Tautomerism of Purines. 2. Amino–Imino Tautomerism in 1-Alkyladenines[1]

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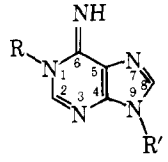
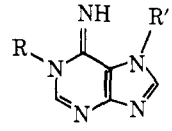
Abstract: The tautomeric states of several 1-alkyladenines (Ia ⇌ IIa ⇌ III, R = Me to *n*-heptyl) and of a 1,N^6-dialkyladenine (IVa) have been investigated. It is shown from UV and IR spectroscopy that, in all cases, the imino forms predominate in a nonpolar environment. However, the content of the amino form (III) increases with the medium dielectric constant, this being interpreted in terms of its much higher dipole moment. In aqueous solution, the amino form predominates (K = [Ia]/[III] ≃ 10^{-2} at 12 °C and $\Delta H_{Ia \rightarrow III}$ = 3.1 ± 0.1 kcal M^{-1}), and is shown by ^{13}C NMR spectroscopy to protonate mainly at the N_9 position. The mechanism of the Ia ⇌ III interconversion has been studied by temp-jump relaxation in D_2O solution. The interconversion is acid and base catalyzed, no kinetic contribution from a direct proton transfer mechanism not involving dissociation to ions being detected. Finally, the kinetics of the amino–imino tautomerism of 9-substituted adenines, together with some of their possible biological implications, are tentatively discussed in light of this work.

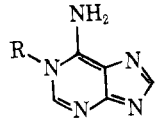
When 1-methyladenine (Ia ⇌ IIa ⇌ III, R = CH_3) was first prepared[2] in 1960, it was described as existing in aqueous solution under an *imino* (Ia or IIa, R = CH_3) rather than an *amino* tautomeric form (III):



Ia, R = alkyl; R′ = H
Ib, R = R′ = alkyl



IIa, R = alkyl; R′ = H
IIb, R = R′ = alkyl



III, R = alkyl

Although this assignment was based on somewhat fragmentary evidence (cf. the discussion below), few new sound data have appeared on this point despite current efforts. Thus, a UV and dipole moment study of tautomerism of ring *N*-alkyladenines was inconclusive in the case of the 1-alkyl compounds because of their very low solubility in suitable solvents,[3] whereas the IR spectrum in the solid state has been considered as consistent with an imino structure.[4] A theoretical investigation also suggests that the imino structures are intrinsically more stable than the amino one in this system.[5] However, in a recent monograph on the NMR of purine compounds, Townsend[6] describes evidence for a predominant amino structure in Me_2SO solution. Nevertheless, a full report of these experiments has not yet appeared in the literature; thus, the most recent reviews[7] on this field still describe 1-methyladenine as being predominantly an imino base, a rather rare situation in compounds capable of tautomerizing to an amino form, and a probably unique one in aminopurine chemistry.

This somewhat confusing situation, together with the recently increasing interest in the hormonal role of these substances[8] (which should ultimately depend upon their tautomeric state in solution), has encouraged us to reinvestigate spectroscopically their tautomeric structure, both in neutral and cationic form, and the results of this study are reported herein. Moreover, quantitative information about the kinetics and thermodynamics of the amino–imino tautomerism in water has been obtained from temp-jump relaxation, a technique well adapted to the study of tautomerism in aqueous solution.[9-11] In addition to their inherent interest, the kinetic results provide some insight on the dynamics of formation and decay of the rare imino form of the 9-substituted adenines from the more common amino form, a piece of information which is likely to be relevant to the theory of spontaneous mutations.[12]

Experimental Section

All compounds used in this work were tested for purity using analytical TLC on silica gel and cellulose. Samples for TLC on silica gel were prepared by dissolving the substance in a suitable buffer in which it existed under neutral form; elution was then carried out either with methanolic ammonia, ethanolic ammonia, or 1:1 mixtures of ethanolic ammonia and ethyl acetate, depending on the polarity of the substance. Analytical TLC on cellulose plates (Merck) was performed in solvents A (1-butanol–acetic acid–water, 4:1:5), B (2-propanol–aqueous ammonia–water, 7:1:2), and C (water-saturated 1-butanol). All compounds were homogeneous in these analytical experiments.

Synthesis of 1-Alkyladenines. The various 1-alkyladenines (Ia ⇌ II ⇌ III, with R = Me, *n*-propyl, *n*-butyl, *n*-pentyl, and *n*-heptyl) were prepared according to the two-step procedure first described by Jones and Robins[13] for the preparation of 1-methyladenine, and subsequently used for obtaining various other 1-alkyladenines.[8,14] In the original procedure, adenosine is alkylated with an alkyl iodide in dimethylacetamide (DMA) to yield the corresponding 1-alkyladenosine, which is further converted to the desired aglycon by acid hydrolysis of the glycosidic linkage. This second step should be performed under carefully controlled conditions, since the cleavage of the glycosidic bond is accompanied by much slower acid-catalyzed ring opening of the resultant 1-alkyladenine.[15] The course of both the alkylation and the hydrolysis step could be followed conveniently either by TLC, or by injecting a small amount of the reaction mixture into a UV cell containing pH 10 buffer and recording the spectrum. In all cases, the desired products were readily identified because of the similarity of their pK and UV spectra with those of the parent compound 1-methyladenine.

The procedure used is described in detail only for 1-propyl- and 1-heptyladenine, which have been of particular interest in this work.

1-Propyladenine. A mixture of 3.5 g of adenosine and 8 cm^3 of propyl iodide in 45 cm^3 of DMA was heated with stirring at 70 °C for 48 h in a water bath and then evaporated to dryness under vacuum. HCl (40 cm^3, 1 N) was added to the brown paste thus obtained, and the mixture was stirred in a steam bath for 30 min, after which complete hydrolysis of the glycosidic linkage occurred. Ammonium perchlorate (3.8 g) was then added to the filtered hot solution (charcoal); the mixture was neutralized to pH 8 and chilled for 2 days, during which crude 1-propyladenine perchlorate had precipitated. The product was twice crystallized from ethanol (charcoal), affording 700 mg of analytically pure 1-propyladenine perchlorate, mp 269 °C dec. Concentration of the mother liquors afforded an additional 250-mg crop. The fairly water-soluble free base was obtained by pouring a concentrated solution of the perchlorate in warm water on a column of a weakly basic anion exchanger (OH^- form) and then evaporating the effluent solution to dryness. The analytically pure base could be

crystallized from water in relatively low yield (mp 229 °C; lit.[16] 232–233 °C). Anal. Calcd for the perchlorate: C, 34.59; H, 4.32; N, 25.23; Cl, 12.79. Found: C, 34.42; H, 4.26; N, 25.34; Cl, 12.60.

An additional check[17] for the structure of 1-alkyladenine is provided by their rearrangement to 6-alkylaminopurines (Dimroth rearrangement) which can be synthesized by an unambiguous route. Thus, a sample of 1-propyladenine in 0.1 M sodium carbonate solution was heated for 5 h in a steam bath. After this time, the wavelength of maximum absorption recorded in 0.1 N HCl had been shifted from 259 to 270 nm, and was identical with that of 6-propylaminopurine, obtained[18] independently through the reaction of *n*-propylamine with 6-methylthiopurine.[19] TLC on the reaction mixture revealed two products, with no trace of 1-propyladenine. The major product was 6-propylaminopurine, since its chromatographic behavior in a variety of solvent systems was identical with that of the authentic material. The structure of the minor product, which has a low R_f in solvents A, B, and C, was not further investigated.

1-Heptyladenine. Adenosine (4.9 g) and heptyl iodide (16 cm^3) in 60 cm^3 of DMA were stirred at 75 °C for 48 h. The mixture was evaporated to dryness and then hydrolyzed with 50 cm^3 of 1 N HCl at 100 °C (30 min). The hot solution was then filtered (charcoal) and crude 1-heptyladenine chloride, which could be recrystallized from ethanol, deposited on cooling (yield 1.5 g after one crystallization). The analytically pure hydrochloride melted at 262 °C dec. Anal. Calcd: C, 53.43; H, 7.42; N, 25.97; Cl, 13.17. Found: C, 53.56; H, 7.50; N, 26.13; Cl, 13.04.

When a solution of the pure hydrochloride in warm water was neutralized with concentrated ammonia, quantitative precipitation of the analytically pure free base occurred (mp 223–224 °C).

Synthesis of Reference Compounds. The compound 9-methyl-7,8-dihydro-9*H*-imidazo[2,1-*i*]purine[20] (IVb) was selected as a nontautomeric model for amino structure III. It was prepared, along with the corresponding unsubstituted derivative IVa, according to published procedures,[21] except that we used 6-methylthiopurine[19] as a starting material rather than 6-chloropurine. The substitution of the methylthio group by aminoethanol (for IVa) or methylaminoethanol (for IVb) was carried out in refluxing 1-butanol and required a somewhat longer reaction time than reported for the similar reaction with 6-chloropurine.[21] IVa was purified as the free base according to ref 21a. Free base IVb was extracted from the obtained hydrochloride and recrystallized according to the procedure described for the 9-ethyl analogue.[21b] The analytically pure product, which darkens above 270 °C but does not melt below 300 °C, was sublimed with difficulty and characterized by its mass spectrum (18 eV). Principal peaks (the numbers in parentheses are the relative intensity) are: 175 (1000) molecular peak; 174 (306) $M^+ - H$; 160 (64) $M^+ - CH_3$; 148 (26) $M^+ - HCN$; 120 (272) purine molecular ion.

