# Purines. XLIV.[1] A Kinetic Study of the Dimroth Rearrangement of the Marine Sponge Purine 1,9-Dimethyl-8-oxoadenine and Related Compounds

Tozo FUJII,* Tohru SAITO, and Shigeji MORI

*Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi, Kanazawa 920, Japan.* *Received March 19, 1990*

**The reaction rates in the Dimroth rearrangements of the marine sponge base 1,9-dimethyl-8-oxoadenine (1) and related compounds such as 1,9-dimethyladenine (6a) and 8-bromo-1,9-dimethyladenine (6b) were measured in $H_2O$ at various pH's and ionic strength 1.0 at 40 °C. In all cases, attack of hydroxide ion on the protonated species of the substrate at the 2-position was faster than that on the neutral species by a factor of 100—1400. In the reaction of the protonated species, the relative ease of undergoing Dimroth rearrangement was in the order of 6b > 6a > 1. The same order of reactivity was found to hold for the neutral species.**

**Keywords** 8-substituted 1,9-dimethyladenine; adenine ring-opening; Dimroth rearrangement; 8-substituted $N^6$,9-dimethyladenine; acid dissociation constant; kinetic study; UV spectrophotometric determination

Purine derivatives found in various marine sponges have been rapidly increasing in number and in kind as well.[1,2] The title compound, 1,9-dimethyl-8-oxoadenine (6-imino-1,9-dimethyl-8-oxopurine) (**1**), represents a new addition to this group. It was isolated, but only in the form of the $N^6$-acetyl derivative (**2**), by Cimino *et al.* in 1985 from the English Channel sponge *Hymeniacidon sanguinea* GRANT.[3] Although the new acetyl derivative **2** was fully characterized by means of spectroscopic and X-ray crystallographic analyses, the parent base **1** itself remained uncharacterized because of the difficulty in separating **1** and co-occurring 1-methyladenine (spongopurine[4]) (**3**) from each other at the free base level.[3] The problem of securing **1** for characterization was recently solved by us by means of chemical synthesis.[1,5] In the course of this synthetic study, we found that both the target 8-oxoadenine **1** and the intermediate 8-bromo-1,9-dimethyladenine (**6b**) were susceptible to Dimroth rearrangement[6] to give the corresponding $N^6$-methyl isomers (**5** and **7b**) under alkaline conditions, reflecting their 1-substituted adenine structures. The Dimroth rearrangement of the 8-oxoadenine **1** (1 N aqueous NaOH, reflux, 1 h) seemed to require more vigorous reaction conditions than that of the 8-bromoadenine **6b** (1N aqueous NaOH, 55 °C, 35 min).[1,5] In order to investigate the effect of the 8-oxo and 8-bromo functions on the Dimroth rearrangement of the 1,9-dimethyladenine system (**6a**), we carried out a kinetic study using **1** and **6b**, together with the reference compound 1,9-dimethyladenine (**6a**), as the substrates in the present work.

The systems of reactions that produce the $N^6$-methyl isomers **5** and **7** from the 1-methyl isomers **1** and **6** through the nonisolable intermediates **4** and **8** are shown in Charts 1 and 2, respectively. These schemes are analogous to those that have been proposed for the Dimroth rearrangement of 1,9-disubstituted adenines.[6,7] At the inception of the kinetic study, the acid dissociation constants of the conjugate acids of the substrate bases **1**, **6a**, and **6b** were determined by means of ultraviolet (UV) spectrophotometry at 40 °C and ionic strength 1.0. It may be seen from Table I that the 8-bromo and 8-oxo functions affect the acid dissociation constant of the reference compound **6a** · $HClO_4$ in a striking manner. The observed decreases in base strength of **6b** and

