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PURINES—VI[1]

RATE STUDY AND MECHANISM OF THE DIMROTH REARRANGEMENT OF 1-ALKOXY-9-ALKYLADENINES AND 1-ALKYL-9-METHYLADENINES[2]

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Abstract—The rates of the Dimroth rearrangements of 1-alkoxy-9-alkyladenines (I) and 1-alkyl-9-methyladenines (V) have been measured. It is shown that 1-alkoxy derivatives (I) undergo rearrangement to give isomeric 6-alkoxyamino derivatives (III) through isolable monocyclic intermediates (II) and that these intermediates also suffer hydrolysis leading to deformylated derivatives (IV) in competition with their reclosure to III. In the 1-alkyl series, no intermediates have been detected and thus the observed first-order rate constants for the rearrangements may be regarded as those for the ring-opening of V. Comparison of the rate constants for 1-methoxy-9-methyladenine (Ia) and 1,9-dimethyladenine (Va) reveals that at pH 7·60 and above Va rearranges more rapidly than Ia, although the latter undergoes ring-opening *ca.* 30 times as fast as the former. In each of both compounds ring-opening proceeds at a rate proportional to hydroxide ion concentration below pH 8·5 and above pH 11, with a plateau between the two values. These results are consistent with attack by the hydroxide ion on both the protonated and neutral species of the adenine derivatives.

PREVIOUS work[3,4] in this series has suggested that the Dimroth rearrangement of 1-alkoxy-9-alkyladenine (type I) to 6-alkoxyamino-9-alkylpurine (type III) readily proceeds through isolable N′-alkoxy-1-alkyl-5-formamidoimidazole-4-carboxamidine (type II), presumed to be an intermediate in the rearrangement, which also gives N′-alkoxy-1-alkyl-5-aminoimidazole-4-carboxamidine (type IV), the deformylated product. The characterization of the intermediate and the by-product has encouraged us to investigate kinetically the Dimroth rearrangement in the adenine series since only a few kinetic results[5–11] with 1-alkyladenine derivatives have been reported, whereas the studies on the pyrimidine series have been made most extensively.[12] The present paper reports a rate study concerned with 1-alkoxy-9-alkyladenines (I) and 1-alkyl-9-methyladenines (V) and presents probable mechanisms for the Dimroth rearrangement of such derivatives.

Preparation of compounds

Among the test compounds selected for the kinetic study, 1-methoxy-9-methyladenine (Ia), 1-ethoxy-9-methyladenine (Ib), 1-ethoxy-9-ethyladenine (Ic), 1,9-dimethyladenine (Va), 1-ethyl-9-methyladenine (Vb), and 1-propyl-9-methyladenine (Vc) were used in the form of the perchlorate salts since the corresponding free bases were hygroscopic[13] and/or difficult to purify and the perchlorate ion, which is transparent in the UV, caused no interference in the spectrophotometric determination of the organic components in reaction mixtures. The perchlorates of Ia and Ic and the ring-opened compounds, N′-methoxy-1-methyl- (IIa), N′-ethoxy-1-methyl- (IIb), and N′-ethoxy-1-ethyl-5-formamidoimidazole-4-carboxamidine (IIc), were prepared

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according to the procedures reported previously.[4, 13] The perchlorate of Ib was obtained from the corresponding hydriodide[14, 15] by treating it with ammonium perchlorate. Similar treatment of the hydriodides of Va, b, c, derived from 9-methylation[16, 17] of 1-alkyladenines or 1-alkylation[5, 16, 18] of 9-methyladenine, produced the corresponding perchlorates. The Dimroth rearrangement of Va, b, c at neutral or high pH and purification of the products gave analytical samples of 6-methylamino- (VIIa), 6-ethylamino- (VIIb), and 6-propylamino-9-methylpurine (VIIc).

Table 1 collects the UV spectral data and acid dissociation constants of some of the compounds thus prepared. These data are in harmony with those[9, 10, 16, 18, 19] of the known 1,9- and N^6,9-disubstituted adenines, although the pK_a values for Va, b, c are somewhat higher than the values[9, 10] recorded for 1-substituted adenosines.

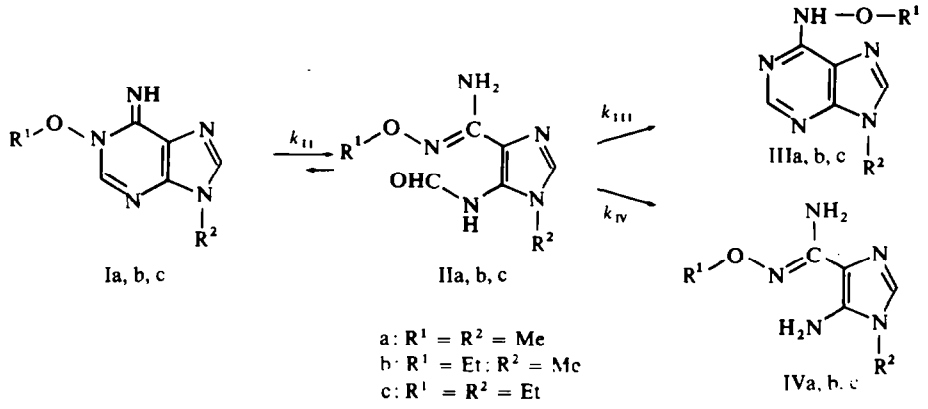
TABLE 1. ACID DISSOCIATION CONSTANTS AND UV SPECTRA OF SUBSTITUTED 9-ALKYLADENINES