1,9-Dimethyladenine Perchlorate. This was prepared according to published procedures,[22] except that we used 9-methyladenine[23] as a starting material. The very water-soluble hydrochloride, used in ^{13}C NMR measurements, was obtained by stirring a suspension of the required amount of perchlorate in water with a large excess of a strongly basic anion exchange resin in the Cl^- form (Merck Lewatit MP 7080). The free base was obtained from the aqueous hydrochloride by neutralization followed by chloroform extraction. The chloroform extract was evaporated to dryness; the resulting solid was vacuum dried over phosphorus pentoxide without heating, and was used as such.

1,7-Dibenzyladenine. A few optical density (OD) units of this product in the form of a concentrated methanolic solution, prepared by N. J. Leonard,[24] were a gift from Dr. M. Dorée (cf. ref 8). This analytically pure solution was used as such for spectroscopic measurements.

3-Methyladenine,[25] 3-benzyladenine,[26] and 7-methyladenine[26] were prepared according to classical routes. **9-Butyladenine** was obtained through sublimation of an equimolar mixture of adenine and tetrabutylammonium hydroxide.[23]

For some compounds, replacement of the labile hydrogen atoms by deuterium was needed to help infrared band assignment. This was achieved by dissolving the sample in a large excess of methanol-d_1 (CH_3OD), allowing it to stand for 24 h, and evaporating the solvent under vacuum.

Solvents. All solvents used in this work were spectrograde and were used without special purification. However, spectrograde chloroform and methylene chloride were soaked for 48 h in the dark with concentrated aqueous $NaHCO_3$ to remove any acidic impurities, then dried over calcium sulfate, refluxed over phosphorus pentoxide, and finally distilled from phosphorus pentoxide; these solvents were used as soon as possible after preparation.

Table I. Thermodynamic Data[a] Used in This Work

Compound	pK_1 (proton gain)	pK_2 (proton loss)
1-Methyladenine	7.11 ± 0.05	11.0[b] (20 °C)
	7.35 ± 0.03 (12 °C ± 0.2)	
1-Heptyladenine	7.00 ± 0.05	
Ib	9.03 ± 0.05	
IIb	6.50 ± 0.10	
IVa	7.0[c]	11.5 ± 0.2
IVb	6.83 ± 0.04	
1-Methyladenosine[d] (Ib, R = CH_3; R′ = ribose)	8.55 ± 0.03	

[a] Measured spectrophotometrically at 25 ± 0.1 °C unless otherwise specified and corrected to zero ionic strength. [b] Reference 2, ionic strength 0.05. [c] Reference 21a, temperature and ionic strength not specified. [d] Measured potentiometrically at 25 ± 0.1 °C and corrected to zero ionic strength.

UV Spectroscopy. All UV spectra were recorded using a Cary 118 UV spectrophotometer. The spectra of substituted adenines in various solvents were obtained by diluting known aliquots of a concentrated ethanolic or methanolic solution of the compound in a UV cell containing a known volume of solvent. The content of ethanol or methanol in the final solution never exceeded 1%, except for 1,7-dibenzyladenine, for which it was 5%. Conventional 1-cm quartz cells were used whenever possible; however, shorter optical paths down to 1 mm were used in the case of UV absorbing solvents (chloroform, methylene chloride, dioxane) in order to record the spectrum down to the shortest possible wavelength.

pK values recorded in Table I were measured spectroscopically[10] using conventional 1-cm thermostated cells. The temperature was measured in the sample cell and was 25 ± 0.2 °C, unless otherwise specified. The pK values were extrapolated to zero ionic strength using the Debye–Hückel formula. Approximate pK values in deuterium oxide were obtained by adding 0.5 pK unit to the corresponding pK measured at the same temperature in ordinary water.[10]

IR Spectroscopy. The IR spectra were recorded on a Perkin-Elmer 225 spectrophotometer fitted with 2-cm "infrasil" cells (Hellma). Spectra of the 1-alkyladenine were usually taken in saturated solution at 40 °C, the exact concentration being deduced from the UV spectra of the solutions. Spectra of samples in which the exchangeable hydrogen atoms had been replaced by deuterium were run in D_2O-saturated chloroform to prevent dedeuteration of the very dilute solute by traces of water present in solution. The solubility of water in chloroform (0.07% by weight at 23 °C) is considered low enough so as not to significantly affect the solvent properties.

^{13}C NMR Spectra. The ^{13}C NMR spectra were recorded using a Jeol PFT 100 Fourier transform NMR spectrometer. The chemical shifts were measured for aqueous solutions of the compounds (0.5 to 1 M) contained in 10-mm o.d. tubes, with respect to external Me_2SO-Me_2SO-d_6 (for lock) contained in a coaxial cylindrical cell (4 mm o.d.). The bulk susceptibility correction was assumed to be constant from one sample to another, and was therefore not made. Peak attribution was helped by off-resonance measurements which allowed an easy identification of the quaternary carbon atom resonances.

Temp-Jump Relaxation Measurements. Temp-jump experiments were performed as in our previous works,[9-11] using a commercial Joule-heated double beam temp-jump spectrophotometer (Messanlagen Studiengesellschaft) interfaced to a PDP-11 computer (Digital Equipment Corp.), which allowed an improvement of the signal-to-noise ratio by summing three to five relaxation curves at a time. The detailed procedure used to compute the relaxation times (τ) and amplitude (A) from the observed relaxation curves has been described elsewhere.[9,10] The pH is monitored using a G 202 C Radiometer glass electrode, fitted to a Radiometer PHM 64 research pH meter. The pD in deuterium oxide was evaluated by adding 0.4 unit to the reading of the pH meter standardized in ordinary water.[27]

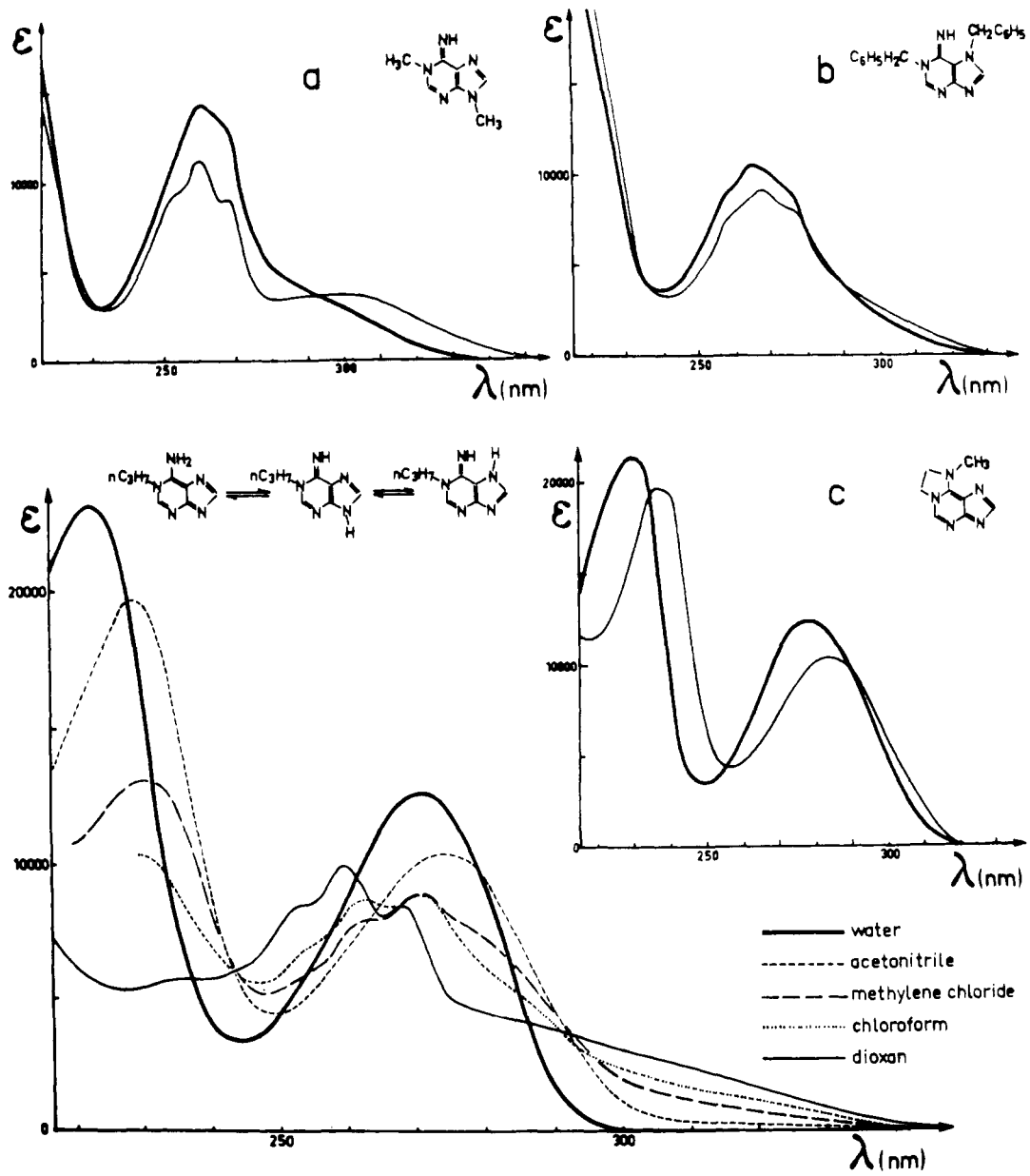
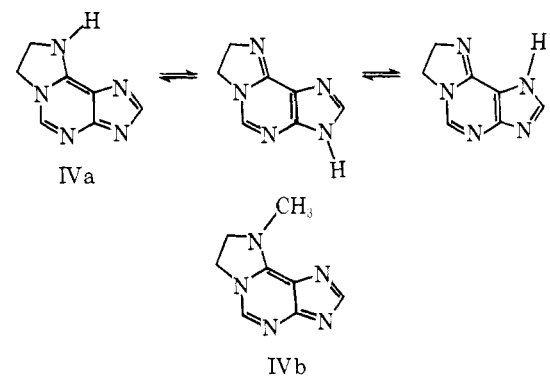


