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Purines. XVII.[1] Kinetic Studies of the Base-Catalyzed Conversion of 1-Alkyladenosines into N-Alkyladenosines: Effect of Substituents on the Rearrangement Rate[2]

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The rates of the Dimroth rearrangements of 1-alkyladenosines (Ie, f) and 1-alkyl-9-methyladenines (Ia—d) at various pH's and ionic strength 1.0 at 40° have been measured. It has been shown that all reactions obey good pseudo-first-order kinetics and follow the rate law given by $k^{(0)}_{obsd}=k^{(0)}_{ionic}[I\cdot H^+][OH^-]+k^{(0)}_{neut}[I][OH^-]$ where $k^{(0)}_{obsd}$ is the observed limiting specific rate for zero buffer concentration; $[I\cdot H^+]$ is the fraction of the base protonated at each pH; [I], the fraction present as free base. Comparison between 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 90—1100 and that the former is affected by the electronic property of a substituent at the 1-position, whereas the latter is influenced by a steric factor. The rate of the rearrangement is enhanced by the benzyl group at the 1-position at near neutrality and by the β-D-ribofuranosyl group at the 9-position at all pH's examined.

The isomerization proceeding by ring fission and subsequent recyclization whereby a heterocyclic nitrogen and its attached substituent exchange places with an α-amino or α-imino group has been known as the Dimroth rearrangement.[4] 1-Alkyladenines and their derivatives usually undergo this type of conversion in base to produce isomeric N^6-substituted derivatives.[4,5] Macon and Wolfenden[6a] found that the rearrangement of 1-methyladenosine (Ie) to N-methyladenosine (IIIe) at 25° and various pH's follows pseudo-first-order kinetics wherein no intermediate is detectable. Their results were best interpreted in terms of a mechanism involving a rate-determining initial ring-opening (Ie→IIe), caused by attack of hydroxide ion on both the protonated and neutral species of Ie, and a subsequent rapid ring-closure (IIe→IIIe), paralleling our recent experience[7] in a rate study of the Dimroth rearrangement of 1,9-dimethyladenine (Ia) at 40°.

In related work, we found that 1-alkoxyadenine derivatives also rearrange to N^6-alkoxy isomers, but through readily isolable monocyclic intermediates (type II: RO for R^1), and that

1) Paper XVI in this series, T. Fujii, S. Kawakatsu, and T. Itaya, *Chem. Pharm. Bull.* (Tokyo), **22**, 2466 (1974).
2) Presented in part at the 36th Meeting of Hokuriku Branch, Pharmaceutical Society of Japan, Kanazawa, June 16, 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, p. 313.
5) *a*) M.J. Robins and E.M. Trip, *Biochemistry*, **12**, 2179 (1973); *b*) M.H. Wilson and J.A. McCloskey, *J. Org. Chem.*, **38**, 2247 (1973); *c*) T. Fujii, F. Tanaka, K. Mohri, and T. Itaya, *Chem. Pharm. Bull.* (Tokyo), **22**, 2211 (1974), and references cited therein.
6) *a*) J.B. Macon and R. Wolfenden, *Biochemistry*, **7**, 3453 (1968); *b*) E.R. Garrett and P.J. Mehta, *J. Am. Chem. Soc.*, **94**, 8542 (1972).
7) T. Itaya, F. Tanaka, and T. Fujii, *Tetrahedron*, **28**, 535 (1972).

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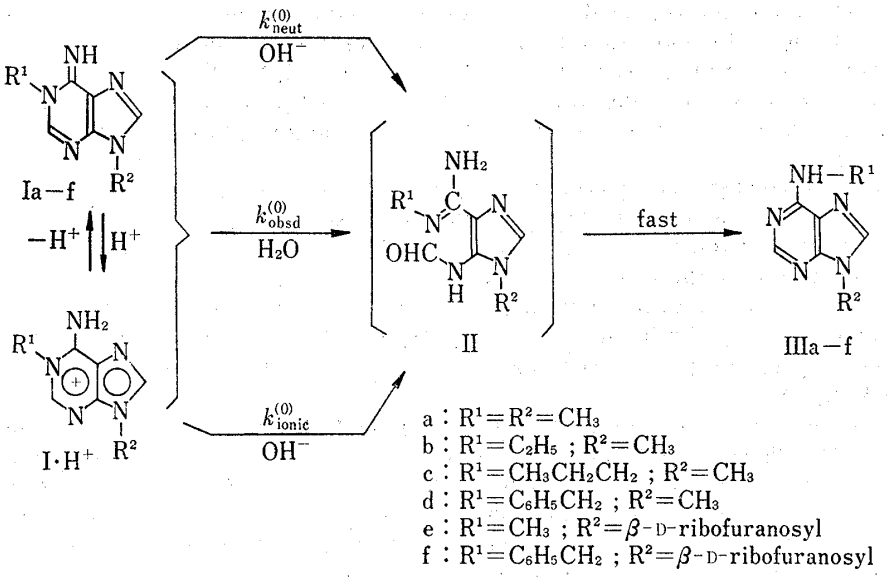