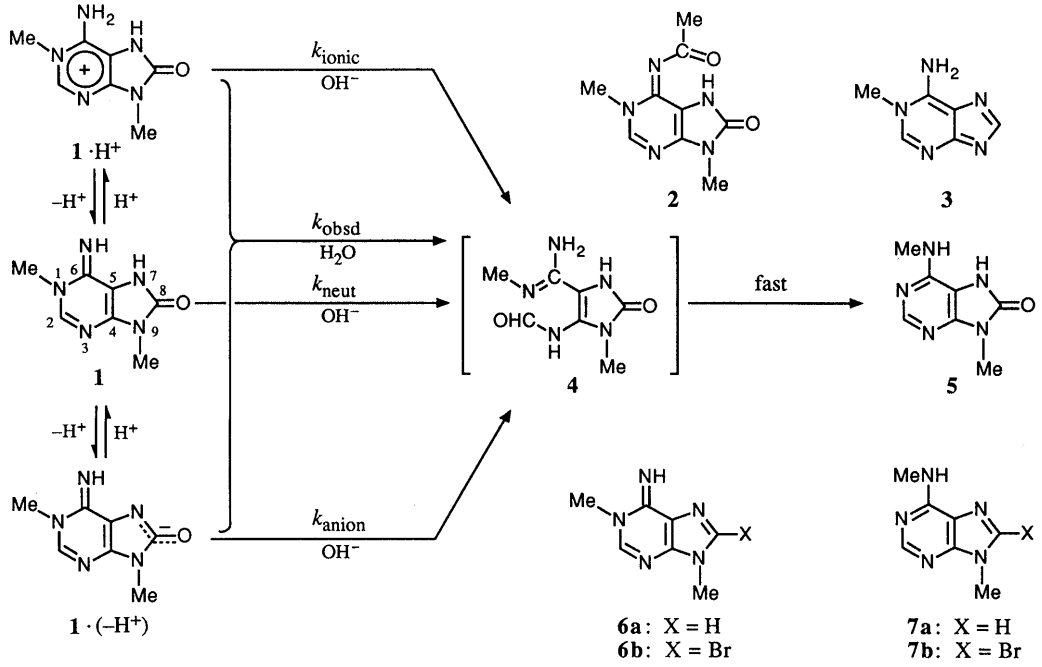


Chart 1

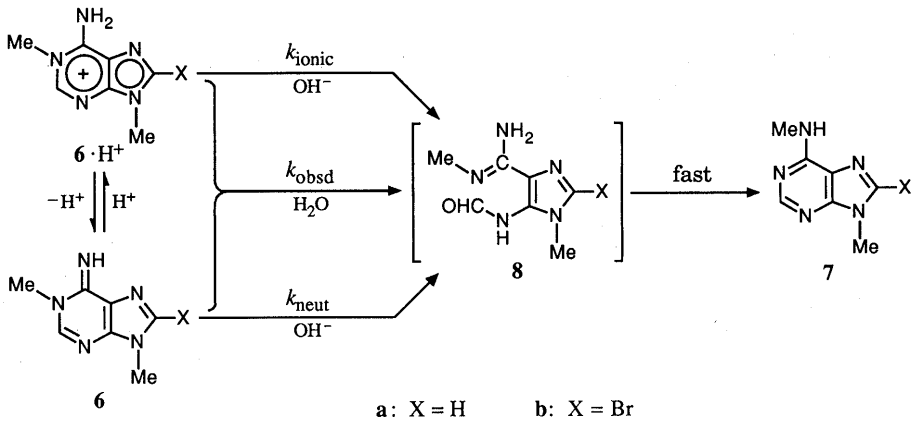


Chart 2

TABLE I. Acid Dissociation Constants of 1,9-Dimethyladenines (**1**, **6a**, and **6b**) and the Rate Constants for Their Rearrangements to $N^6$,9-Dimethyladenines (**5**, **7a**, and **7b**) at 40 °C and Ionic Strength 1.0

| Compound | | $pK_a$[b] | Pseudo-first-order rate constant, $k_{obsd} \times 10^5$ ($min^{-1}$)[a] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | pH value | | | | | |
| No. | 8-Substituent | | 7.00 | 7.65 | 9.00 | 10.00 | 11.00 | 11.42 |
| **6a**·$HClO_4$ | H | 8.97±0.03[c] | 1.95 | 8.23 | 68.5 | 138 | 292 | 457 |
| **6b** | Br | 8.54±0.02 | 4.11 | 14.9 | 111 | 225 | 600 | 961 |
| **1** | oxo | 6.90±0.06[d] | 0.501 | 0.619 | 0.774 | 1.27 | 10.6 | 23.8 |