Figure 1. UV spectra of 1-propyladenine in different solvents compared with the spectra of nontautomerizable model compounds: (a) 1,9-dimethyladenine; (b) 1,7-dibenzyladenine; (c) compound IVb.

The temp-jump magnitude was measured by recording the relaxation amplitude observed with a suitable calibration solution for various values of the high voltage applied to the heating capacitor. These amplitude values were then converted to optical density changes, and compared with spectral changes produced by static modification of the temperature of the same solution contained in the thermostated cell of a UV spectrophotometer. An aqueous solution of cresol red ($c = 3 \times 10^{-5}$ M) and Tris buffer ($c = 0.2$ M, pH 8.3), which gives rise to a very large relaxation signal, was found to be suitable for these calibration experiments. The heating time (τ_h) was computed from the measured resistance of the sample cell (R) and from the value of the discharging capacitor (C) according to the expression $\tau_h = RC/2$. Standard experimental conditions used in this work were: initial temperature (t_i) = 6 ± 0.2 °C; final temperature (t_f) = 12 ± 0.5 °C; heating time (τ_h) = 2.7 μs; spectral bandwidth of the optical detection = 5 nm.

Results

UV Spectroscopy. Irrespective of the solvent used, the UV spectra of the model *imino* compounds, 1,9-dimethyladenine (Ib, R = R′ = CH_3) and 1,7-dibenzyladenine (IIb, R = R′ = $CH_2C_6H_5$), are found to be rather similar to each other, but



IVa

IVb

to differ drastically from the spectrum of IVb, which has a fixed *amino* structure (Figure 1, a–c).

Thus, only one well-defined band, with considerable fine structure, is observed in the spectra of Ib and IIb in the 215–350-nm wavelength range (Figure 1, a and b). Moreover, an important tail is found at the low-energy end of both spectra (A-type spectrum). By contrast, the spectrum of amino com-

pound IVb shows two well-defined bands in this spectral region (Figure 1c). The intensity of the short-wavelength band is approximately twice that of the long-wavelength one, and no fine structure is observed in either band (B-type spectrum). It is apparent in Figure 1 that 1-propyladenine shows an A-type spectrum in dioxane, indicating an imino structure in that solvent, and a B-type spectrum in water, thereby strongly supporting a predominant amino form in aqueous solution. In going from dioxane (ϵ 2.2) to chloroform (ϵ 4.8), methylene chloride (ϵ 8.9), acetonitrile (ϵ 37.5), and water (ϵ 78), a smooth transition from an A-type to a B-type spectrum is observed, suggesting that the position of the amino–imino tautomeric equilibrium is chiefly governed by the dielectric constant of the medium. No clear-cut isosbestic points are observed, presumably because three species (Ia $\rightleftharpoons$ IIa $\rightleftharpoons$ III) are present at equilibrium. Moreover, the exact position and intensity of the bands arising from each tautomer are likely to vary slightly when changing solvent (Figure 1 a–c).

All the 1-alkyladenines we investigated (Ia $\rightleftharpoons$ IIa $\rightleftharpoons$ III, R = methyl, *n*-propyl, *n*-butyl, *n*-pentyl, and *n*-heptyl), as well as compound IVa which can similarly tautomerize either to amino or to imino forms, showed spectral features very similar to 1-propyladenine; this indicates that the balance of the tautomeric equilibrium is rather insensitive to the length of the chain branched at N_1 and even to a further substitution of the exocyclic nitrogen atom.

It is clear from these observations that an unambiguous attribution of the correct tautomeric structure of 1-alkyladenines can be made on the basis of UV spectra alone. However, because of the conflicting reports on this point in literature,[2-7] we searched for some independent check for our conclusions. IR spectroscopy was found to provide strong evidence for a predominant *imino* form in chloroform solutions, whereas p*K* comparisons were used to show that the *amino* form is the most abundant one in water.

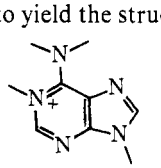
IR Evidence for a Predominant Imino Form in Chloroform. As previously stated,[3] the solubility of 1-alkyladenine in nonpolar solvents is very low, thereby making useful structural studies difficult. Nevertheless, the solubility increases slightly with the side-chain length, and we found that 1-heptyladenine was soluble enough in chloroform (ca. 5×10^{-4} M at 40 °C) for useful IR spectra to be recorded in the NH stretch region (3570–3250 cm^{-1}) where the high transparency of the solvent allowed a rather long optical path to be used (2 cm). The spectrum was also run in the more polar solvent methylene chloride, in which 1-heptyladenine showed similar solubility. However, in that case, the solvent cutoff does not allow spectral recording below 3350 cm^{-1} with a 2-cm optical path, thereby making the observation of the entire NH stretching range impossible. Nevertheless, we found it useful to present this truncated spectrum for the purpose of comparison.

The spectrum of 1-heptyladenine in the NH stretch region recorded in chloroform shows four identifiable bands (Figure 2a). All these bands disappear upon deuteration, and thus may probably all be attributed to NH stretching modes. The rather intense band (ϵ 125 ± 8 M^{-1} cm^{-1}) observed at 3446 cm^{-1} is readily attributed to the H atom fixed on the imidazole ring of imino tautomer Ia or IIa; thus, frequencies ranging from 3430 to 3450 cm^{-1} have been reported for the imidazole NH group in many purines in chloroform solution,[28] and, more specifically, this band occurs at 3440 cm^{-1} in 1-methylhypoxanthine, a compound closely related to tautomeric structures Ia and IIa through the replacement of the exocyclic =NH group by a carbonyl group (Figure 2b). Next, the weak band ($\epsilon \sim 16 \pm 3$ M^{-1} cm^{-1}) observed at 3308 cm^{-1} is readily attributed to the =NH vibrator in imino forms Ia or IIa. Imino compounds of this type are known to show a weak NH stretching band in this frequency range;[29] moreover, in the spectrum of model compound Ib only one band is observed in the NH stretching region, its frequency (3310 cm^{-1}) and intensity (ϵ 23 M^{-1} cm^{-1}) being quite close to that of the band under study (Figure 2c). The two remaining bands at 3508 and 3399 cm^{-1} occur in the frequency range usually observed for the antisymmetric and symmetric stretching modes, respectively, of the NH_2 vibrator in heterocyclic amines,[30] and are therefore attributed to amino tautomer III. This assignment is supported by the following arguments. (a) The lower frequency component is the most intense, as almost invariably observed for the antisymmetric and symmetric stretching modes.[30,31] (b) The spacing between the two bands (109 cm^{-1}) compares well with that observed with adenines substituted at N_3, N_7, or N_9, all of which show only two bands in the NH stretch region as expected from their known amino structure;[3,7] thus, we found that this spacing is 111 cm^{-1} (3525 and 3414 cm^{-1}) in chloroform solutions of 9-butyladenine, 114 cm^{-1} (3524 and 3410 cm^{-1}) for 3-benzyladenine (Figure 2d), and 110 cm^{-1} (3520 and 3410 cm^{-1}) for 7-methyladenine.