Compound	pK_a[a]		UV Spectra							
			Solvent E[b]		Solvent A[c]		Solvent N[d]		Solvent B[e]	
	at 20°	at 40°[f]	λ_{max} (mμ)	$\varepsilon \times 10^{-3}$	λ_{max} (mμ)	$\varepsilon \times 10^{-3}$	λ_{max} (mμ)	$\varepsilon \times 10^{-3}$	λ_{max} (mμ)	$\varepsilon \times 10^{-3}$
Ia · $HClO_4$[g]	8·55 ± 0·04	8·44 ± 0·04	259	12·3	260	11·9	260	11·9	258	13·0
Ib · $HClO_4$	8·61 ± 0·05	.....	259	12·7	260	12·3	260	12·3	258	12·9
Va · $HClO_4$	9·08 ± 0·07	8·94 ± 0·05	261	13·5	261	13·6	261	13·3	261	14·1
Vb · $HClO_4$	9·16 ± 0·09	—	261	12·3	261	12·7	261	12·7	261	13·3
Vc · $HClO_4$	9·15 ± 0·09		261	12·8	261	12·8	261	12·6	261	13·2
VIIa	4·02 ± 0·03	—	268	14·7	265	16·0	268	15·1	268	15·1
VIIb	4·08 ± 0·05	.....	270	15·8	266	16·8	270	16·4	270	16·4
VIIc	4·14 ± 0·11	—	270	16·2	266	17·3	270	17·0	270	17·0

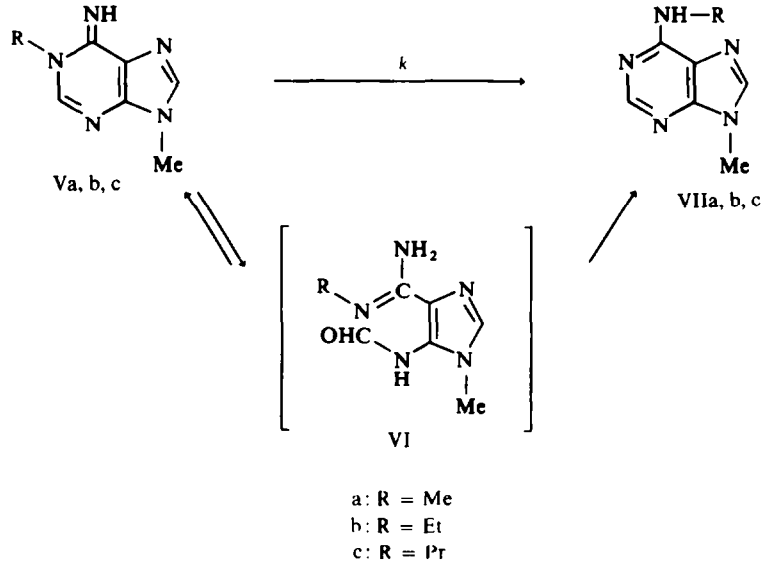
[a] Determined by UV spectrophotometry.[32]
[b] 95% EtOH aq.
[c] 0·1 N HCl (pH 1).
[d] 0·005 M phosphate buffer (pH 7).
[e] 0·1 N NaOH aq (pH 13).
[f] Measured on solutions in 0·1 M buffers (KH_2PO_4—Na_2HPO_4, KH_2PO_4—$Na_2B_4O_7$, and $NaHCO_3$—Na_2CO_3) at ionic strength 0·50; analytical wavelength: 295 mμ.
[g] From Ref. 13 except for the pK_a value at 40°.

Kinetic study

Schemes 1 and 2, respectively, represent the systems of reactions supported by the chemical evidence[3, 4, 9, 12] for the Dimroth rearrangements of 1-alkoxy-9-alkyladenines (I) and 1-alkyl-9-methyladenines (V). Rates of the various reactions (I → II, IV ← II → III, and V → VII) at 40° and different pH's were followed by UV spectrophotometry. Since it was found that all the components in the reaction mixtures were stable at pH 5·8 at room temperature and that their UV spectra at this particular pH were distinguishable from each other as exemplified in Figs 1 and 2, mixtures were rapidly quenched and diluted with citrate buffer (pH 5·80) to stop the reactions, and then absorbances of the resulting solutions were measured at appropriate analytical wavelengths[20] to determine the concentrations of each of the components.



SCHEME 1



SCHEME 2

In the rearrangement of Ia, b, c at pH 7·60 and above, the first step (I → II) of the consecutive reactions (I → II → III), which were accompanied by the simultaneous reaction (II → IV), was so rapid that it was possible to treat it separately from the second step (analytical wavelength: 260 mμ). Cyclization of IIa, b, c to IIIa, b, c and deformylation to IVa, b, c occurred simultaneously and analysis of the resulting three-component system was performed by measuring optical densities at 252 and 268 mμ (Fig. 1). In the reaction of Ia at pH 6·18, however, the rate of the first step was comparable