*a*) The values at pH 11.42 are those obtained by extrapolating the plot of the rate constant (at 0.05 and 0.1 M buffer concentration) *versus* buffer concentration to 0.02 M buffer concentration, and the others are those of the rate constants determined at 0.02 M buffer concentration. *b*) Determined at 40 °C and ionic strength 1.0. *c*) The reported value[7c] is 8.96±0.04. *d*) Besides this basic $pK_a$, the acidic $pK_a$ was roughly estimated to be *ca.* 12.

**1** by 0.43 and 2.07 $pK_a$ units, relative to **6a**, are mainly attributable to the electron-withdrawing inductive effects ($-I$ effects) of the 8-bromo and 8-oxo groups[8] on the cyclic amidine system centering at the C(6) atom. In the case of **1**, an acidic $pK_a$ was roughly estimated to lie around 12, but we were unable to obtain the exact figure.

We then followed the rearrangements of **6a**, **6b**, and **1** in 0.02—0.1 M buffer solutions of pH 7.00 to 11.42 at ionic strength 1.0 at 40 °C by measuring the increases in UV absorption at 268 nm, 274 nm, and an appropriate wavelength in the range of 275—284 nm, which occur on formation of the rearranged products **7a**, **7b**, and **5**, respectively. The semilogarithmic plots of mole fractions of the residual substrates against time indicated that these rearrangement reactions obey fairly good pseudo-first-order kinetics at all pH's. Table I includes the rate constants ($k_{obsd}$) obtained from such plots. As a good approximation, the listed rate constants may be regarded as the limiting rate constants for zero buffer concentration because we have already found that the catalytic coefficients of the buffer components are small in this type of reaction.[7c,d] It may be seen that in all cases the reaction rate increases with increasing pH of the reaction medium.

Figure 1 shows pH–rate profiles, which were obtained by plotting the rate constants for the rearrangements of **6a** and **6b** as functions of pH. As in previous, similar cases,[7] theoretical pH–rate profiles may be calculated from Eqs. 1 and 2, where $v$ is the reaction rate; $[\mathbf{6a}]_{total}$ and $[\mathbf{6b}]_{total}$, the total concentrations of **6a** and its protonated species and of **6b** and its protonated species; $[\mathbf{6a}\cdot H^+]$ and $[\mathbf{6b}\cdot H^+]$,

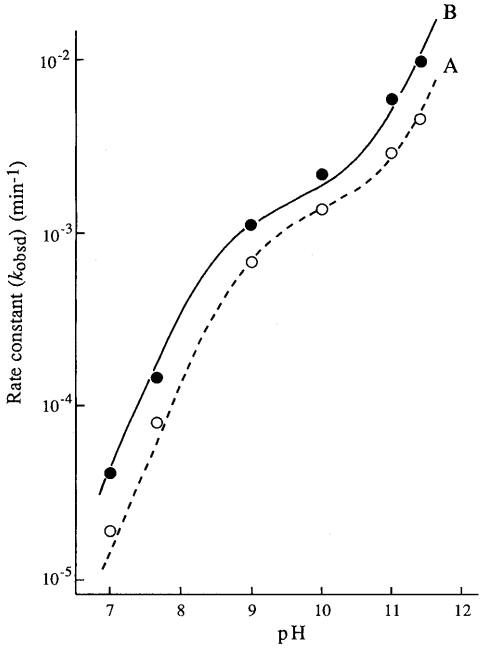


Fig. 1. pH–Rate Profiles for the Rearrangements of **6a** to **7a** (○) and of **6b** to **7b** (●) at 40 °C and Ionic Strength 1.0

the concentrations of the protonated species of **6a** and of **6b**; [**6a**] and [**6b**], the concentrations of the neutral species of **6a** and of **6b**; $[OH^-]$, hydroxide ion concentration;

$$v = k_{obsd}[\mathbf{6a}]_{total} = k_{ionic}[\mathbf{6a}\cdot H^+][OH^-] + k_{neut}[\mathbf{6a}][OH^-] \quad (1)$$

$$v = k_{obsd}[\mathbf{6b}]_{total} = k_{ionic}[\mathbf{6b}\cdot H^+][OH^-] + k_{neut}[\mathbf{6b}][OH^-] \quad (2)$$