Chart 1

the intermediates suffer the competitive hydrolysis leading to deformylated derivatives.[5c,7–9] It was also shown that at pH 7.60 and above at 40° Ia rearranges more rapidly than 1-methoxy-9-methyladenine, although the latter undergoes ring-opening *ca.* 30 times as fast as the former.[7] The speeding up of the ring-opening step and the slowing down of the recyclization step observed for the 1-methoxy derivative could be attributed directly to the electron-withdrawing nature of the substituent at the 1-position.

On the other hand, a rough comparison of the reported rate constants[6,10] for the rearrangement of Ie and its analogs at various temperatures and pH's with those[7] of Ia has suggested that the β-D-ribofuranosyl group at the 9-position may have a rate-promoting effect. In view of the actual or potential utility[5c,8,9] of this rearrangement in synthetic operations and chemical modification of adenine derivatives and nucleic acids, it is important to learn more about the effect of 1- and 9-substituents on the reaction rate. We now report comparative kinetic studies on the rearrangement of a series of 1-alkyl-9-methyladenines (Ia—d) and 1-alkyladenosines (Ie, f) in aqueous solution.

Experimental

All melting points were taken on a Yamato MP-1 capillary apparatus and are corrected. Paper chromatographies were developed as described previously.[8b] Ultraviolet (UV) spectra were measured with a Hitachi Model 323 spectrophotometer on solutions in 95% aq. ethanol, 0.1 N aq. HCl (pH 1), 0.005 M phosphate buffer (pH 7), and 0.1 N aq. NaOH (pH 13). Spectrophotometric determinations were carried out

8) *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. Sato, and T. Itaya, *Chem. Pharm. Bull.* (Tokyo), **19**, 1731 (1971); *d*) T. Fujii, T. Itaya, and S. Moro, *ibid.*, **20**, 1818 (1972); *e*) T. Fujii, C.C. Wu, T. Itaya, S. Moro, and T. Saito, *ibid.*, **21**, 1676 (1973).

9) Since our first paper[8a] was published, these reactions have been utilized by many workers. See, for example, *a*) A. Yamazaki, I. Kumashiro, and T. Takenishi, *Chem. Pharm. Bull.* (Tokyo), **17**, 1128 (1969); *b*) T. Ueda, M. Imazawa, K. Miura, R. Iwata, and K. Odajima, *Tetrahedron Letters*, **1971**, 2507; *c*) J.A. Montgomery and H.J. Thomas, *Chem. Commun.*, **1969**, 458; *d*) *Idem*, *J. Med. Chem.*, **15**, 182 (1972); *e*) R.B. Meyer, Jr., D.A. Shuman, R.K. Robins, J.P. Miller, and L.N. Simon, *ibid.*, **16**, 1319 (1973); *f*) T. Fujii, T. Itaya, K. Mohri, and T. Saito, *J. C. S. Chem. Comm.*, **1973**, 917.

10) *a*) P. Brookes, A. Dipple, and P.D. Lawley, *J. Chem. Soc.* (C), **1968**, 2026; *b*) P.D. Lawley and P. Brookes, *Biochem. J.*, **89**, 127 (1963).

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with a Hitachi Model 181 spectrophotometer; pH's were measured on a Hitachi-Horiba F-5 pH meter. Elemental analyses were performed by Mr. Y. Itatani and Miss S. Toyoshima at Kanazawa University.

Materials——Analytically pure samples of the perchlorates of 1-methyl- (Ia), 1-ethyl- (Ib), and 1-propyl-9-methyladenine (Ic) and of the free bases of N-methyl- (IIIa), N-ethyl- (IIIb), and N-propyl-9-methyladenine (IIIc) were prepared according to the procedure reported previously.[7] Other compounds were obtained in the manner described below. Table I collects the acid dissociation constants of some of the compounds thus prepared.