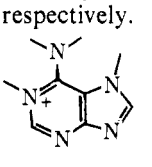
On going from chloroform to the more polar solvent methylene chloride, an 8–10-cm^{-1} shift to lower frequencies is observed for all bands (Figure 2a′), as expected from the higher dielectric constant of the medium.[32] Moreover, the weak band from the imino =NH stretch is now unobservable because of the solvent cutoff. However, the most impressive modification of the spectrum is the considerable increase of the intensity of the two bands attributed to amino form III with respect to the imidazole NH stretch from imino forms Ia or IIa (Figure 2a′), this being immediately interpreted in terms of an increased proportion of amino tautomer III. Assuming the extinction coefficient of the NH_2 symmetric and antisymmetric stretch to be nearly equal in structure III and in 9-butyladenine, it is possible to estimate roughly the proportion of the amino form in both solvents. It is thus found that III amounts to 20–25% of the total 1-heptyladenine in chloroform, and to ca. 50% in methylene chloride. It is clear from Figure 1 that these estimates agree qualitatively with those based on the intensity of the 225–230-nm band in the UV spectrum, which has been shown to be characteristic of the *amino* form. This consistency further supports the correctness of our IR assignments.

Structure of Cationic 1-Alkyladenines and p*K* Evidence for a Predominant Amino Form in Water. It is well established[33] that valuable semiquantitative information about tautomeric equilibria in water can be gained from a comparison of the dissociation constant (corresponding to a proton gain) of the tautomeric compound with those of the two related "tautomers" in which the labile hydrogen atom has been replaced by an alkyl group, provided these three compounds yield cations of similar structure upon protonation. It is thus evident that information about the tautomerism of 1-alkyladenines in water can be gained from the data in Table I, once the structure of the various corresponding cations has been determined.

This structure is unambiguous for the model imino compounds, 1,9-dimethyladenine (Ib, R = R′ = CH_3) and 1,7-dibenzyladenine (IIb, R = R′ = $CH_2C_6H_5$), since in this case protonation will certainly take place on the very basic imino group to yield the structures V and VI, respectively.



V



VI

However, the protonation site is less evident for the third model compound, IVb, and for the 1-alkyladenines themselves. In this case, the cation could conceivably assume either structure V, VI, or even VII, although this last structure is unlikely in view of the accumulated evidence.[34] It is clear from the UV data in Table II that the spectra of the cations derived from 1-alkyladenines or from IVb are very similar to the

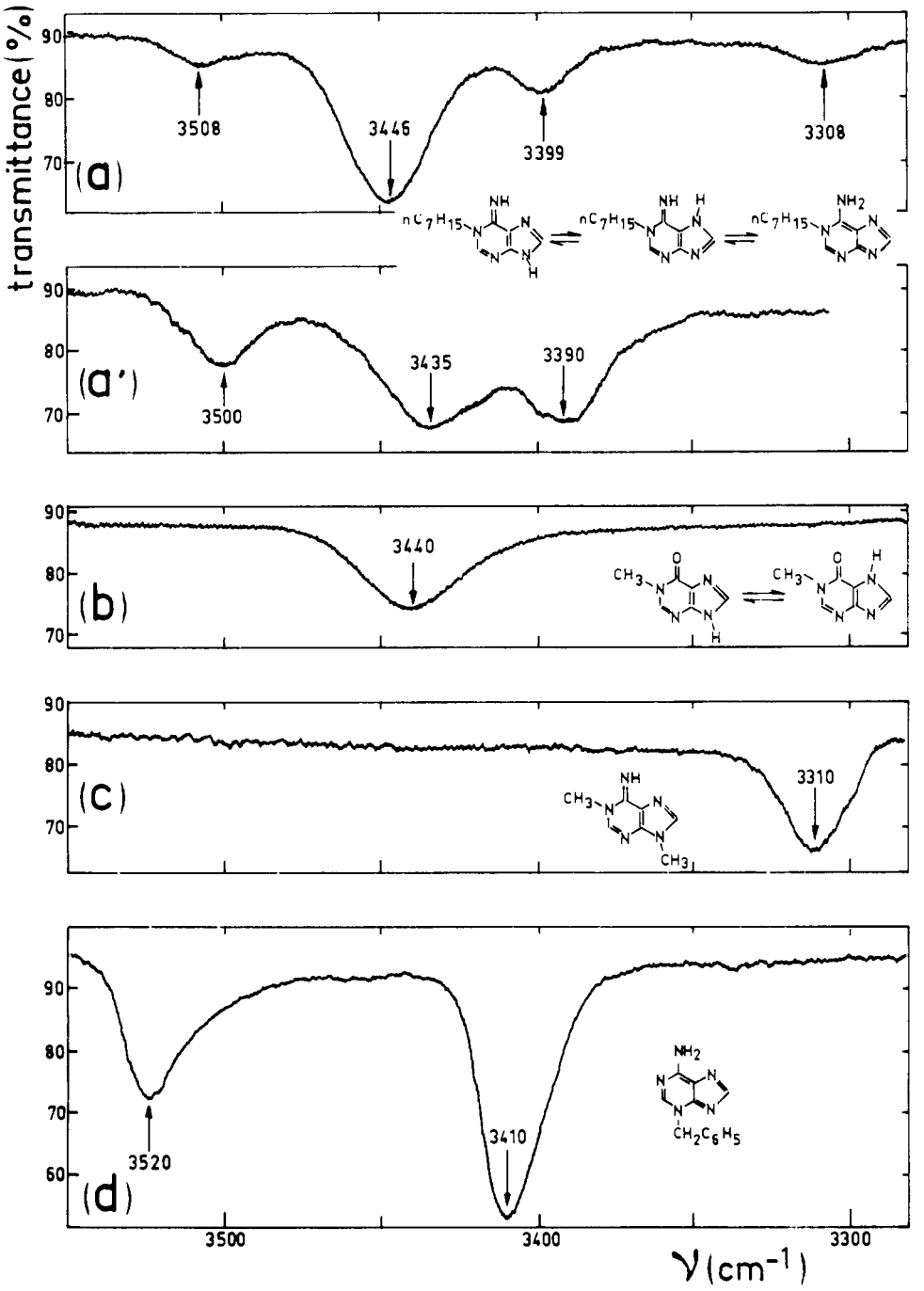
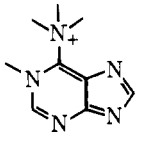


Figure 2. IR spectra in the NH stretch region of the following compounds (in chloroform solution unless otherwise stated): (a) 1-heptyladenine, 5×10^{-4} M; (a') 1-heptyladenine, in methylene chloride solution; (b) 1-methylhypoxanthine, 2×10^{-4} M; (c) 1,9-dimethyladenine, 2×10^{-3} M; (d) 3-benzyladenine, 5×10^{-4} M. All spectra were taken at 40 °C and using 2-cm optical length.



VII

spectrum of cationic Ib, as concerns both the maximum and the minimum of the extinction coefficient and the corresponding wavelengths. By contrast these spectra are very different from that of cationic IIb,[36] or from the spectrum of neutral 1-methylpurine, which should be a good spectral model for structure VII.[34] Therefore, these data clearly suggest that the predominant tautomeric forms of cationic IVb and cationic 1-alkyladenines have the structure V rather than VI or VII.

Table II. Comparison of the UV Spectra of Various Cations (in Water)

Compound	λ_{max} (ϵ_{max}), nm	λ_{min} (ϵ_{min}), nm
1-Alkyladenines, H^+	258–259 (~11 900)	288–229 (~2500)
Ib, H^+ (R = R' = CH_3)	259 (13 000)	232 (2900)
IIb, H^+ (R = R' = $CH_2C_6H_5$)	272 (8800)	244 (6000)
IIb, H^+ (R = R' = CH_3)	270[a] (7200)	
IVb, H^+	267 (14 500)	234 (2100)
1-Methylpurine[b]	275 (6100)	

[a] Reference 35. [b] Neutral molecule (ref 7b).

However unequivocal information is difficult to gain from the UV spectra alone because of band overlapping.

