIa—c) or at pH 7.00 (for IId) and that of the deformylation increases as the pH of the medium is increased in all cases. No appreciable difference in the rate of the ring-closure was found between the N′-methoxy (IIa) and the N′-ethoxy derivative (IIb). The N′-benzyloxy group appeared to have a rate-retarding effect on the ring-closure at pH 10.00 and below, although it seemed to act rather acceleratively at pH 11.00 and above. However, the 1-β-D-ribofuranosyl group exerted a rate-promoting effect on the ring-closure at all pH's. In the deformylation there was no meaningful difference in rate between IIa and IIb, although the latter underwent deformylation at the rate less than a half of that for IIa at pH 7.00 and below; the N′-benzyloxy analog (IIc), more slowly than IIa at all pH's; the nucleoside (IId), on the contrary, more rapidly than the 1-methyl derivative (IIc) at all pH's.

Table IV. Pseudo-First-Order Rate Constants, k_{III} (min^{-1}) and k_{IV} (min^{-1}), for Ring-Closure and Hydrolysis of II in 0.1 M Buffer Solutions at 40° and Ionic Strength 1.0

Substrate		pH value						
		6.20	7.00	7.80	9.00	10.00	11.00	11.40
IIa	$k_{III}\times10^4$	1.4	0.81	0.70	1.6	5.1	10	12
	$k_{IV}\times10^4$	0.19	0.25	0.31	1.1	2.9	12	21
IIb	$k_{III}\times10^4$	1.7	0.97	0.68	1.9	4.5	9.8	9.9
	$k_{IV}\times10^4$	0.07	0.11	0.26	0.89	2.8	9.4	19
IIc	$k_{III}\times10^4$	0.95	0.59	0.48	1.3	3.3	11	16
	$k_{IV}\times10^4$	0.04	0.12	0.19	0.78	2.3	7.9	16
IId	$k_{III}\times10^4$	2.2	1.4	1.6	3.1	8.3	14	16
	$k_{IV}\times10^4$	0.07	0.15	0.30	1.5	6.9	35	56

For determination of the rates of the ring-closure and the deformylation of IIa at zero buffer concentration, the reaction of IIa at 40° and ionic strength 1.0 was performed in 1 N NaOH and in 0.05 M and 0.15 M buffer solutions at seven different pH's defined in Table IV. The rate constants for the ring-closure and the deformylation were obtained in the manner similar to that described for the reactions in 0.1 M buffer solutions. Extrapolation of the plots of the rate constants *vs.* buffer concentration to zero buffer concentration gave the limiting rate constants, $k_{III}^{(0)}$ and $k_{IV}^{(0)}$, at the pH involved. When the limiting rate constant for the ring-closure of IIa is plotted as a function of pH, the pH—rate profileshown in Fig. 4 is obtained. It reveals that the rate of the ring-closure decreases with increasing pH in the acidic region, increases with increasing pH in the alkaline region, tends toward a break at around the acidic pK_a of IIa, and increases again at high pH. One of the kinetically equivalent tentative rate laws, which may agree with the reaction feature, is given by

$$k_{III}^{(0)}[IIa]_{total} = 7.5\times10[IIa][H^+] + 3.6\times10^{-5}[IIa] + 2.3[IIa][OH^-] + 6.2\times10^{-3}[Va][OH^-] \quad (3)$$

where [IIa] and [Va] are the concentrations of the neutral molecule and the conjugate base of IIa; $[IIa]_{total}$, the sum of [IIa] and [Va]; $[H^+]$, hydrogen ion concentration; $[OH^-]$, hydroxide ion concentration. The rate coefficients in the equation [Eq. (3)] were evaluated by fitting Eq. (3) to the experimental data. A theoretical rate profile (curve 2 in Fig. 4) was calculated from Eq. (3) adopting a pK_a of 10.36 for IIa (Table I), pK_w of 13.53 (at 40°),[17] and a negative logarithm of activity coefficient of 0.17 (at 40°)[19] for 1.0 N NaOH.

19) G. Åkerlöf and G. Kegeles, *J. Am. Chem. Soc.*, **62**, 620 (1940).