1-Benzyl-9-methyladenine Perchlorate (Id·$HClO_4$)——A solution of 1-benzyl-9-methyladenine hydriodide (Id·HI)[11] (734 mg, 2 mmoles) in H_2O (70 ml) was passed through a column packed with Amberlite IRA-402 (ClO_4^-) (8 ml) and the column was eluted with H_2O (230 ml). Evaporation of the eluate (300 ml) under vacuum and drying of the resulting residue gave Id·$HClO_4$ (669 mg, 99%) as a colorless solid, mp 221—230° (decomp.). Recrystallization from H_2O produced an analytical sample as colorless pillars, mp 229—230° (decomp.); UV $\lambda_{max}^{95\%\ EtOH}$ 261 nm (ε 12800); $\lambda_{max}^{H_2O}$ (pH 1) 261 (13000); $\lambda_{max}^{H_2O}$ (pH 7) 261 (13000); $\lambda_{max}^{H_2O}$ (pH 13) 260 (13500); pK_a (Table I). *Anal.* Calcd. for $C_{13}H_{14}O_4N_5Cl$: C, 45.96; H, 4.15; N, 20.62. Found: C, 45.71; H, 4.20; N, 20.37.

1-Methyladenosine (Ie)——For preparing this compound the procedure of Jones and Robins[12] was adopted, but with the following modification: a solution of 1-methyladenosine hydriodide (Ie·HI)[12] (12.28 g, 30 mmoles) in H_2O (50 ml) was passed through a column cantaining Amberlite IRA-410 (HCO_3^-) (35 ml) and the column was eluted with H_2O. The eluate (750 ml) was concentrated to dryness *in vacuo* and the residual colorless solid was dried to give the free base (Ie) (8.36 g, 99%), mp 205—206° (decomp.). Recrystallization from methanol afforded colorless needles, mp 217—218° (decomp.) [lit.[12] mp 214—217° (decomp.)]; UV $\lambda_{max}^{95\%\ EtOH}$ 258 nm (ε 13000); $\lambda_{max}^{H_2O}$ (pH 1) 258 (13300); $\lambda_{max}^{H_2O}$ (pH 7) 258 (13300); $\lambda_{max}^{H_2O}$ (pH 13) 259 (14000); pK_a (Table I). *Anal.* Calcd. for $C_{11}H_{15}O_4N_5$: C, 46.97; H, 5.38; N, 24.90. Found: C, 47.14; H, 5.54; N, 24.87.

1-Benzyladenosine Hydrobromide (If·HBr)——The previously reported procedure[10a,11] was slightly modified as follows: a stirred mixture of adenosine (26.7 g, 100 mmoles) and benzyl bromide (34.2 g, 200 mmoles) in N,N-dimethylacetamide (100 ml) was kept at 35° for 72 hr. The solvent was removed by evaporation at 5 mm Hg and below 60°. The residual sirup was washed with several 120-ml portions of dry acetone and dried to give crude If·HBr (43.6 g, almost quantitative) as a colorless solid, mp 130° (decomp.). Rapid recrystallization from hot (below 70°) water yielded colorless prisms, mp 190—192° (decomp.) [lit.[10a] mp 194° (decomp.)]; UV $\lambda_{max}^{95\%\ EtOH}$ 260 nm (ε 13400); $\lambda_{max}^{H_2O}$ (pH 1) 259 (13800); $\lambda_{max}^{H_2O}$ (pH 7) 259 (13700); $\lambda_{max}^{H_2O}$ (pH 13) 260 (13900); pK_a (Table I). *Anal.* Calcd. for $C_{17}H_{20}O_4N_5Br$: C, 46.58; H, 4.60; N, 15.98. Found: C, 46.68; H, 4.78; N, 16.28.

N-Benzyl-9-methyladenine (IIId)——A suspension of Id·HI[11] (3.67 g, 10 mmoles) in 0.2 N aq. NaOH (100 ml) was refluxed for 10 min. The reaction mixture was adjusted to pH 9 with 10% aq. HCl and extracted with three 50-ml portions of benzene. The benzene solutions were combined, dried over anhyd. $MgSO_4$, and evaporated to dryness *in vacuo* to leave IIId (2.36 g, 99%) as a colorless solid, mp 123—125°. Recrystallization from benzene produced colorless minute pillars that melted once at 125—126°, resolidified, and melted again at 136—137° (lit.[13] mp 127—128°); UV $\lambda_{max}^{95\%\ EtOH}$ 270 nm (ε 19400); $\lambda_{max}^{H_2O}$ (pH 1) 268 (19300); $\lambda_{max}^{H_2O}$ (pH 7) 270 (20000); $\lambda_{max}^{H_2O}$ (pH 13) 270 (20000). *Anal.* Calcd. for $C_{13}H_{13}N_5$: C, 65.25; H, 5.48; N, 29.27. Found: C, 65.44; H, 5.65; N, 29.24. This sample did not give any indications of decomposition even when heated at 145—160° for 15 min.