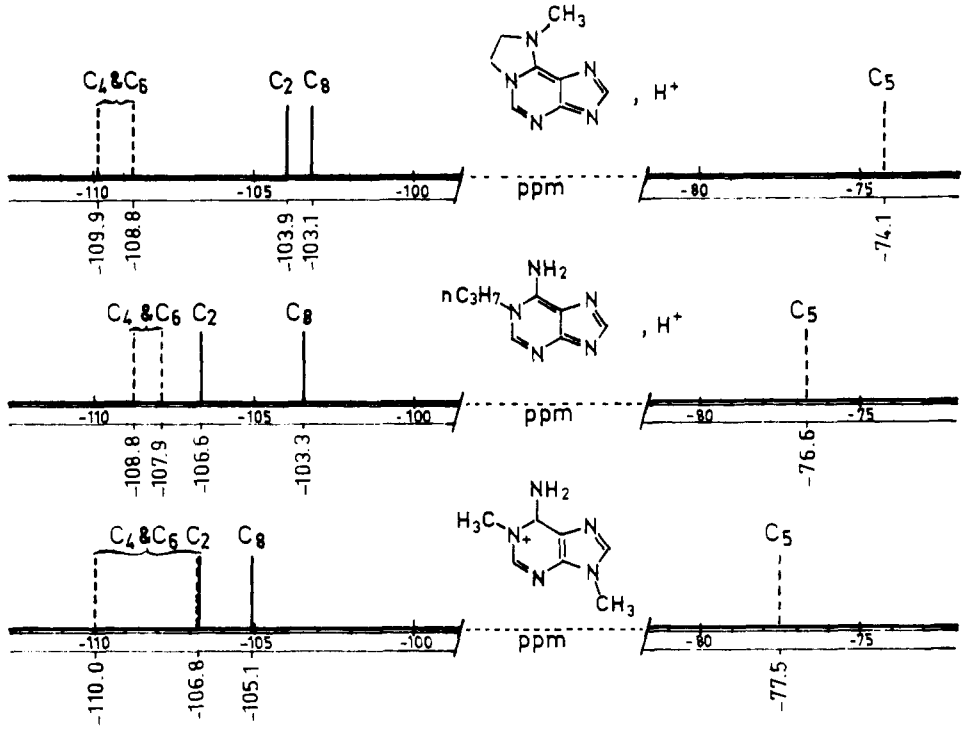


Figure 3. ^{13}C NMR spectra recorded in water for (from top to bottom) cationic IVb, cationic 1-propyladenine, and cationic 1,9-dimethyladenine (the aliphatic resonances are not shown). Chemical shifts are indicated in parts per million upfield from Me_2SO as an external reference. Dashed lines represent quaternary carbon resonances. The respective order of the C_2 and C_8 lines is in all cases inferred from that reported for the protonated adenine nucleotides.[38]

This prompted us to check our structural results by ^{13}C NMR spectroscopy, since it has been observed[37] that protonation (or alkylation) on the imidazole ring of purines invariably causes a 7–12-ppm upfield shift of the resonance line of the bridgehead carbon atom adjacent to the protonation site with respect to its position in the unsubstituted molecule, whereas the other bridgehead carbon atom is much less affected. This generalization has proved useful for the investigation of $N_7H \rightleftharpoons N_9H$ tautomerism in purines,[37c] and also provides a straightforward way of discriminating between structures V and VI in our systems.

The ^{13}C NMR spectra of cationic 1,9-dimethyladenine and IVb and that of cationic 1-propyladenine (1-PrA) are shown schematically in Figure 3 (all chemical shifts are given relative to dimethyl sulfoxide as an external reference, positive shifts being upfield from the reference). Although we made no effort to fully assign the spectra, the two peaks arising from C_2 and C_8 were, in all cases, readily distinguished from others, since they split into doublets under off-resonance measurements. Moreover, in the three spectra, a resonance line was found at a much higher field than the other lines and was attributed to the bridgehead carbon, C_5, by analogy with other purine systems.[37,38] It is then clear from Figure 3 that the spectra of cationic 1-PrA and IVb bear striking similarities to that of 1,9-dimethyladenine, indicating that these two molecules must protonate on N_9. Indeed, if protonation occurred mainly on N_7, and C_5 resonance line would move upfield by ca. 10 ppm with respect to its position in 1,9-dimethyladenine cation, whereas C_4 would similarly shift downfield. Although upfield shifts of the C_5 resonance are actually observed, by ca. 1 and 3.5 ppm in cationic 1-PrA and IVb, respectively, we feel that their magnitudes are too small to indicate extensive protonation on N_7. Furthermore, no large downfield shift is observed in any of the two quaternary resonances which can be attributed to C_4.

We consider that the concordant indications from UV and NMR spectroscopy demonstrate rather convincingly that 1-alkyladenines, as well as model compound IVb, protonate mainly at N_9, thereby leading to cationic structures similar to V.[39] It is then seen from Table I that the pK values of 1-alkyladenines and IVb are rather similar, and are both considerably smaller than that of 1,9-dimethyladenine, thereby indicating that amino form III largely predominates over Ia in aqueous solution. More quantitatively, the ratio [Ia]/[III] must lie around 10^{-2} at 25 °C. Of course, these pK comparisons tell us nothing about the abundance of the third tautomer, IIa, which, upon protonation, must lead to structure VI. However, in a variety of purine systems, in which the imidazole ring bears a hydrogen atom, it has been shown that the N_7H and N_9H tautomers exist in more or less comparable proportion.[9,37c] Therefore, the large predominance of III over Ia also implies that III is the predominant form in water, a conclusion already reached from UV comparisons.

Relaxation Spectrum of Aqueous 1-Methyladenine. When an aqueous solution of neutral 1-methyladenine (concentration range 10^{-3}–10^{-2} M) is submitted to a fast temperature jump and the subsequent optical density changes are recorded as a function of time, in the 290–315-nm wavelength region, two successive phenomena are observed: (a) an "immeasurably fast" rise in optical density with a time constant shorter than that of the heating process; this relaxation phenomenon, the amplitude of which vanishes for $\lambda > 305$ nm, presumably arises from instant variation of the extinction coefficient with temperature, since a similar effect is observed with most of the other purines and pyrimidines (whether tautomerizable or not) we have studied so far; (b) a slower exponential optical density rise, which must originate from a chemical modification in the 1-methyladenine solution. A similar relaxation spectrum was also observed in the same wavelength and concentration range, with the cyclic $1,N^6$-disubstituted compound IVa which can similarly tautomerize to imino forms. By contrast, nontautomeric compound IVb showed no relaxation spectrum other than the "fast" relaxation caused by temperature variation of the extinction coefficient. In the Discussion we shall limit

outselves to the description and analysis of the "slow" relaxation effect.

Despite it being distinctly slower than the heating time, this "slow" process was still too fast ($\tau \simeq 10^{-5}$ s for 1-methyladenine solutions) to be measured very accurately in water. In deuterium oxide, however, the relaxation time was slower by a factor of ca. 3, thereby allowing a more suitable study to be made; thus this solvent was chosen for further studies. Furthermore, we observed a definite drift in the measured relaxation time at constant pH and temperature on standing for long periods; thus, after a few hours in weakly basic medium (pD $\simeq$10) at 6 °C, the relaxation times were shorter by ca. 20–30% with respect to their initial values, at least for the most concentrated solutions we used. This drift probably arises from the slow transformation of some 1-methyladenine to 6-methylaminopurine (Dimroth rearrangement[40]), which has a pK of 10,[7b] and is therefore likely to be an effective catalyst in the reaction under study, as will become apparent in the Discussion. In support of this view, we observed no drift in the relaxation times from the concentrated solutions of cyclic compound IVa which cannot undergo the Dimroth rearrangement, even when standing for 48 h at room temperature at pD $\simeq$10. The kinetic measurements on 1-methyladenine solutions were therefore made as soon as possible after the dissolution of the compound in order to minimize the systematic errors thus introduced.

The relaxation amplitudes observed with 1-methyladenine solution at a given wavelength were found to be independent of pD provided most of the substrate exists under neutral form (pD >9) and to be proportional to the concentration of 1-methyladenine. The wavelength dependence of the amplitude was studied in the 290–315-nm spectral range, and shows a smooth increase of the amplitude when decreasing the wavelength. No useful observations could be made below 290 nm because of the large absorbances of the solutions used.

For a given 1-methyladenine concentration, the relaxation time was found to be rather sensitive to the pD value, a maximum value being reached around pD 10 (Figure 4). It was also very dependent upon the 1-methyladenine concentration for pD < 10, and increasingly less so for higher pD values. The results were therefore tentatively fitted to the following phenomenological law:

$$\tau^{-1} = k_0 + k_{OD^-}\overline{C}_{OD^-} + k_{C^+}\overline{C}_{C^+} \qquad (1)$$

in which $\overline{C}_i$ is the concentration of species i at the final equilibrium temperature (t_f), calculated according to the Appendix of ref 9, and C^+ stands for the cationic form of the substrate. A least-squares fitting of the data from Figure 4 according to eq 1 leads to $k_0 = 1.5 \times 10^4\ s^{-1}$, $k_{OD^-} = (0.6 \pm 0.04) \times 10^{10}\ M^{-1}\ s^{-1}$, and $k_{C^+} = (2.2 \pm 0.2) \times 10^8\ M^{-1}\ s^{-1}$.