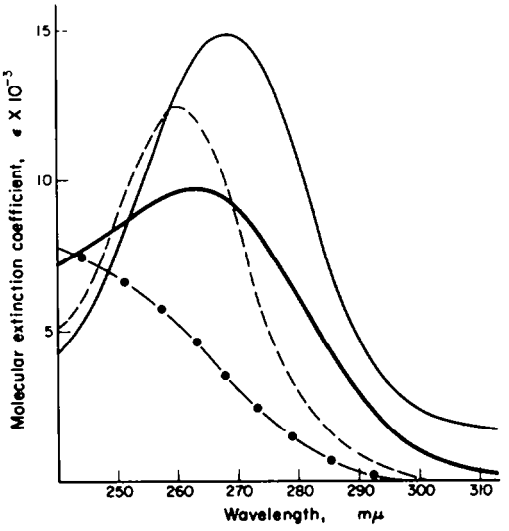


FIG 1. UV spectra of the N-ethoxy derivatives (Ic–IVc) in 0·06 M citrate buffer (pH 5·80) at ionic strength 0·50 at 20°.
– – – – –: Ic · $HClO_4$
–·–·–·–: IIc
———: IIIc
———: IVc

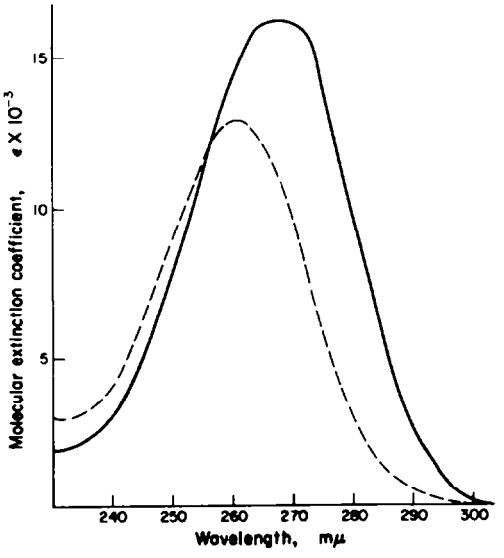


FIG 2. UV spectra of dimethyladenines (Va and VIIa) in 0·06 M citrate buffer (pH 5·80) at ionic strength 0·50 at 20°.
– – – – –: Va · $HClO_4$
———: VIIa

to those of the two simultaneous reactions in the second step. Accordingly, the concentrations of the four components (Ia, IIa, IIIa, and IVa) that coexisted in the reaction mixture had to be estimated on the basis of calculations using absorbances at 260 and 268 mμ and the ratio (k_{III}/k_{IV}) of the rate constants for the simultaneous reactions (IV ← II → III) at the same pH.

Rearrangement in the 1-alkyl series (Va, b, c) at various pH's was followed by the increase in absorption at 275 mμ which occurs upon formation of the rearranged products (VIIa, b, c) (Fig. 2).

RESULTS

A quantitative study of the rearrangement of Ia at pH 6·18 and ionic strength 0·50 at 40° led to the results illustrated in Fig. 3. The observed variation of the concentrations of each of the four components (Ia, IIa, IIIa, and IVa) with time is in good agreement with that calculated from the equations shown in the legend for Fig. 3, which are given by assuming that the rearrangement of Ia into IIIa is composed of consecutive first-order reactions, Ia → IIa → IIIa, and a simultaneous reaction, IIa → IVa, as shown in Scheme 1, where

$$k_{II} = 1{\cdot}5 \times 10^{-4}\ \text{min}^{-1},$$
$$k_{III} = 1{\cdot}4 \times 10^{-4}\ \text{min}^{-1},$$

and

$$k_{IV} = 0{\cdot}5 \times 10^{-4}\ \text{min}^{-1}\ \text{(see below)}.$$

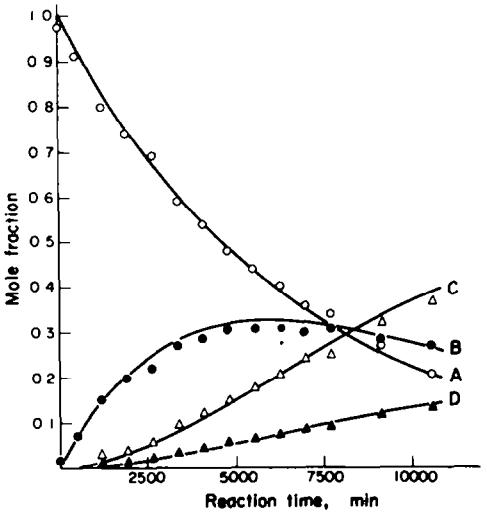


FIG. 3. Variation of the concentrations of the four components with time in the rearrangement of Ia at pH 6·18 and ionic strength 0·50 at 40°: ○, Ia; ●, IIa; △, IIIa; ▲, IVa. Solid lines A, B, C, and D represent mole fractions calculated from the following equations:

Curve A: $[Ia]/[Ia]_0 = \exp(-1{\cdot}5 \times 10^{-4}t)$

Curve B: $[IIa]/[Ia]_0 = (1{\cdot}5/0{\cdot}4)\{\exp(-1{\cdot}5 \times 10^{-4}t) - \exp(-1{\cdot}9 \times 10^{-4}t)\}$

Curve C: $[IIIa]/[Ia]_0 = (1{\cdot}5/1{\cdot}9)\{1 + (1{\cdot}5/0{\cdot}4)\cdot\exp(-1{\cdot}9 \times 10^{-4}t) - (1{\cdot}5/0{\cdot}4)\cdot\exp(-1{\cdot}5 \times 10^{-4}t)\}$

Curve D: $[IVa]/[Ia]_0 = (0{\cdot}4/1{\cdot}9)\{1 + (1{\cdot}5/0{\cdot}4)\cdot\exp(-1{\cdot}9 \times 10^{-4}t) - (1{\cdot}5/0{\cdot}4)\cdot\exp(-1{\cdot}5 \times 10^{-4}t)\}$

where the quantities in brackets represent the concentrations; $[Ia]_0$ is the initial concentration of Ia; and t is the reaction time in min.