N-Methyladenosine (IIIe)——Prepared according to the procedure of Jones and Robins,[12] but with the following modification: a solution of Ie (2.81 g, 10 mmoles) in H_2O (20 ml) was refluxed for 3 hr and then evaporated to dryness *in vacuo*. The resulting solid was recrystallized from methanol to give a colorless solid (1.42 g, 51%), mp 164—167°, shown to be homogeneous by thin-layer chromatography. Further recrystallizations from methanol provided IIIe as colorless needles, mp 213—216° (with previous sintering) (lit.[12] mp 219—221°); UV $\lambda_{max}^{95\%\ EtOH}$ 266 nm (ε 16200); $\lambda_{max}^{H_2O}$ (pH 1) 262 (17700); $\lambda_{max}^{H_2O}$ (pH 7) 266 (16500); $\lambda_{max}^{H_2O}$ (pH 13) 266 (16700). *Anal.* Calcd. for $C_{11}H_{15}O_4N_5$: C, 46.97; H, 5.38; N, 24.90. Found: C, 46.63; H, 5.70; N, 24.52.

N-Benzyladenosine (IIIf)——This compound was prepared in a manner similar to that of Fleysher, *et al.*[14] and recrystallized from methanol to give colorless needles that melted once at 164°, resolidified, and melted again at 182—183° (lit. mp 183°;[14] mp 186—187° with partial melting at 169—170°, followed by resolidification[5a]); UV $\lambda_{max}^{95\%\ EtOH}$ 270 nm (ε 20200); $\lambda_{max}^{H_2O}$ (pH 1) 265 (20300); $\lambda_{max}^{H_2O}$ (pH 7) 269 (20600); $\lambda_{max}^{H_2O}$ (pH 13) 269 (20900). *Anal.* Calcd. for $C_{17}H_{19}O_4N_5$: C, 57.13; H, 5.36; N, 19.60. Found: C, 57.40; H, 5.71; N, 19.39.

Kinetic Studies——The rearrangement reactions of Ia—f to IIIa—f, shown in Chart 1, in aq. solution at various pH's and ionic strength 1.0 at 40° were followed by UV spectrophotometry. Buffer solutions employed for kinetic runs were 0.02, 0.05, and 0.1 M NaH_2PO_4—Na_2HPO_4 (pH 7.00 and 8.00 at 40°); 0.02,

11) N.J. Leonard and T. Fujii, *Proc. Natl. Acad. Sci. U. S.*, **51**, 73 (1964).
12) J.W. Jones and R.K. Robins, *J. Am. Chem. Soc.*, **85**, 193 (1963).
13) T. Kunieda and B. Witkop, *J. Orgm. Chem.*, **35**, 3981 (1970).
14) M.H. Fleysher, A. Bloch, M.T. Hakala, and C.A. Nichol, *J. Med. Chem.*, **12**, 1056 (1969).

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0.05, and 0.1 M $NaHCO_3$—Na_2CO_3 (pH 9.00 and 10.00 at 40°); 0.02, 0.05, and 0.1 M Na_2HPO_4—Na_3PO_4 (pH 11.00 at 40°); 0.05 and 0.1 M Na_2HPO_4—Na_3PO_4 (pH 11.40 at 40°), and were brought to ionic strength 1.0 with KCl.

The substrates (Ia—f), in the form of salt or free base, were separately dissolved in the buffer solutions at a concentration ranged from 3.5×10^{-5} to 4.5×10^{-5} M. Aliquots (*ca.* 3 ml) of these solutions were sealed in small ampoules and placed in a thermoregulated constant temperature bath kept at 40° (accurate to ±0.05°). At intervals the ampoules were removed, cooled, and broken and the optical densities of the contents at 268 nm were determined at room temperature against the blank buffer solutions. During the kinetic runs the pH was found never to vary more than ±0.02 unit. Concentrations of the substrates were calculated in the usual manner[15] by utilizing the molecular extinction coefficients at the analytical wavelength, obtained on solutions of analytically pure samples of the substrates and IIIa—f in the appropriate buffer solutions. Except for the slow reactions of Ia—d at pH 7.00, all rearrangements were followed through at least two half-lives with at least five determinations and good pseudo-first-order kinetics obtained in all cases.