$k_{obsd}$, the observed pseudo-first-order rate constant; $k_{ionic}$ and $k_{neut}$, the rate constants for hydroxide attack on the protonated and the neutral species; the p$K_a$ values of **6a** and **6b** are 8.97 and 8.54 (Table I). When $k_{ionic}$ of 50 and $k_{neut}$ of 0.50 (time in minutes) for Eq. 1 and $k_{ionic}$ of 150 and $k_{neut}$ of 1.25 (time in minutes) for Eq. 2 were adopted, the resulting theoretical pH–rate profiles corresponded to curves A and B plotted in Fig. 1.

In the case of **1**, the rearrangement may involve attack of hydroxide ion on the anionic species [**1**·(−H$^+$)] besides the above two modes of attack, as shown in Chart 1, since **1** has an acidic p$K_a$ value of *ca.* 12 (*vide supra*). Then, a theoretical pH–rate profile may be calculated from Eq. 3,

$$v = k_{obsd}[\mathbf{1}]_{total} = k_{ionic}[\mathbf{1}\cdot H^+][OH^-] + k_{neut}[\mathbf{1}][OH^-] + k_{anion}[\mathbf{1}\cdot(-H^+)][OH^-] \quad (3)$$

where [**1**·(−H$^+$)] is the concentration of the anionic species of **1**; $k_{anion}$, the rate constant for hydroxide attack on the anionic species; the other symbols and quantities correspond to those in Eqs. 1 and 2; the basic p$K_a$ of **1** is 6.90 (Table I) and the acidic p$K_a$ is taken as 12. When $k_{ionic}$ of 35 and $k_{neut}$ of 0.025 were adopted by assuming $k_{anion}$ is negligibly small,[9] the resulting theoretical pH–rate profile corresponded to the curve plotted in Fig. 2. This is roughly in agreement with a pH–rate profile obtained by plotting the rate constants ($k_{obsd}$) for the rearrangement of **1** (Table I) as a function of pH.

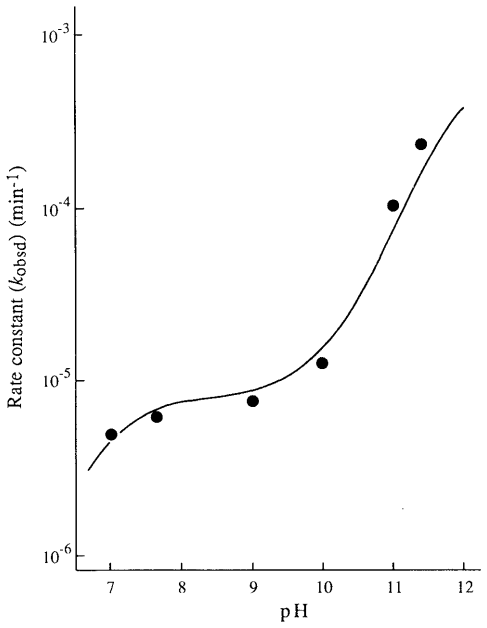


Fig. 2. pH–Rate Profile for the Rearrangement of **1** to **5** at 40 °C and Ionic Strength 1.0

TABLE II. Effect of Substituents at the 8-Position on the Dimroth Rearrangement of the Protonated and Neutral Species of 1,9-Dimethyladenine (**6a**) at 40 °C and Ionic Strength 1.0

| Substrate | | Second-order rate constant (M$^{-1}$ min$^{-1}$) | | | |
|---|---|---|---|---|---|
| | | Ionic species | | Neutral species | |
| No. | 8-Substituent | $k_{ionic}$ [a] | $k_{rel}$ [b] | $k_{neut}$ [a] | $k_{rel}$ [c] |
| **6a** | H | 50 (50)[d] | 1 | 0.50 (0.55)[d] | 1 |
| **6b** | Br | 150 | 3 | 1.25 | 2.5 |
| **1** | oxo | 35 | 0.7 | 0.025 | 0.05 |

*a*) Refer to those in Eqs. 1—3. *b*) Relative to the $k_{ionic}$ value for **6a**. *c*) Relative to the $k_{neut}$ value for **6a**. *d*) Taken from ref. 7c.