Discussion

Amino–Imino Tautomerism in 1-Alkyladenine. From our spectroscopic results, it appears that the 1-alkyladenines exist predominantly under their *imino form,* Ia or IIa, in nonpolar solvents, no clear-cut distinction being possible between these two species with the techniques we used. Furthermore, the effect of the dielectric constant of the medium upon the amino–imino equilibrium suggests that these conslusions will a fortiori hold in vacuo so that the theoretically reached result[5] that the imino forms are intrinsically more stable than the amino form is certainly correct. However, the reverse is true in highly polar media such as water or acetonitrile, and this is in line with Townsend's report that the amino structure predominates in dimethyl sulfoxide.[6] By contrast, Brookes and Lawley's assignment of an imino structure to aqueous 1-methyladenine must be wrong.[2] These authors felt that 1-methyladenine is too basic [pK_1 (proton gain) = 7.2] with respect to the other ring N-alkyladenines to exist under a similar amino form; they believed that their pK data were nicely interpreted in terms of an imino structure, the first pK (pK_1) being attributed to the very basic exocyclic =NH group, while another observed pK [pK_2 (proton loss) = 11.0] would arise from the ionizable proton on the imidazole ring of the imino forms. This whole argument, however, is clearly inconsistent, since assuming that amino form III is actually a minor tautomeric species leads to the conclusion that it must be even more basic (and also more acidic) than the whole tautomeric mixture, according to the following straightforward relationships:

$$pK_1^{III} = pK_1 + \log\,(1 + [\text{imine}]/[\text{amine}])$$

$$pK_2^{III} = pK_2 - \log\,(1 + [\text{imine}]/[\text{amine}])$$

Such a misleading conclusion underlines the difficulty of using pK data to discriminate between two possible tautomeric states unless the pK values of related alkylated "tautomers", such as Ib, IIb, or IVb, are known for further comparison.

What is responsible for the observed shift in the amino–imino equilibrium position when changing the solvent? It must first be pointed out that such solvent dependence is not rare in the literature; thus, quite drastic changes in the tautomeric ratio have been observed in some cases in going from the aqueous solution to the vapor phase,[41] and more modest medium changes have frequently been found to influence the equilibrium position when the tautomeric equilibrium is rather balanced.[10,41] In several of these examples, it has been possible to account semiquantitatively for the observed dependence of the tautomeric equilibrium constant upon the medium in terms of a simple electrostatic theory, the most polar tautomer receiving an extra stabilization when the dielectric constant is raised.[10,41,42] In the present case, the dipole moment computed from CNDO calculations[43] for III is extremely high (8.9 D) while those of tautomers Ia (5.9 D) and IIa (3.8 D) are significantly lower,[5] especially if attention is paid to the fact that the energy of a dipole interacting with a dielectric continuum varies as the *square* of the dipole magnitude. This is likely to be the main factor favoring the amino form when the dielectric constant of the medium is raised. In fact, the 1-methyladenine tautomerism is very similar, in this respect, to the amino–imino tautomerism of 3-methylcytosine which we discussed in a recent paper.[10] In that case, we were able to show that the logarithm of the tautomeric ratio was a linear function of the solvent parameter $(\epsilon - 1)/(2\epsilon + 1)$ as indeed expected from naive electrostatic theory. No such treatment is possible here because of the difficulty of measuring precisely the equilibrium constants, as three species are actually involved. Nevertheless, there is little doubt that the same electrostatic interaction governs the equilibrium position.

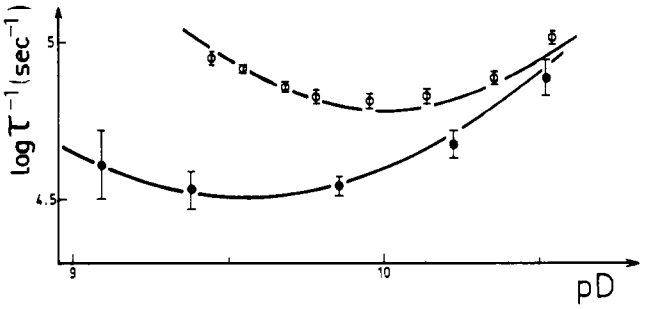
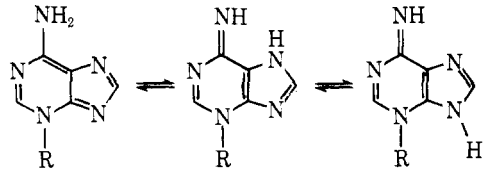


Figure 4. Plot of the relaxation times (τ) observed with solutions of neutral 1-methyladenine in heavy water vs. pD (measured at the temperature before temp-jump, t_i = 6 °C): 1-methyladenine concentration = 7.75×10^{-3} M (upper curve) and 1.12×10^{-3} M (lower curve); magnitude of the temperature jump = 6 °C; λ 300 nm.

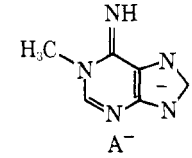
It is interesting at this point to compare the tautomeric preference of 1-alkyladenines with that of the related family, 3-alkyladenines.



In both classes of compounds, the amino tautomer has a "fixed" double bond arrangement contrasting with the "Kekulé type" formula of the amino forms of 7- and 9-alkyladenines, in which the consequent resonance undoubtedly contributes to the well-known predominance of the amino form.[3,7] Nevertheless, despite their formal structural similarity, it is evident on the basis of their IR spectra that 1-alkyladenines and 3-alkyladenines behave quite differently with respect to amino–imino tautomerism. Thus, as we pointed out under Results, 3-methyladenine and 3-benzyladenine exist virtually entirely in the amino form in chloroform solution (Figure 2d). No band is observed around 3440–3450 cm^{-1}, where the imidazole NH stretch from the imino form is likely to be observed;[28] on this basis, we can state that the amount of imino form present is certainly less than, say, 5%. As a further proof of a predominant amino form, we were able to observe that, upon partial deuteration of 3-methyladenine, a new band situated exactly midway between the antisymmetric and symmetric stretch (3468 cm^{-1}) appears in the NH stretching region; this band arises from the NHD vibrator and its observation has been considered as clear-cut evidence for an amino structure.[44] When the solvent is changed, the UV spectra of 3-methyladenine are little modified; it appears unlikely, therefore, that the predominant tautomer changes. Moreover, we observed no relaxation phenomenon which could be attributed to a tautomeric equilibrium in aqueous solutions of 3-methyladenine. From all these observations, it may be concluded, in agreement with earlier reports,[3,45] that in the 3-alkyladenine system, the equilibrium strongly favors the amino form irrespective of the environment. The greater propensity of 1-alkyladenines with respect to 3-alkyladenines to exist in an imino form must result from a quite subtle energetic balance; nevertheless, it is very satisfactorily accounted for by CNDO/2 calculations[5] and can probably, once again, be traced back to the very large charge separation in the amino form of 1-alkyladenine, which specifically disfavors this structure.

Amino–Imino Interconversion Kinetics. The observation that the amplitude of the 1-methyladenine relaxation is independent of pD and proportional to the substrate concentration suggests that it arises from the interconversion of two tautomeric forms of 1-methyladenine. That no relaxation is observed with a solution of IVb, which is structurally related to the predominant form of aqueous 1-methyladenine (III) but cannot undergo tautomerism, further substantiates this view. Now, which are these interconverting forms? Information on this point can in principle be obtained from the wavelength dependence of the amplitude, since this dependence should parallel the difference of the spectra of the two exchanging tautomers, which, in turn, can be approximated by the spectra of the corresponding "fixed" derivatives, Ib, IIb, and IVb.[9-11] Unfortunately, this criterion was not very useful here, because of the close similarities of the spectra of 1,9-dimethyladenine and 1,7-dibenzyladenine in the wavelength range accessible for meaasurements. By contrast, the study of the observed kinetic law itself allows an unambiguous assignment of the relaxation process to the Ia $\rightleftharpoons$ III reequilibration. To establish this point, the kinetic law expected for such a process will be derived and compared with the observed one (eq 1).

The interconversion of Ia and III can a priori proceed via their common protonated species (denoted C_V^+) which has been shown to be the major form of cationic 1-methyladenine, or via their common anion (A^-), which predominates in very



basic 1-methyladenine solutions.[2] Thus, the interconversion is likely to be acid and base catalyzed. The three acidic species present in the solution (the hydronium ion, the cation C_V^+, and water acting as an acid) and the three basic species (the deuteroxide ion, A^-, and water acting as a base) can a priori catalyze the interconversion according to the following kinetic schemes (Schemes I–VI).