Spectrometric Determination of Acid Dissociation Constants of Ia—f (Table I)——The p*K*a's at 40° and ionic strength 1.0 were determined according to the general procedure of Albert and Serjeant.[16a] Buffer solutions used were at 1/9 M concentration and the ionic strength was maintained at 1.0 with KCl. The pH regions covered by individual buffer systems at 40° were 5.5—8.5, NaH_2PO_4—Na_2HPO_4; 8.8—10.0, $NaHCO_3$—Na_2CO_3; 11.3, Na_2HPO_4—Na_3PO_4. Weighed samples of the 1,9-disubstituted adenine derivatives were dissolved in 1 M aq. KCl at a concentration ranged from 1.5×10^{-3} to 2.5×10^{-3} M. The sample solutions were quickly diluted tenfold with the appropriate buffer solutions kept at 40°, and the optical densities of the resulting solutions at 300 nm at 40° were measured, with as little delay as possible, against blank solutions prepared by diluting 1 M aq. KCl with the corresponding buffer solutions by a factor of 10. The pH's of these blank solutions were separately determined at 40°.

Table I. Acid Dissociation Constants of 1,9-Disubstituted Adenines

Compound	pK_a at 40° and ionic strength 1.0
Ia·$HClO_4$	8.96±0.04[a]
Ib·$HClO_4$	9.02±0.04
Ic·$HClO_4$	9.00±0.05
Id·$HClO_4$	8.34±0.04
Ie	8.55±0.07
If·HBr	7.93±0.03

a) At ionic strength 0.5, pK_a 8.94 ±0.05.[7]

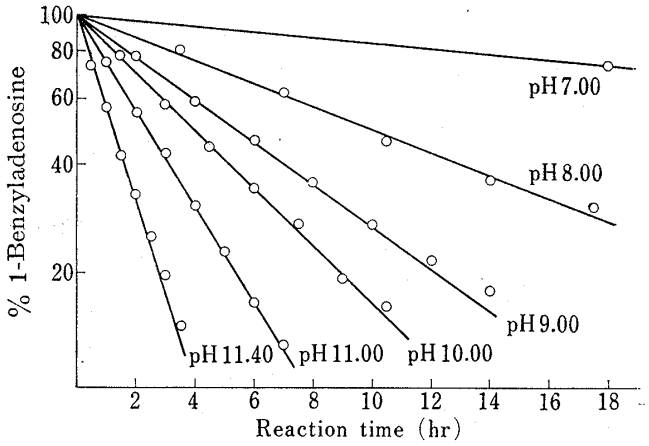

Fig. 1. First-Order Plot for Rearrangement of If to IIIf in 0.05 M Buffer Solution at Various pH's and Ionic Strength 1.0 at 40°

Results

In a typical experiment, the rearrangement of 1-benzyladenosine (If) in 0.05M buffer solution at ionic strength 1.0 and various pH's at 40° was followed by the increase in UV absorption at 268 nm which occurs upon formation of N-benzyladenosine (IIIf). The semi-logarithmic plots of mole fractions of the residual substrate (If) against time, illustrated in Fig. 1, show that the reaction obeys fairly good pseudo-first-order kinetics at all pH's through at least two half-lives. A similar treatment of data obtained from the rearrangement in 0.02 and 0.1M buffer solutions led to the finding that the rate constant increases with increasing

15) H.H. Jaffé and M. Orchin, "Theory and Applications of Ultraviolet Spectroscopy," John Wiley & Sons, Inc., New York, 1962, pp. 556—560.

16) *a*) A. Albert and E.P. Serjeant, "Ionization Constants of Acids and Bases," Methuen & Co., London, 1962, pp. 69—92; *b*) *Idem, ibid.*, p. 140.

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buffer concentration at constant pH. Extrapolation of the plot of the rate constant *vs.* buffer concentration to zero buffer concentration gave the limiting rate constant, $k^{(0)}_{obsd}$, at the pH involved.[17] Kinetic runs of the other substrates (Ia—e) were also handled in a similar manner. In all cases the catalytic coefficients of the buffer components were found to be fairly small: the difference between the rate constants at 0.02 M and zero buffer concentration was within less than 3% of the former, suggesting that as a good approximation in a certain case the former may be regarded as the next best substitute for the latter.