Thus, it becomes quite evident that the ring-opened compound (IIa) isolated during the course of the reaction is actually an intermediate in the Dimroth rearrangement.

At pH 7·60 and above rates of the first step (Ia → IIa) could be measured separately from the second step (IVa ← IIa → IIIa) and good first-order kinetics were observed at all pH's for at least two half-times. The results are summarized in Table 2. Although the conversion of Ia into IIa has been considered to be reversible,[3,4] formation of Ia in the reverse experiment was extremely small, indicating that equilibrium between Ia and IIa must lie almost completely on the side of IIa. Therefore, it is permissible as a good approximation to regard the first step as practically irreversible.

TABLE 2. RATES OF THE RING-OPENING OF 1-ALKOXY-9-ALKYLADENINES (Ia, b, c) AT 40° AND IONIC STRENGTH 0·50

Substrate	Pseudo-first-order rate constant, k_{II} (min^{-1}) × 10^4 pH value							
	6·18	7·60	7·98	9·44	10·49	10·99	11·36	11·72
Ia	1·5	27	48	180	220	250	380	470
Ib	—	—	—	—	—	—	—	410
Ic	—	—	—	—	—	—	—	380

The ring-opening reactions of Ib and Ic at pH 11·72 were also followed in a similar manner. It may be seen from Table 2 that there is no appreciable difference in the rate constants of 1-methoxy and 1-ethoxy derivatives and that the rate of ring-opening increases as the pH of the medium is raised.

In the reaction of the intermediates (IIa, b, c) at various pH's at 40° and ionic strength 0·50, reversion of II to I was negligibly small. Thus, variation of the concentrations of the three components, II, III, and IV, with time was followed: decrease of the concentration of II and increase of those of III and IV*, respectively, obeyed good pseudo-first-order kinetics at all pH's over at least 75% reaction. As shown in Table 3, the rates of each of the simultaneous reactions (IVa ← IIa → IIIa) in the alkaline region increase with increasing pH value. However, the ratio of k_{III} to k_{IV} decreases with increasing pH, becoming less than 1 at pH 11·72. The rate constants observed for IIb and IIc at pH 11·72 were also comparable to those recorded for IIa. Comparison of k_{III} (for IIa) with k_{II} (for Ia) (Table 2) at pH 7·60 and above reveals that in the 1-methoxy derivative (Ia) the rate of the ring-opening step is 27–64 times that of the cyclization step.

Rearrangement of the 1-alkyl derivatives (Va, b, c) to the N^6-alkyl derivatives (VIIa, b, c) (Scheme 2) was also followed in a similar way. Good first-order rate plots were obtained at all pH's for at least two half-times. It may be seen from Table 4 that the

* In the previous experiment[3,4] in which a solution of IIc in H_2O was refluxed near neutrality for 5 hr, paper chromatographies were incapable of differentiating IVc from IIIc or IIc. Formation of IVc, however, was revealed by means of TLC [Merck silica gel GF_{254}, $CHCl_3$—EtOH (8:1) or AcOEt—EtOH (8:1) or benzene—EtOH (6:1)].

TABLE 3. RATE CONSTANTS, k_{III} (min^{-1}) AND k_{IV} (min^{-1}), FOR THE RING-CLOSURE AND DEFORMYLATION OF THE INTERMEDIATES (IIa, b, c) AT 40° AND IONIC STRENGTH 0·50

Substrate		Pseudo-first-order rate constant pH value					
		6·18	7·60	9·44	10·49	10·99	11·72
IIa	$k_{III} \times 10^4$:	1·4	0·73	2·8	6·3	9·2	13
	$k_{IV} \times 10^4$:	0·5	0·37	2·0	4·4	7·8	18
IIb	$k_{III} \times 10^4$:	—	—	—	—	—	11
	$k_{IV} \times 10^4$:	—	—	—	—	—	19
IIc	$k_{III} \times 10^4$:	—	—	—	—	—	11
	$k_{IV} \times 10^4$:	—	—	—	—	—	7·0

rate constant, k, increases as the pH of the medium is increased. At pH 11·72 the 1-methyl derivative (Va) rearranges somewhat faster than 1-ethyl (Vb) or 1-propyl derivative (Vc). Since no intermediate (VI) was detectable during the course of the reaction, the ring-opening step (V → VI) seems to be rate-determining. This presents a striking contrast to the case of the 1-alkoxy derivatives (I). Accordingly, the observed pseudo-first-order rate constant (k) may be regarded as that for ring-opening of V.