Scheme I. Base Catalysis by OD^-

$$\text{Ia} + OD^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} A^- \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{III} + OD^-$$

Scheme II. Base Catalysis by A^- (Base Autocatalysis)

$$\text{Ia} + A^- \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \text{III} + A^-$$

Scheme III. Base Catalysis by the Water Molecule

$$\text{Ia} + D_2O \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} A^- + D_3O^+ \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} \text{III} + D_2O$$

Scheme IV. Acid Catalysis by D_3O^+

$$\text{Ia} + D_3O^+ \underset{k_{-1'}}{\overset{k_{1'}}{\rightleftharpoons}} C_V^+ \underset{k_{-2'}}{\overset{k_{2'}}{\rightleftharpoons}} \text{III} + D_3O^+$$

Scheme V. Acid Catalysis by C_V^+ (Acid Autocatalysis)

$$\text{Ia} + C_V^+ \underset{k_{-3'}}{\overset{k_{3'}}{\rightleftharpoons}} \text{III} + C_V^+$$

Scheme VI. Acid Catalysis by the Water Molecule

$$\text{Ia} + D_2O \underset{k_{-4'}}{\overset{k_{4'}}{\rightleftharpoons}} C_V^+ + OD^- \underset{k_{-5'}}{\overset{k_{5'}}{\rightleftharpoons}} \text{III} + D_2O$$

The overall reequilibration rate constant can be expressed, to a good approximation,[9,10] as:

$$\tau^{-1} = \sum_{i=1}^{6} \tau_i^{-1}$$

where τ_i^{-1} is the reequilibration rate along Scheme i. The τ_i^{-1}, in turn, can be expressed in a straightforward way in terms of the elementary rate constants and of the concentration of the catalysis.[9-11] Furthermore, an a priori guess of the order of magnitude of these contributions can be made by merely considering the pD and the substrate concentration if the pK values of the tautomeric substance are known, since the values of the rate constants for the thermodynamically favorable rate constants in Schemes I–VI appear, from previous work from this laboratory[9-11] or others,[46] to be highly predictable. It is thus found that, at the pD and concentrations we used, only Schemes I, V, and VI will make an appreciable contribution to the interconversion rate law; the expressions for these contributions can be written:

$$\text{Scheme I} \quad \tau_1^{-1} = \frac{k_1k_2 + k_{-1}k_{-2}}{k_{-1} + k_2}\bar{C}_{OD^-} = \frac{k_1k_{-2}}{k_{av}^{OD^-}}\bar{C}_{OD^-}$$

$$\text{Scheme V} \quad \tau_5^{-1} = (k_3' + k_{-3}')\bar{C}_{C_V^+} \qquad (2)$$

$$\text{Scheme VI} \quad \tau_6^{-1} = \frac{k_4'k_5' + k_{-4}'k_{-5}'}{k_{-4}' + k_5'} = \frac{k_{-4}'k_5'}{k_{-4}' + k_5'} \times \left[\frac{K_W}{K_{Ia}^1} + \frac{K_W}{K_{III}^1}\right]$$

In these expressions, K_{Ia}^{I} and K_{III}^{I} stand for the dissociation constants (proton gain) of tautomers Ia and III, respectively, K_W is the autoprotolysis constant of water, and $k_{av}^{OD^-} = (k_1\bar{C}_{Ia} + k_{-2}\bar{C}_{III})/(\bar{C}_{III} + \bar{C}_{Ia})$ is the "average" rate constant for reaction of Ia and III with OD^-.

It is clear that the three contributions in eq 2 account qualitatively for the observed phenomenological law (eq 1). We shall now compare the magnitude expected for the different terms in eq 2 with their observed counterparts.

Scheme I Contribution. k_1 and k_{-2} are expected to have the same order of magnitude, being both diffusion controlled or nearly so ($\sim 10^{10}$ M^{-1} s^{-1}). Since, morover, $C_{III} \gg C_{Ia}$, the relationship $k_{av}^{OD^-} \simeq k_{-2}$ must hold, so that the contribution of Scheme I to the rate law simply reduces to $k_1\bar{C}_{OD^-}$. Therefore, k_{OD^-} in eq 1 must equal k_1; the observed value (0.6×10^{10} M^{-1} s^{-1}) has indeed the expected order of magnitude for the diffusion-controlled reaction of a neutral acid with the deuteroxide ion.[10,11]

Scheme V Contribution. Comparison of eq 1 and 2 shows that if our kinetic assignment is correct, k_{C^+} in eq 1 must represent the sum ($k_3' + k_{-3}'$), subsequently referred to as the "autocatalytic rate constant." In our previous works,[9-11] we observed autocatalytic rate constants ranging from 1×10^8 to 5×10^8 M^{-1} s^{-1}. More specifically, a value of 2.2×10^8 M^{-1} s^{-1} was observed for the amino-imino equilibrium in 3-methylcytosine, which was similarly studied in D_2O solution. Thus, the value observed for k_{C^+}, $(2.2 \pm 0.2) \times 10^8$ M^{-1} s^{-1}, has indeed the expected magnitude.

It should be emphasized that the observation of a "normal" autocatalysis by the 1-methyladeninium cation demonstrates that its major form is the common protonated form of the two exchanging tautomers. Since, moreover, this major form has been shown to have structure V, these two tautomers *must* be Ia and III. No important autocatalysis would be expected, for instance, for a relaxation corresponding to the IIa $\rightleftharpoons$ III reequilibration, because the N_7 protonated form (VI) which would be the active species for such autocatalysis is only a very minor form in the 1-methyladeninium cation.

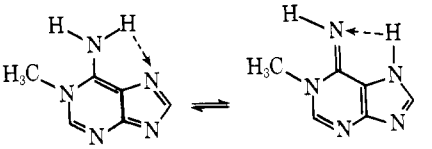
Scheme VI Contribution. Since amino form III greatly predominates over imino form Ia, the relationship $K_{Ia}^{I} = KK_{III}^{I} \ll K_{III}^{I}$, in which K is the tautomeric ratio ($K = [Ia]/[III]$), must hold. The contribution of Scheme VI thus reduces to:

$$\tau_6^{-1} = [(k_{-4}'k_5')/(k_{-4}' + k_5')](K_W/KK_{III}^{I})$$

In this expression, (K_W/KK_{III}^{I}) represents the basicity constant of the minor tautomer, Ia, and k_{-4}' and k_5' are the rate constants for reaction of the two different acidic sites of the cation with the deuteroxide ion. These two rate constants are expected to have the same order or magnitude, being diffusion controlled or nearly so.[10,11] We shall assume a value of 10^{10} M^{-1} s^{-1} for each of them. Furthermore, the ratio K_W/K_{III}^{I} can be computed from the ion product of water and the dissociation constant (proton gain) of 1-methyladenine at the relevant temperature, so that equating the expression of τ_6^{-1} to the observed value in eq 1 (1.5×10^4 s^{-1}) allows an estimate of the tautomeric ratio, K. The value thus obtained, i.e. $K \simeq 1.8 \times 10^{-2}$, agrees satisfactorily with that deduced above from pK comparisons, in view of the simplifying assumptions in both approaches. This consistency further supports our kinetic assignment.

It should be emphasized that, since the ratio $(k_{-4}'k_5')/(k_{-4}' + k_5')$ is not likely to vary much from one system to another, the magnitude of the k_0 term in eq 1 is governed chiefly by the basicity constant of the minor tautomer. This constant is expected to increase by a factor of 2-3 in going from deuterium oxide to ordinary water,[10] thereby accounting for observed faster reequilibration kinetics in this solvent. These findings are in line with our previously reached conclusion[10] that if the rare form is basic enough, the rate law will contain a large, predictable constant term which will make the interconversion rapid even in the absence of any added catalysis, since the rare form rapidly reacts with water acting as an acid to return to the more common tautomeric form. A similar conclusion holds if the rare form is *acidic* enough to rapidly react with water acting as a base.[11] Some possible consequences are discussed in the Conclusion. At this time, we should merely like to point out that no *direct* proton transfer, from one tautomeric site to the other without intermediate dissociation, is needed to account for the observed kinetics; this confirms our previously reached conclusion that such transfers are uncommon and require rather special conditions as concerns the proximity and relative orientation of the two sites.[11]

Although the Ia $\rightleftharpoons$ III reequilibration fully accounts for the observed kinetics, attention should nevertheless be paid to the fact that, most probably, the aqueous solutions of 1-methyladenine contain *three* tautomeric species, i.e. Ia, IIa, and III, so that in principle two relaxation times should be observed. Our failute to observe a second relaxation process presumably arises from one of the following situations. (a) The IIa form is present in very low proportion relative to Ia and/or the enthalpy of the IIa $\rightleftharpoons$ III tautomeric equilibrium is very small with respect to the Ia $\rightleftharpoons$ III enthalpy. These possibilities are considered unlikely since the two forms Ia and IIa are expected to be more or less equivalent energetically.[9,37c] (b) The two relaxation times lie close together and what we observe is in fact a mixture of relaxation modes. Again, this appears unlikely since reasonably "good" exponential signals were observed; moreover, the two relaxation phenomena are likely to depend differently upon pH, and thus such a "mixing" would not subsist throughout the whole pH range. (c) Another possibility is that the IIa $\rightleftharpoons$ III interconversion is "ultrafast" because of the close proximity of the exchanging sites, so that the corresponding relaxation would be imbedded in the initial "rapid" optical density rise:



Such types of very fast proton transfers *not* involving intermediate dissociation to ions but rather direct proton jump from one site to the other have been clearly characterized in the case of α-substituted pyridines,[11] and it is probable that here the geometrical arrangement of the proton donor and proton acceptor sites is even more favorable for such a direct transfer. Indeed, this arrangement is rather similar to that found in the α-salicylate ion, in which an interconversion rate constant higher than 10^8 s^{-1} have been reported.[47] Clearly, further work would be necessary to elucidate this interesting point.