When the limiting rate constant for the deformylation of IIa is plotted as a function of pH, the pH—rate profile shown in Fig. 4 is obtained. If in this reaction a mechanism similar to that[20] proposed for the alkaline hydrolysis of amides is operative, the rate equation will be given by

$$k_{OH^-} = \frac{k_1 K_w}{K_a + [H^+]} \times \frac{k_2 K_x + k_3 K_y [OH^-]}{k_{-1} + k_2 K_x + k_3 K_y [OH^-]} \quad (4)$$

where k_{OH^-} is the pseudo-first-order rate constant for alkaline hydrolysis of IIa at zero buffer concentration; $[H^+]$, concentration of hydrogen ion; $[OH^-]$, concentration of hydroxide ion; K_a, acid dissociation constant of IIa; K_w, ionic product of H_2O; k_1, k_{-1}, k_2, k_3, K_x, and K_y, the rate constants and the equilibrium constants for the reactions depicted in Chart 2. Since only a little change in the hydrolysis rate of IIa was observed as the pH of the medium varied at near neutrality, the reaction in such a region may be regarded as being largely due to the attack of H_2O on the undissociated molecule of IIa. Hence the rate equation for the deformylation of IIa in the range of neutral pH is expressed by

$$k_{H_2O} = k_4 \frac{[H^+]}{K_a + [H^+]} \quad (5)$$

where k_{H_2O} is the apparent first-order rate constant for the hydrolysis of IIa by H_2O; k_4, the rate constant for the H_2O attack on the undissociated molecule of IIa. For the deformylation of IIa, therefore, can be given the equation:

$$k_{IV}^{(0)} = k_{OH^-} + k_{H_2O} = \frac{k_1 K_w}{K_a + [H^+]} \times \frac{k_2 K_x + k_3 K_y [OH^-]}{k_{-1} + k_2 K_x + k_3 K_y [OH^-]} + k_4 \frac{[H^+]}{K_a + [H^+]} \quad (6)$$

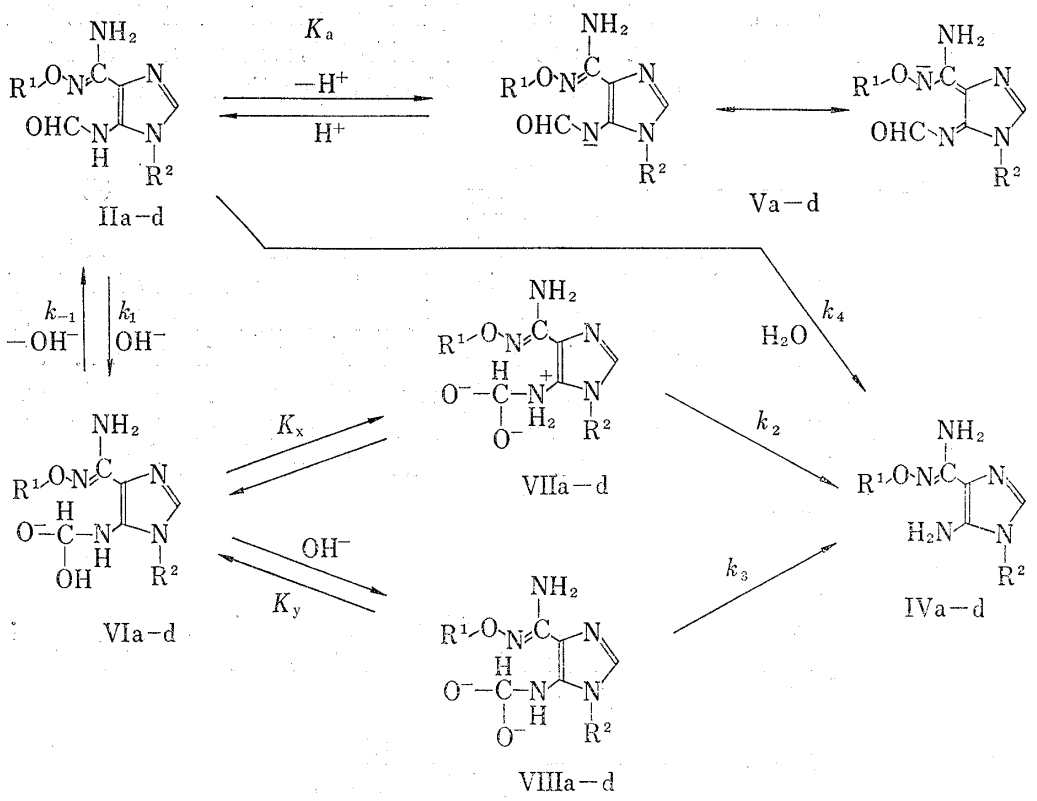
At pH 6.20, 7.00, and 7.80, where k_{OH^-} may be negligibly small and $K_a \ll [H^+]$, k_4 approximates to $k_{IV}^{(0)}$ itself. The other parameters for Eq. (6) are determined in a manner similar to that employed by Eriksson and Holst.[21] The use of the parameter values thus obtained [$k_1 = 19$ liters mole^{-1} min^{-1}; $k_2 K_x / k_{-1} = 0.025$; $k_3 K_y / k_{-1} = 26$ liters mole^{-1}; $k_4 = 1.7 \times 10^{-5}$ min^{-1}, acidic pK_a of 10.36 for IIa (Table I), and pK_w of 13.53 (at 40°)[17]] leads to the construction of curve 3 in Fig. 4.