TABLE 4. RATES OF THE REARRANGEMENT OF 1-ALKYL-9-METHYLADENINES (Va, b, c) TO 6-ALKYLAMINO-9-METHYLPURINES (VIIa, b, c) AT 40° AND IONIC STRENGTH 0·50

Substrate	Pseudo-first-order rate constant, k (min^{-1}) × 10^4 pH value								
	6·18	7·60	7·98	8·08	9·44	10·49	10·99	11·36	11·72
Va	0·042	0·94	1·9	2·5	13	21	30	53	58
Vb	—	—	—	—	—	—	—	—	39
Vc	—	—	—	—	—	—	—	—	39

In the ring-opening steps of both the 1-alkoxy and 1-alkyl series, no appreciable change in rate was observed when buffer concentrations were varied at constant ionic strength. In Fig. 4 the rate constants obtained for ring-opening of Ia and Va are plotted as a function of pH. Similar to the rearrangement of 1-methyladenosine reported by Macon and Wolfenden,[9] this type of rate profile appears to be consistent with a mechanism involving attack of the hydroxide ion on the protonated species of Ia or Va, superseded in importance at high pH by hydroxide attack on the neutral species. Theoretical rate profiles were calculated from the rate equations, substituting for $[AH^+]$ the fraction of the base protonated at each pH value, substituting for [A] the fraction present as free base, and adopting pK_a's of 8·44 for Ia and 8·94 for Va, which were measured at 40° at ionic strength 0·50, and ionic product of water (pK_w) of 13·53 (at 40°).[21] The ring-opening reactions of Ia and Va follow the rate laws:

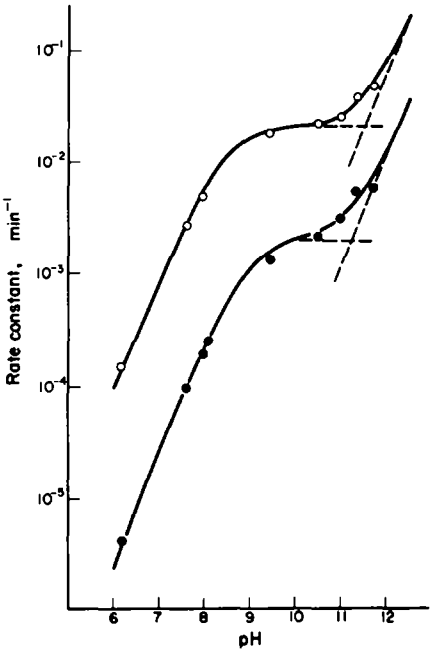


FIG. 4. Effect of pH on rates of the ring-opening of Ia and of the rearrangement of Va; ○, Ia; ●, Va. Solid and dashed lines are calculated from the rate equations presented in the text.

$$k_{II} = 2500\,[AH^+]\,[OH^-] + 2{\cdot}0\;\;[A]\,[OH^-]$$
$$k \;\;= \;\;80\,[AH^+]\,[OH^-] + 0{\cdot}35\,[A]\,[OH^-]$$

The solid lines in Fig. 4 are calculated from these equations, whereas the dashed lines represent the contributions of the two modes of attack.

Support for the postulated mechanism has been furnished by a study of ionic-strength effect. As shown in Table 5, at pH 7·98 an increase in ionic strength from

TABLE 5. INFLUENCE OF IONIC STRENGTH ON RATES OF THE RING-OPENING OF Ia AND OF THE REARRANGEMENT OF Va IN 0·1 M PHOSPHATE BUFFERS AT 40°

Substrate	pH	Ionic strength[a]	Rate constant, min^{-1}	
			$k_{II} \times 10^4$	$k \times 10^4$
Ia	7·98	0·50	48	—
	7·98	2·50	33	—
	11·36	0·50	380	—
	11·36	2·50	360	—
Va	7·98	0·50	—	1·9
	7·98	2·50	—	0·99
	11·36	0·50	—	53
	11·36	2·50	—	46

[a] Adjusted with KCl.

0·50 to 2·50 resulted in a 31% decrease in the rate of ring-opening of Ia and a 48% decrease in the rate of rearrangement of Va. This indicates that a reaction of cation, AH^+, with anion, OH^-, is dominant in the region of pH lower than the pK_a value of the conjugate acid of the base.[22] On the other hand, little change was observed at pH 11·36 for both bases, suggesting that at high pH contribution of a reaction between a neutral molecule, A, and anion, OH^-, must be great.[22]

DISCUSSION

The rates of rearrangement of 1,9-dimethyladenine (Va) at 40° at various pH's, shown in Table 4, may be compared with those[6] reported for 1-methyladenosine 5'-phosphate and 1-methyldeoxyadenosine 5'-phosphate at 37°. This suggests that effect of 9-substituents on the rate may be small. It is interesting that among the 1-alkyl derivatives (V) tested the methyl derivative (Va) underwent rearrangement more rapidly than did the higher homologs in contrast to the results[23] obtained for 1-alkyl-1,2-dihydro-2-iminopyrimidines at pH 14 and 40°. As in the case of 1-methyladenosine,[9] no intermediate (VI) (Scheme 2) was detected throughout the reaction. Thus, it is reasonable to assume that the ring-opening step is rate-determining, and to regard the observed rate constants as those for ring-opening of V.

The results shown in Fig. 3 and Tables 2 and 3 reveal that 1-alkoxy-9-alkyladenines (I) undergo rearrangement to give the isomeric 6-alkoxyamino derivatives (III) through the isolable intermediates (II), as illustrated in Scheme 1, and that the intermediates also suffer hydrolysis leading to the deformylated products (IV) in competition with their reclosure to III. Contrary to the case of 1-alkyl-9-substituted-adenines, the rate-determining stage is ring-closure of II; the rate of ring-opening of I is 27–64 times that of reclosure of II.