Table II lists the second-order rate constants thus calculated for the hydroxide attack on the protonated and the neutral species of the three substrates. It may be seen that the present $k_{ionic}$ and $k_{neut}$ values obtained with **6a** are virtually identical with those reported previously,[7c] indicating their high reliability. Introduction of a bromo or an oxo group into the 8-position still preserves the characteristic feature that the hydroxide attack on the protonated species is much faster than that on the neutral species (by a factor of 100—1400). In the reaction of the protonated species, however, the 8-bromo derivative **6b** rearranges 3 times as fast as the reference compound **6a**, whereas the 8-oxo derivative **1** rearranges more slowly than **6a** by a factor of 0.7. The rearrangement of the neutral species also follows this trend. Such rate-enhancement by the 8-bromo group in both species may be explained in terms of its electron-withdrawing inductive effect (−*I* effect)[8] on the 2-position where hydroxide attack (Chart 2) is to occur. The rate-retardation by the 8-oxo group in both species may be attributable to its C(5)-amido [or C(8)-OH] character, exerting an electron-donating resonance effect (+*R* effect)[8] on the 2-position (Chart 1).

In conclusion, the above results have shed light on the instability of the marine sponge base **1** to alkaline hydrolysis. Since the free base **1** itself has not yet been isolated from natural sources, it is hoped that the search for **1** as a natural product will be facilitated by the present and previous,[1,5] chemical and spectral characterization of synthetic **1**.

## Experimental

**General Notes** Spectrophotometric determinations were carried out with a Hitachi model 320 spectrophotometer. For the measurements of pH values, a Toa HM-18ET pH meter equipped with a Toa type GST-155C glass electrode was used.

**Materials** The substrates and their $N^6$-methyl isomers selected for the kinetic study were taken from stocks which had been prepared according to published procedures: 1,9-dimethyl-8-oxoadenine (**1**)[1]; 1,9-dimethyladenine perchlorate (**6a**·HClO$_4$)[7b]; 8-bromo-1,9-dimethyladenine (**6b**)[1]; $N^6$,9-dimethyl-8-oxoadenine (**5**)[1]; $N^6$, 9-dimethyladenine (**7a**)[7b]; 8-bromo-$N^6$,9-dimethyladenine (**7b**).[1]

**Spectrometric Determination of Acid Dissociation Constants** The p$K_a$'s of **1**, **6a**·HClO$_4$, and **6b** at 40 °C and ionic strength 1.0 were determined in a manner similar to that described previously.[7c] The results are listed in Table I.

**Kinetic Procedure** The Dimroth rearrangement reactions of **1**, **6a**·HClO$_4$, and **6b** to **5**, **7a**, and **7b**, as shown in Charts 1 and 2, in aqueous solution at various pH's and ionic strength 1.0 at 40 °C were followed by UV spectrophotometry. Buffer solutions employed for kinetic runs were 0.02 M $NaH_2PO_4$–$Na_2HPO_4$ (pH 7.00 and 7.65 at 40 °C); 0.02 M $NaHCO_3$–$Na_2CO_3$ (pH 9.00 and 10.00 at 40 °C); 0.02 M $Na_2HPO_4$–$Na_3PO_4$ (pH 11.00 at 40 °C); 0.05 and 0.1 M $Na_2HPO_4$–$Na_3PO_4$ (pH 11.42 at 40 °C), and were brought to ionic strength 1.0 with KCl.