Discussion

The results shown in Fig. 4 (curve 1) and Table III reveal that all compounds (Ia—d) undergo ring-opening to give the corresponding monocyclic intermediates (IIa—d) by following the rate law given by Eq. (1), being consistent with the previous result[6] obtained with Ia under slightly different reaction conditions. In the ring-opening of I attack of hydroxide ion on the protonated species ($I \cdot H^+$) is dominant in the region of pH lower than the pK_a of I, superseded in importance at high pH by hydroxide attack on the neutral species and the rate of the former is faster than that of the latter by a factor of 560—1200 (Table III). Comparison of the second-order rate constants in Table III with the corresponding rate constants for the ring-opening of the 1-alkyladenine derivatives[1] has confirmed the previously claimed generality[6] that the electron-withdrawing 1-alkoxy group is more effective than the 1-alkyl group for speeding up both modes of the ring-opening of the 1-substituted adenine derivatives.

Acidity of ordinary amides is very weak and even *p*-nitroformanilide, which ranks among amides of strong acidity, has been reported[22] to have such a high pK_a value of 12.5 at 30° or

20) B.C. Challis and J.A. Challis, "The Chemistry of Amides," ed. by J. Zabicky, Interscience Publishers, Inc., New York, 1970, pp. 816—824.
21) S.O. Eriksson and C. Holst, *Acta Chem. Scand.*, **20**, 1892 (1966).
22) R.H. DeWolfe and R.C. Newcomb, *J. Org. Chem.*, **36**, 3870 (1971).



a : $R^1=R^2=CH_3$
b : $R^1=C_2H_5$; $R^2=CH_3$
c : $R^1=C_6H_5CH_2$; $R^2=CH_3$
d : $R^1=C_6H_5CH_2$; $R^2=\beta$-D-ribofuranosyl

Chart 2

12.8 at 15°. A remarkable feature of the formamides (II) is demonstrated by their extraordinarily strong acidity. The pK_a's of 9.93 to 10.60 in Table I almost compete with those for the anilides with strongly electron-withdrawing acyl moiety such as trifluoroacetanilide (pK_a 9.51,[21] 9.54[23]) and trichloroacetanilide (pK_a 9.98[21]). They can be assigned to the acidic ones of the formamido group of II because neither the deformylated product (IV) of II nor the N-methylamide analog, N′-ethoxy-1-ethyl-5-(N-methylformamido)imidazole-4-carboxamidine,[24] has been proved to have pK_a in this region. The unusually strong acidity of the formamido group may be attributed to the electron-withdrawing character of the imidiazole ring[25] and the stabilized conjugate base (V) through the resonance as depicted in Chart 2.

The β-D-ribofuranosyl group at the 1-position of II further causes to lower the acidic pK_a of II. On the contrary, the O-benzyl group at the amidoxime moiety of II raises the acidic pK_a of II although the electronic effect of the benzyl group can be realized in the lowered basic pK_a of IIc. The reason for such an unexpected effect of the benzyl group on the acidic pK_a is not clear, but one logical explanation is that the bulky benzyl group disturbs the coplanarity of the two side chains at the 4- and 5-position to destabilize the conjugate base (V).

If the general mechanism of alkaline hydrolysis of amides[20] is applicable to the deformylation of II, the hydrolysis of II is considered to proceed *via* the tetrahedral intermediate (VI), followed by equilibrium formation of the dipolar ions (VII and VIII) which break down rapidly to the product (IV) as shown in Chart 2. Assuming that the conjugate base of amide (type

23) P.M. Mader, *J. Am. Chem. Soc.*, **87**, 3191 (1965).
24) To be published elsewhere.
25) H.A. Staab, *Angew. Chem.*, **74**, 407 (1962).