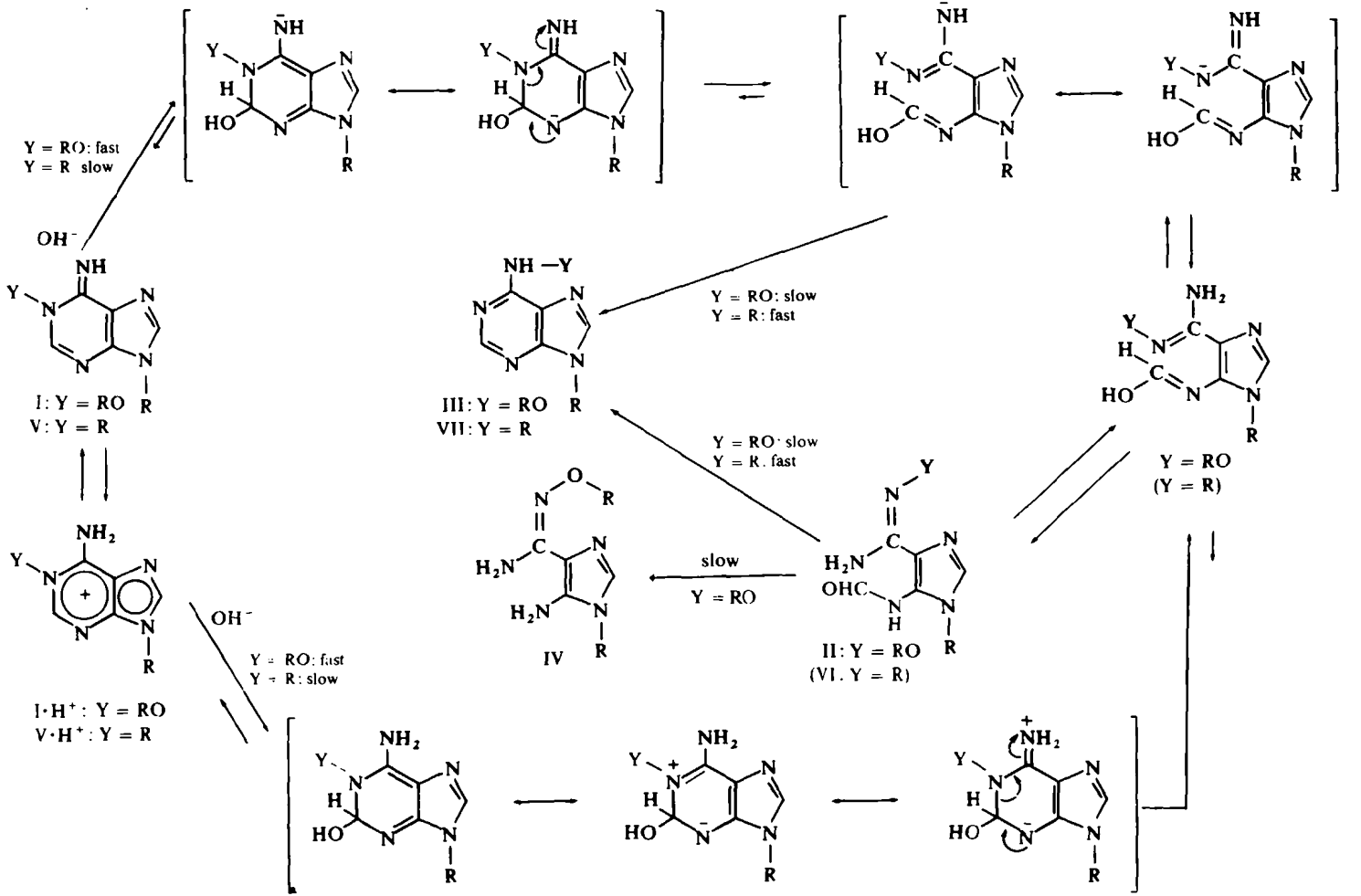
In the pH range examined for Ia at 40°, the ratio of k_{II} to the sum of k_{III} and k_{IV} becomes greatest at pH 9·44, and maximum yield (91%) and optimum reaction time (4 hr) for the preparation of IIa can be calculated from the following equation:

$$c = c_0 [k_{II}/(k_{III} + k_{IV} - k_{II})] \{\exp(-k_{II}t) - \exp[-(k_{III} + k_{IV})t]\}$$

where c is the concentration of IIa; c_0 is the initial concentration of Ia; and t, the reaction time in min.

Comparison of k with k_{III} and k_{II} discloses that at pH 7·60 and above the 1-methyl derivative (Va) rearranges 1·3–4·6 times as fast as the 1-methoxy derivative (Ia), although the latter undergoes ring-opening 7–29 times more rapidly than the former. Since no appreciable effect of variation in size in the $N_{(1)}$-substituent was observed for both series, this marked difference in the rates of ring-opening could be attributed to an electronic factor encouraging attack by the hydroxide ion or by water at the 2-position and discouraging reversal of the first step, 1,9-disubstituted-adenine ⇄ intermediate. Thus, the speeding up of ring-opening in the 1-alkoxy series (I) could be ascribed purely to the electron-withdrawing properties of such alkoxyl groups. An analogous discussion may apply to the slowing down of the second step in the N-alkoxy series since this step appears to be initiated by an intramolecular nucleophilic attack by the N-substituted amidine group on the formamido carbonyl group.