The substrates **1**, **6a**·HClO$_4$, and **6b** were separately dissolved in the buffer solutions at a concentration of $4.35\times10^{-5}$—$4.6\times10^{-5}$ M. Aliquots (*ca.* 5 ml) of the resulting solutions were sealed in small ampules and placed in a thermoregulated constant-temperature bath kept at 40 °C (accurate to ±0.05 °C). At intervals, the ampules were removed, cooled, and broken, and the optical densities of the contents at 268 nm (for the reaction **6a**·HClO$_4$→**7a**), 274 nm (**6b**→**7b**), or 275 nm (for **1**→**5** at pH 7.00 and 7.65), 276 nm (at pH 9.00), 280 nm (at pH 10.00), or 284 nm (at pH 11.00 and 11.42) were determined at room temperature against blank buffer

solutions. During the kinetic runs the pH was never found to vary by more than $\pm 0.02$ unit. The concentrations of the unaltered substrates were calculated in the usual manner[10] by utilizing the molecular extinction coefficients at the analytical wavelength, obtained from solutions of analytically pure samples of the substrates and the rearranged isomers **7a**, **7b**, and **5** in the appropriate buffer solutions. All rearrangements except for slow ones were followed through at least two half-lives with at least five determinations, and good pseudo-first-order kinetics were obtained in all cases. The results are included in Table I.

**Acknowledgment** We are pleased to acknowledge the support of this work by a Grant-in-Aid for Scientific Research (C) (No. 01571148) from the Ministry of Education, Science and Culture, Japan.

**References and Notes**

1) Paper XLIII in this series, T. Fujii, T. Saito, and S. Mori, *Chem. Pharm. Bull.*, **38**, 2146 (1990).
2) Apart from the sponge purine derivatives cited in ref.1, the isolation of mycalisines A and B, unusual nucleosides with the 7-deazapurine nucleus, from a marine sponge *Mycale* sp. has been reported: Y. Kato, N. Fusetani, S. Matsunaga, and K. Hashimoto, *Tetrahedron Lett.*, **26**, 3483 (1985).
3) G. Cimino, A. De Giulio, S. De Rosa, S. De Stefano, R. Puliti, C. A. Mattia, and L. Mazzarella, *J. Nat. Prod.*, **48**, 523 (1985).
4) *a*) D. Ackermann and P. H. List, *Naturwissenschaften*, **48**, 74 (1961); *b*) *Idem*, *Z. Physiol. Chem.*, **323**, 192 (1961).
5) T. Fujii, T. Saito, and S. Mori, *Heterocycles*, **27**, 1145 (1988).
6) For reviews, see *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; *c*) T. Fujii, T. Itaya, and T. Saito, *Yuki Gosei Kagaku Kyokai Shi*, **41**, 1193 (1983).
7) *a*) J. B. Macon and R. Wolfenden, *Biochemistry*, **7**, 3453 (1968); *b*) T. Itaya, F. Tanaka, and T. Fujii, *Tetrahedron*, **28**, 535 (1972); *c*) T. Fujii, T. Itaya, and T. Saito, *Chem. Pharm. Bull.*, **23**, 54 (1975); *d*) T. Itaya, T. Saito, S. Kawakatsu, and T. Fujii, *ibid.*, **23**, 2643 (1975); *e*) T. Fujii, T. Itaya, T. Saito, and S. Kawakatsu, *ibid.*, **32**, 4842 (1984); *f*) T. Fujii and T. Saito, *ibid.*, **33**, 3635 (1985); *g*) T. Fujii, T. Saito, and N. Terahara, *ibid.*, **34**, 1094 (1986).
8) *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, New York, 1955, Chapter 14; *b*) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," Methuen, London, 1962, Chapter 8; *c*) E. S. Gould, "Mechanism and Structure in Organic Chemistry," Henry Holt and Co., 1959, Chapter 7; *d*) N. S. Isaacs, "Physical Organic Chemistry," Longman Scientific & Technical, Harlow, 1987, Chapter 4.
9) This assumption is based on a consideration of the electron-donating resonance effect ($+R$ effect) of the negatively charged imidazole moiety on the 2-position where hydroxide attack is to occur.
10) H. H. Jaffé and M. Orchin, "Theory and Applications of Ultraviolet Spectroscopy," John Wiley & Sons, New York, 1962, pp. 556—560.