Table II assembles the limiting rate constants for zero buffer concentration for the rearrangement of Ia—f. It may be seen that in all cases the reaction rate increases as the pH of the medium is increased. At pH 10 and below the *n*-alkyl homologs (Ia, b, c) underwent rearrangement in a comparable manner, whereas at pH 11 and above the 1-ethyl (Ib) and 1-propyl derivative (Ic) rearranged more slowly than did the 1-methyl derivative (Ia), in agreement with our previous finding[7] under similar conditions but at ionic strength 0.5 at higher buffer concentration. The 1-benzyl derivative (Id) underwent rearrangement 1.3—3.5 times as fast as the 1-methyl derivative (Ia) at pH 10 and below, whereas raising pH to pH 11 and above reversed the situation. Nucleoside Ie also rearranged 2.1—3.3 times more rapidly than the corresponding 9-methyl analog (Ia), but at all pH's. At pH 9 and below the compound most susceptible to rearrangement was If, a compound which carries both the benzyl and the β-D-ribofuranosyl group; but at pH 10 and above it was nucleoside Ie, a compound which possesses the smallest alkyl group at the 1-position.

Table II. The Limiting Rate Constants for Zero Buffer Concentration for the Rearrangement of Ia—f to IIIa—f at 40° and Ionic Strength 1.0

Substrate	Pseudo-first-order rate constant, $k^{(0)}_{obsd} \times 10^4$, min^{-1} pH value					
	7.00	8.00	9.00	10.00	11.00	11.40
Ia·$HClO_4$	0.22	1.3	6.2	14	32	45
Ib·$HClO_4$	0.19	1.4	7.7	15	25	35
Ic·$HClO_4$	0.24	1.4	8.3	16	27	35
Id·$HClO_4$	0.74	4.6	14	18	28	42
Ie	0.72	4.2	19	36	67	114
If·HBr	2.7	11	21	29	47	92

It may be seen from Table I that the benzyl and the β-D-ribofuranosyl group also separately affect the acid dissociation constant of a compound of type I in a striking manner. When determined spectrophotometrically at 40° and ionic strength 1.0, the *n*-alkyl homologs (Ia, b, c) gave pK_a's comparable to each other, whereas the 1-benzyl derivatives (Id, f) showed pK_a values lower than those of the corresponding 1-methyl derivatives (Ia, e) by *ca.* 0.6 pK_a unit; the nucleosides (Ie, f) had pK_a's lower than those of the corresponding 9-methyl derivatives (Ia, d) by *ca.* 0.4 unit. In the case of 1-benzyladenosine (If) the observed effects of both substituents were operating cumulatively,[18a] resulting in a decrease of *ca.* 1 pK_a unit against the pK_a of 1,9-dimethyladenine (Ia).

When the limiting rate constant for the rearrangement of Id (Table II) is plotted as a function of pH, the pH—rate profile shown in Fig. 2 is obtained. If in this reaction a mecha-

17) L.P. Hammett, "Physical Organic Chemistry. Reaction Rates, Equilibria, and Mechanisms," 2nd ed., McGraw-Hill Book Co., New York, 1970, pp. 340—342.

18) *a*) H.C. Brown, D.H. McDaniel, and O. Häfliger, "Determination of Organic Structures by Physical Methods," ed. by E.A. Braude and F.C. Nachod, Academic Press Inc., New York, 1955, pp. 579—580; *b*) *Idem, ibid.*, pp. 581—582, p. 584.

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nism similar to that[6a,7] proposed for the Dimroth rearrangement of Ia,e is operative, the reaction rate v will be given by

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

where $[Id]_{total}$ is the total concentration of Id; $[Id\cdot H^+]$, the concentration of the protonated species of Id; [Id], the concentration of the neutral species of Id; $[OH^-]$, hydroxide ion concentration; $k^{(0)}_{ionic}$ and $k^{(0)}_{neut}$, 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 8.34 for Id (Table I) and ionic product of water (pK_w) of 13.53 (at 40°).[19] It corresponds to the curve plotted in Fig. 2 if $k^{(0)}_{ionic}=255$ and $k^{(0)}_{neut}=0.37$, 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 in the reaction of the protonated species there is no appreciable difference in rate between 1-alkyl-9-methyladenines (Ia, b, c), although the 1-benzyl analog (Id) rearranges *ca.* 5 times as fast as any of them; the nucleoside (Ie), 5 times more rapidly than Ia. Furthermore, the rate-promoting effect of the benzyl group is roughly multiplied by that of the ribofuranosyl group in the case of If. In contrast, the neutral species of Ia—f all rearrange only at an extremely slower rate. The higher alkyl groups and even the benzyl group 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.