Both in the 1-alkoxy and 1-alkyl series, the rate of ring-opening increases with increasing pH and tends toward a break above the pK_a of the base, increasing again



SCHEME 3*

* Compound number in parentheses represents a transient molecule which cannot be isolated.

at high pH's (Fig. 4). The study of ionic-strength effect on the rate (Table 5) suggests that in each of both series ring-opening proceeds through attack by hydroxide ion on the protonated base and hydroxide attack on the neutral species, (as postulated by Taylor and Loeffler[24] for the conversion of 1-substituted-7-methyladenines into the isomeric N^6-substituted derivatives), becomes dominant at high pH. The ratio of the rate constants for hydroxide attack on the protonated and neutral species of Va, 80:0·35, may be compared with that of 220:1·7 observed by Macon and Wolfenden[9] for the rearrangement of 1-methyladenosine at 25°. The much greater ratio, 2500:2, observed for Ia appears to reflect the marked effect of the electron-withdrawing 1-methoxyl group on the reactivity of the $C_{(2)}$ atom especially in the protonated species.

On the basis of the results and discussion described, it may be possible to formulate the most plausible mechanism for the Dimroth rearrangement of the 1-alkoxy (I) and 1-alkyl series (V) as shown in Scheme 3.

In conclusion, the present results confirm that 1-alkoxyl groups of 9-substituted adenines make the ring-opening extremely easy and retard the reclosure to the rearranged products (III), facilitating isolation of the intermediates (II). Although several methods for cleaving the adenine ring either in the pyrimidine or in the imidazole moiety have been known,[25-29] the reaction conditions employed seem to be more rigorous than those of our method. Thus, the intentional introduction of an alkoxyl group to the adenine ring at the 1-position[13,14] and successive induction of the ring-opening at neutral pH[3,4] may be useful as a new method for chemical modification of adenine derivatives and nucleic acids.

EXPERIMENTAL

All m.ps were obtained on a Yamato MP-1 capillary apparatus and are corrected. Paper chromatographies were developed as described previously.[13] UV spectra were measured on a Hitachi EPS-20 spectrophotometer. See also Ref 13 for details of instrumentation and measurement.

Materials

1-Ethoxy-9-methyladenine perchlorate (Ib·$HClO_4$). To a soln of the hydriodide[14,15] (3·21 g, 10 mmoles) of Ib in H_2O (160 ml) was added 15% NH_4ClO_4 aq (8 ml). The resulting ppts were filtered, washed with a little H_2O, and dried. The filtrate was concentrated *in vacuo* to *ca.* 30 ml and 15% NH_4ClO_4 aq (4 ml) added to give an additional amount (0·32 g) of Ib·$HClO_4$. Total yield, 2·78 g (95%). Recrystallization from 50% EtOH aq gave colourless prisms, m.p. 272–274° (dec): UV (Table 1): pK_a(Table 1). (Found: C, 32·74: H, 4·23: N, 23·73. $C_8H_{12}O_5N_5Cl$ requires: C, 32·72: H, 4·12: N, 23·85%).

1,9-Dimethyladenine perchlorate (Va · $HClO_4$). A mixture of 1-methyladenine[16,18] (224 mg, 1·5 mmoles), MeI (1·42 g, 10 mmoles), and $AcNMe_2$ (10 ml) was stirred at 60–65° for 11 hr. The ppts formed were filtered, washed with a little EtOH, and dried to give crude Va·HI (215 mg), m.p. 277–278° (dec.) The hydriodide was dissolved in H_2O (5 ml) and 15% NH_4ClO_4 aq (2 ml) added to yield Va·$HClO_4$ (119 mg). The filtrate, originating from the filtration of the crude hydriodide, was evaporated *in vacuo* and the resulting residue was treated with 15% NH_4ClO_4 aq in a similar manner to produce a second crop (29 mg). The crude perchlorate was recrystallized from H_2O (2 ml) as colourless pillars (105 mg, 27% based on the 1-methyladenine used), m.p. 300–301° (dec), shown to be homogeneous by means of paper electrophoresis using 0·013 M phosphate buffer (pH 7·2). Further recrystallization from H_2O gave an analytical sample, m.p. 303–304° (dec): UV (Table 1): pK_a (Table 1). (Found: C, 31·93: H, 3·79: N, 26·64. $C_7H_{10}O_4N_5Cl$ requires: C, 31·89: H, 3·82: N, 26·57%).

1-Ethyl-9-methyladenine perchlorate (Vb·$HClO_4$). A mixture of 9-methyladenine[13,16,30] (746 mg, 5 mmoles), EtI (3·90 g, 25 mmoles), and $AcNMe_2$ (10 ml) was stirred at 75–80° for 7 hr. The ppts that resulted were filtered, washed with a little EtOH, and recrystallized from H_2O to yield the *hydriodide* (980 mg, 64%) of Vb as colourless pillars. Recrystallization from H_2O provided an analytical sample, m.p. 291–292°

(dec). (Found: C, 31·68; H, 4·04; N, 22·94. $C_8H_{12}N_5I$ requires: C, 31·49; H, 3·97; N, 22·95%). Methylation of 1-ethyladenine* with MeI in a similar way also gave Vb·HI (66%).

To a warm soln of the hydriodide (1·89 g, 6·19 mmoles) in H_2O (20 ml) was added 15% NH_4ClO_4 aq (7·3 ml). The perchlorate produced in a yield of 97% was recrystallized from H_2O to give colourless plates, m.p. 309–310° (dec): UV (Table 1): pK_a (Table 1). (Found: C, 34·55; H, 4·45; N, 25·22. $C_8H_{12}O_4N_5Cl$ requires: C, 34·60; H, 4·36; N, 25·22%).