V) does not react[26] and that the steady-state approximation can be applied[21] to the concentration of the tetrahedral intermediate (type VI), we can derive Eq. (4) for the alkaline hydrolysis of II. Taking into account the contribution of attack of H_2O on the undissociated molecule of II, we can get Eq. (6) for the hydrolysis of II. The observed rate constants for the deformylation of IIa at zero buffer concentration at 40° and ionic strength 1.0 are in fairly good agreement with the calculated values from Eq. (6) by the use of the parameter values described already. Hence it is reasonable to consider that the deformylation of IIa follows the reaction sequence shown in Chart 2. Curve 3 in Fig. 4 shows that in the deformylation of IIa attack of H_2O on the neutral molecule of IIa (corresponding to curve 3A) is dominant between pH 6 and 9.1, superseded in importance by the course that involves VIIa (Chart 2) (corresponding to curve 3B) between pH 9.1 and 10.5, and the course through VIIIa (corresponding to curve 3C) becomes most important above pH 10.5.

In the rate study on the ring-closure of IIa to IIIa, it is hard to claim that the theoretical pH—rate profile (curve 2 in Fig. 4) derived from Eq. (3) is well consistent with the observed one. At present, therefore, we wish to refrain from discussing the detailed mechanism of this reaction step.

Comparison of the rate constants for the individual compounds in Table IV indicates that the reaction at around pH 6 is suitable for the selective formation of III from II in 0.1 M buffer solution at 40° and ionic stength 1.0 and that the reaction in the high pH region is good for the preferential synthesis of IV.

It has become apparent from Tables III and IV that all the reaction stages shown in Chart 1 are accelerated by the β-D-ribofuranosyl group. At present it is not clear whether the rate-promoting effect of the ribofuranosyl group is due to the electron-withdrawing property[27] and/or the catalytic action[28] of its hydroxyl group(s). From a practical point of view the rate enhancement brought by the ribofuranosyl substituent will be advantageous when the ring-opening (I→II) and especially the rearrangement (I→II→III) or the deformylation (I→II→IV) procedure are undertaken to modify the adenine ring in nucleic acids.

In the reaction sequence, I→II and consecutive III←II→IV, which were carried out in 0.1 M buffer concentration, the maximum concentration of II, $[II]_{max}$, is determined by

$$[II]_{max} = [I]_0[k_{II}/(k_{III}+k_{IV}-k_{II})]\{\exp(-k_{II}t_{max})-\exp[-(k_{III}+k_{IV})t_{max}]\} \quad (7)$$

where $[I]_0$ is the initial concentration of I; k_{II}, the observed rate constant for the ring-opening of I; $t_{max}=[k_{II}-(k_{III}+k_{IV})]^{-1}[\ln k_{II}-\ln(k_{III}+k_{IV})]$. At an optimum pH (9.00) the maximum formation of IIa is estimated at 93% from Eq. (7), while in the case of IIc the same maximum yield is obtained at pH 7.80. Such an optimum-pH-lowering effect of the 1-benzyloxy group would be favorable when the ring-opening modification of adenine moiety is applied to nucleic acids. Moreover, the sum of k_{III} and k_{IV} for IIc is 62—86% of that for IIa at pH 11.00 and below as shown in Table IV, indicating that the benzyloxy group is capable of stabilizing II once formed. Considering these two effects, the utility of the benzyloxy group will be best appreciated when the modification process of the adenine ring is designed to be interrupted at the intermediate stage.

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26) S.S. Biechler and R.W. Taft, Jr., *J. Am. Chem. Soc.*, **79**, 4927 (1957).
27) C.D. Jardetzky and O. Jardetzky, *J. Am. Chem. Soc.*, **82**, 222 (1960).
28) *a*) T.C. Bruice and S.J. Benkovic, "Bioorganic Mechanisms," Vol. I, W.A. Benjamin, Inc., New York, 1966, pp. 146—166; *b*) *Idem, ibid.*, Vol. II, pp. 37—48; *c*) D.A.R. Happer and J. Vaughan, "The Chemistry of the Hydroxyl Group," ed. by S. Patai, Interscience Publishers, London, 1971, pp. 397—405, pp. 416—418; *d*) Ref. 20, pp. 833—841.