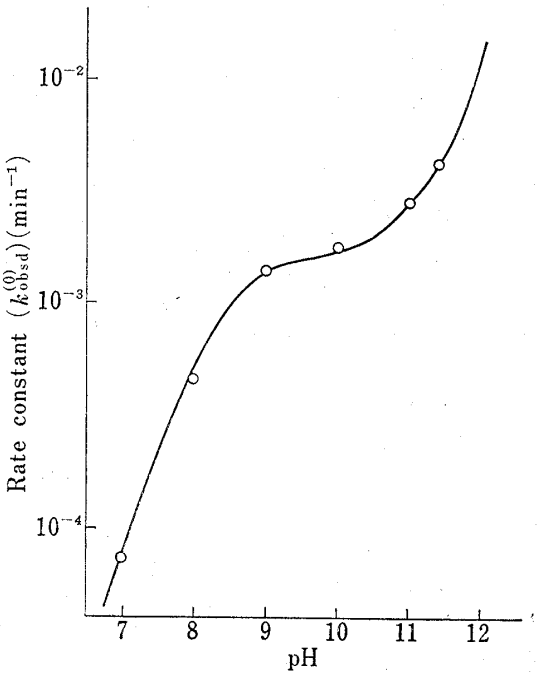

Fig. 2. Limiting pH–Rate Profile for the Rearrangement of Id to IIId at 40° and Ionic Strength 1.0

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

Substrate	Second-order rate constants,[a] liters mole^{-1} min^{-1} $k^{(0)}_{ionic}$	$k^{(0)}_{neut}$
1,9-Dimethyladenine (Ia)	50	0.55
1-Ethyl-9-methyladenine (Ib)	53	0.23
1-Propyl-9-methyladenine (Ic)	55	0.25
1-Benzyl-9-methyladenine (Id)	255	0.37
1-Methyladenosine (Ie)	250	1.4
1-Benzyladenosine (If)	900	0.80

a) Refer to those in Eq. (1).

Discussion

Of the 1,9-disubstituted adenines in Table I, the *n*-alkyl homologs (Ia, b, c) are the strongest bases and their pK_a's are closely similar, reflecting little difference in the electron-

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

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donating properties of the *n*-alkyl groups. The same situation has been found for a series of monoalkylamines[16b] or 1-alkyl-1,2-dihydro-2-iminopyrimidines.[4a] On the other hand, the observed decreases in the base strength of compounds Id,e,f are separately attributed to the electron-withdrawing nature, relative to a *n*-alkyl group, of the benzyl group[18b,20] and of the β-D-ribofuranosyl group.[21] This is paralleled by a similar decrease (0.55 pH unit) in pK_a of 1-substituted 1,2-dihydro-2-iminopyrimidine as the substituent is changed from the methyl to the benzyl group[4a] and by that recorded for a set of 1-methylcytosine and cytidine (0.45 pH unit)[22a] and for a set of 1,5-dimethylcytosine and 5-methylcytidine (0.48 pH unit).[22b] Although no direct evidence is available at present for the site of protonation in the conjugate acids of Ia—f,[23] we prefer to add a proton to the N^6 atom on the consideration of resonance structures. In addition, we have recently found that in the reaction of 9-ethyl-1-methyladenine with methyl iodide[24] and of 1-alkoxy-9-alkyladenine with alkyl halide[1,25] in N,N-dimethylacetamide alkylation occurs predominantly at the N^6-position. This finding and the results of more recent work of Singer, *et al.*[26] on alkylation of 1-methyladenosine also seem to be in favor of the assigned structure ($I \cdot H^+$) in Chart 1.

The results shown in Fig. 2 and Table III reveal that all compounds (Ia—f) undergo rearrangement to give the corresponding N^6-alkyl isomers (IIIa—f) by following the rate law given by Eq. (1), being consistent with the previous results[6a,7] obtained with Ia and Ie under slightly different reaction conditions. Since no intermediate (type II) (Chart 1) was detectable throughout the rearrangement reaction at various pH's, it is reasonable to assume that the ring-opening step (I→II) is rate-determining. Thus, it follows that 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.