1-*Propyl-9-methyladenine perchlorate* (Vc·$HClO_4$). First, the *hydriodide* of Vc was prepared in 36% yield by heating a mixture of 9-methyladenine[13,16,30] and an excess of PrI in $AcNMe_2$ at 90–95° for 8 hr. An analytical sample was obtained by recrystallization from H_2O as colourless needles, m.p. 266–268° (dec). (Found: C, 34·01; H, 4·39; N, 22·08. $C_9H_{14}N_5I$ requires: C, 33·87; H, 4·42; N, 21·95%).

The *perchlorate* of Vc was obtained from the crude hydriodide in 59% yield (based on the 9-methyladenine used) by treating it as described above for the salt of Vb, analysis sample from H_2O as colourless plates, m.p. 282–283° (dec): UV (Table 1): pK_a (Table 1). (Found: C, 37·34; H, 4·71; N, 23·86. $C_9H_{14}O_4N_5Cl$ requires: C, 37·06; H, 4·84; N, 24·01%).

6-*Methylamino-9-methylpurine* (VIIa). A soln of Va·$HClO_4$ (791 mg, 3 mmoles) in H_2O (100 ml) was passed through a column of Amberlite IRA-402 (HCO_3^-) (10 ml) and the column eluted with H_2O. The eluate (400 ml) was concentrated to 30 ml, refluxed for 3 hr, and evaporated *in vacuo* to dryness, and the residue extracted with boiling benzene (20 ml). On cooling, the benzene soln separated almost colourless plates (267 mg, 54%), m.p. 184–186°. Recrystallization from benzene gave an analytical sample of VIIa, m.p. 185–186° (lit.[30] m.p. 190–191°); UV (Table 1); pK_a (Table 1). (Found: C, 51·55; H, 5·51; N, 42·62. $C_7H_9N_5$ requires: C, 51·52; H, 5·56; N, 42·92%).

6-*Ethylamino-9-methylpurine* (VIIb). A mixture of Vb·HI (610 mg, 2 mmoles) in 0·066 M phosphate buffer (pH 7·5) (70 ml) was refluxed for 2 hr. The soln was evaporated *in vacuo* to dryness, leaving a solid. The residue was well dried and extracted with four successive 50-ml portions of boiling benzene. Evaporation of the solvent from the extracts and recrystallization of the residue from benzene gave VIIb (179 mg, 51%) as colourless prisms, m.p. 156–157° (lit.[30] m.p. 157–158°): UV (Table 1): pK_a (Table 1). (Found: C, 54·21; H, 6·21; N, 39·76. $C_8H_{11}N_5$ requires: C, 54·22; H, 6·26; N, 39·52%).

6-*Propylamino-9-methylpurine* (VIIc). A soln of Vc·HI (638 mg, 2 mmoles) in 0·2 N NaOH aq (20 ml) was heated in a water bath kept at 95–100° for 10 min. The mixture was evaporated *in vacuo* to dryness and the crystalline residue extracted with two successive 100-ml portions of boiling ligroin (b.p. 80–110°). Evaporation of solvent from the combined extracts left a solid (358 mg, 94%), m.p. 128–130°, shown to be homogeneous on TLC. Recrystallization from ligroin yielded colourless plates of the same m.p. (lit.[30] m.p. 130–131°): UV (Table 1): pK_a (Table 1). (Found: C, 56·84; H, 6·84; N, 36·43. $C_9H_{13}N_5$ requires: C, 56·53; H, 6·85; N, 36·63%).

Kinetic studies

Buffer solns used for reactions of Ia, b, c, IIa, b, c, and Va, b, c were 0·1 M KH_2PO_4—Na_2HPO_4 (pH 6·18, 7·60, 7·98 at 40°), 0·08 M sodium diethylbarbiturate—HCl (pH 8·08 at 40°), 0·2 M Na_2CO_3—$NaHCO_3$ (pH 9·44 at 40°), 0·18 M Na_2CO_3—$NaHCO_3$ (pH 10·49 at 40°), and 0·1 M Na_2HPO_4—Na_3PO_4 (pH 10·99, 11·36, 11·72 at 40°), each of which contained a sufficient amount of KCl to make the ionic strength 0·50.

The substrates were separately dissolved in the buffer solns at 5×10^{-4} M concentration. Approximately 3-ml aliquots of these solns were sealed in small ampoules and placed in a thermoregulated constant temperature bath (accurate to ± 0·05°). At intervals the ampoules were removed, cooled, and broken and the contents diluted with 0·06 M citrate buffer (pH 5·80 at 20°, adjusted to ionic strength 0·50 with KCl) by a factor of 10. The optical densities of the resulting solns at appropriate analytical wavelengths were determined with a Hitachi EPU-2A spectrophotometer at 20°. Concentrations of the components were calculated in the usual manner[20] by utilizing the molecular extinction coefficients obtained on solns of analytical samples in mixed solvents prepared by diluting the requisite buffers by a factor of 10 with the above-mentioned citrate buffer. Runs at different ionic strength or at different buffer concentration were worked up similarly.

The pH determinations were carried out before and after the reactions with a Hitachi-Horiba F-5 pH meter and showed that change in pH was negligibly small.

Except for the slow reaction of Va at pH 6·18, all the reactions were followed for at least two half-times with at least five measurements and good first-order kinetics obtained in all cases.

* This compound, m.p. 252–254° (dec) (lit.[31] m.p. 265–266°), has been prepared by us from adenosine according to a procedure patterned after that[18] of Jones and Robins.

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