Probably the most striking aspect of these two modes of hydroxide attack is that the attack on the protonated species is much faster than on the neutral species (by a factor of 90—1100) (Table III) and that the former is influenced by an electronic factor of a 1-substituent, whereas the latter, by a steric factor. In the reaction of the neutral species, for example, higher *n*-alkyl groups at the 1-position retarded the attack by hydroxide ion at the 2-position. Since there could be little difference, as discussed already, in the electron-donating properties of such alkyl groups, the observed retardation is probably owing to their steric bulk. Furthermore, the rate-retarding effect of the benzyl group observed for a set of Ia and Id or a set of Ie and If, contrary to the finding of Brookes, *et al.*[10a] that in 0.1 N sodium hydroxide at 37° If undergoes rearrangement somewhat faster than Ie, also appears to be a result of a balance of the steric and electronic effects, being in favor of the former. In sharp contrast, the roughly similar rates for the reactions of the protonated *n*-alkyl homologs (Ia, b, c) and the rate enhancement observed for the protonated 1-benzyl derivatives (Id, f) suggest that the electronic property of a 1-substituent affects the hydroxide attack on $I \cdot H^+$ at the

20) E.S. Gould, "Mechanism and Structure in Organic Chemistry," Henry Holt & Co., New York, 1959, pp. 206—207.
21) *a*) C.D. Jardetzky and O. Jardetzky, *J. Am. Chem. Soc.*, **82**, 222 (1960); *b*) R.P. Panzica, R.J. Rousseau, R.K. Robins, and L.B. Townsend, *ibid.*, **94**, 4708 (1972).
22) *a*) J.J. Fox and D. Shugar, *Biochim. Biophys. Acta*, **9**, 369 (1952); *b*) J.J. Fox, D.V. Praag, I. Wempen, I.L. Doerr, L. Cheong, J.E. Knoll, M.L. Eidinoff, A. Bendich, and G.B. Brown, *J. Am. Chem. Soc.*, **81**, 178 (1959).
23) The nuclear magnetic resonance spectra of the salts of these compounds in deuterated dimethyl sulfoxide generally showed a very broad two-proton peak of NH_2 ot rwo NH's in the −0.4—1.2 τ region, which gave little information on this matter.
24) Y. Taguchi, Bach. Pharm. Sci. Thesis, Kanazawa University, March, 1974.
25) T. Fujii, S. Kawakatsu, K. Kyo, and T. Itaya, Abstracts of Papers, 37th Meeting of Hokuriku Branch, Pharmaceutical Society of Japan, Toyama, Oct. 27, 1973, p. 18.
26) B. Singer, L. Sun, and H. Fraenkel-Conrat, *Biochemisrty*, **13**, 1913 (1974).

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2-position more significantly than does its steric bulk. One logical explanation for such a marked contrast is that the protonated species, being cations activated at the 2-position, are so reactive toward hydroxide anion that the steric factor around the $C_{(2)}$ atom is no longer as important.

It is also particularly noteworthy that the β-D-ribofuranosyl group at the 9-position accelerates the ring-opening of both the neutral and protonated species, but to a somewhat further extent for the latter. At present it is as yet uncertain whether this is attributed purely to the electron-withdrawing effect of the ribosyl group[21] and/or intramolecular participation[22,27] of the 5′-OH group. However, a rough comparison of the rates for the rearrangement of Ie in Table II with those[10b] of 1-methyladenosine 5′-phosphate or 1-methyldeoxyadenosine 5′-phosphate at 37° and the slightly faster rearrangement rate reported[28] for 1-(γ,γ-dimethylallyl)adenosine over its 5′-β-cyanoethyl phosphate suggest that the 5′-OH group may have a certain role to play at any rate in the accelerated ring-opening.

It may be seen from Table I that an electron-withdrawing group at the 1-position and the ribofuranosyl group at the 9-position separately lower the pK_a of I and this causes the population of the reactive protonated species at near neutrality to decrease. In practice, however, the advantage of rate enhancement brought by these substituents is much more great than compensating such a disadvantage, as shown in Table II. The biological implication of the facilitation of the Dimroth rearrangement by the β-D-ribofuranosyl group is not clear.

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27) *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.

28) W.A.H. Grimm and N.J. Leonard, *Biochemistry*, **6**, 3625 (1967).

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