Determination of the Ia $\rightleftharpoons$ III Thermodynamic Parameters. As pointed out before, for $\lambda > 305$ nm, the amplitude of the "rapid" relaxation effect becomes negligible and, therefore, in that wavelength range, the whole variation of the 1-methyladenine extinction coefficient ($\epsilon_{1\text{-MeA}}$) with temperature arises from the Ia $\rightleftharpoons$ III interconversion. If we make the further assumption that the extinction coefficient of form III (ϵ_{III}) contributes negligibly to $\epsilon_{1\text{-MeA}}$ in that wavelength range, a plot of ln $\epsilon_{1\text{-MeA}}$ vs. $(1/T)$ must be linear with a slope $-\Delta H/R$, and the value $\epsilon_{1\text{-MeA}}$ affords an estimate for the tautomeric ratio (K) according to the formula $K = [Ia]/[III] = \epsilon_{1\text{-MeA}}/\epsilon_{Ia} \simeq \epsilon_{1\text{-MeA}}/\epsilon_{1,9\text{-Me}_2\text{A}}$. The constancy of the values of ΔH and K thus obtained vs. the wavelength provides a test for the validity of neglecting the contribution of ϵ_{III} to $\epsilon_{1\text{-MeA}}$. Measurements of the temperature dependence of $\epsilon_{1\text{-MeA}}$ in the 300-350-nm

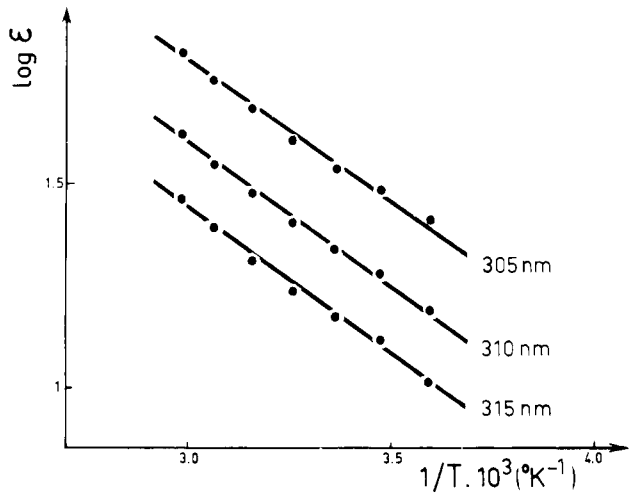
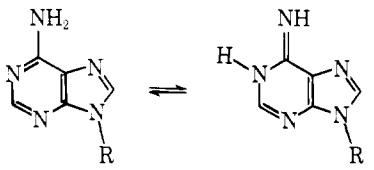


Figure 5. Plot of the 1-methyladenine extinction coefficient vs. $(1/T)$ for different wavelengths; pD (as measured at 25 °C) = 8.6 (cf. text).

range were performed in concentrated solutions ($c \simeq 10^{-2}$ M) from 5 to 65 °C under conditions similar to those used in temp-jump experiments (D_2O was used as a solvent; the pD was fixed to 8.6 at 25 °C). It is seen from Figure 5 that the ln $\epsilon_{1\text{-MeA}}$ vs. $(1/T)$ plots obtained at 305, 310, and 315 nm are indeed parallel, yielding $\Delta H = 3.1 \pm 0.1$ kcal mol^{-1}. The estimate for K was reasonably independent of the wavelength chosen, its value ($K = (1.25 \pm 0.2) \times 10^{-2}$ at 25 °C, or $(1.05 \pm 0.2) \times 10^{-2}$ at 12 °C) being in good agreement with our previous values based on pK comparisons.

Conclusion

Aside from the elucidation of the tautomeric state of 1-alkyladenines, this paper has provided a further example of a system in which the tautomeric interconversion is very fast even in the absence of any added catalysts, because the rare form is basic enough to rapidly react with water acting as an acid. Since the efficiency of this water-catalyzed interconversion depends only upon the magnitude of the basicity constant of the minor tautomer, the same conclusion will hold for the amino–imino equilibrium of 9-substituted adenines (VIIIa ⇌ VIIIb), in which imino form VIIIb is structurally related to Ia and must show a similar basicity:



VIIIa VIIIb

It may thus be predicted that, at neutral pH, the lifetime of the rare tautomer is limited mainly by its rapid reaction with water[48] according to the reaction (cf. Scheme VI above):

$$\text{VIIIb} + H_2O \rightleftharpoons (\text{cation}) + OH^-$$

As an example, we shall consider the biologically important compound adenosine (VIII, R = ribose), which is a constituent of nucleic acids. The minor imino form VIIIb is important in the theory of spontaneous mutation.[12] Assuming that the rate constant for the reverse reaction above is 10^{10} M^{-1} s^{-1} and using the pK value of 1-methyladenosine[49] (Ib, R = CH_3; R′ = ribose) as an approximation for the pK of VIIIb, we find that the forward rate constant is 3.5×10^4 s^{-1}, corresponding to a lifetime of ca. 30 μs for the rare form at 25 °C. Of course, if suitable catalysts (i.e., phosphate ions) are present, the lifetime of the rare form will be even shorter. A comparable kinetic picture has been given for the tautomerization of cytosine and cytidine to their rare imino forms.[10] Furthermore, we have shown that the rare enol forms of uracil and uridine are also expected to be quite short-lived species, because they are *acidic* enough to rapidly return to the major dioxo form through reaction with water acting as a base.[11]

The rare forms of the bases have coding properties different from those of the "normal" tautomers (e.g., the rare imino form of adenosine will pair with cytidine rather than with thymidine) and it has been suggested long ago[12] that the possible occurrence of these rare forms during DNA replication will lead to pairing error, i.e. to mutation. However, it is now known that the number of such errors (10^{-8} to 10^{-11} per nucleotide and generation) is very much smaller than the relatively high ratio of the "abnormal" tautomers of the bases (ca. 10^{-5} to 10^{-4}, or even more),[10,50,51] and this discrepancy has long been puzzling.[52] A recent hypothesis,[53] based on studies on some highly purified DNA polymerase systems, assumes that DNA replication is in fact always a two-step process: the addition of a new nucleotide unit to the growing chain by DNA polymerase is followed by a checking step. The delay between the two steps would allow the newly formed base pair to breathe and to get exposed to the solvent, thereby providing any base accidently incorporated as a rare form during the first step with the possibility of returning to its more common form before the second step. It would then be recognized as mispaired and excised before polymerization proceeds to the next nucleotide unit. Although the kinetic implications of this model have not been discussed, it obviously requires that the lifetimes of the rare forms be short with respect to the delay between the two steps, and a fortiori with respect also to the overall time, the only experimentally accessible value, needed to add a new nucleotide unit to the growing chain. Indeed, this time can be quite short: about 700 μs in vivo in the replication of the genome of *Escherichia coli*,[54] and perhaps 2 ms for the replication in vitro of a DNA template by purified DNA polymerase III.[55] Yet, it is evident from our work that, due to their rapid reaction with water, the lifetimes of the rare forms are shorter than this, by one or two orders of magnitude. Thus, if this replication model is correct, it is presumably the presence of water, an always present catalyst not requiring any preliminary diffusion step, which is the main factor allowing the replication process to combine a very high rate and a low error level, two conditions necessary for duplicating, in a short time and with an acceptable mutation rate, the genetic message.

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Hydrogen Bonding between Nucleic Acid Bases and Carboxylic Acids

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Abstract: High-resolution proton magnetic resonance (^{1}H NMR) studies on adenine derivatives in chloroform reveal a restricted rotation of the amino group in 9-ethyladenine and in 9-methyl-6-methylaminopurine. ^{1}H NMR studies of butyric acid–6-ethyladenine mixtures provide evidence for H-bonding interactions and for double proton magnetization transfer between amino and hydroxylic groups. Measurements of equilibrium constants (K) by ^{1}H NMR and absorbance experiments in $CDCl_3$ at 303 K for association of nucleic acid bases with butyric acid (used as a model of side chain for glutamic or aspartic acid) lead to the following preferential order of associations: 2-dimethylamino-9-methylguanine (K = 660) > 1-cyclohexylcytosine (K = 270) > 9-ethyladenine (K = 160) > 1-cyclohexyluracil (K = 80). Comparative studies of interactions with methylamino derivatives of adenine allow the computation of association constants for the two types of 1:1 complexes with two hydrogen bonds formed between 9-ethyladenine and butyric acid, K_1/K_7 = 2.8 (1 and 7 refer to the position of the nitrogen atom which is bound to the OH group). The results are discussed with respect to the contribution of charge transfer, new repartition of electronic density, and new geometry of monomers in the complexed state superimposed on direct Coulombic interactions.

Among the fundamental mechanisms which can contribute to the specificity of recognition of nucleic acids by proteins and enzymes, direct interactions through hydrogen bonds might be of great importance. Some work has already been devoted to such studies in chloroform[1,2a,b] and cyclohexane.[2a,b] Association constants between amino acid side chains and 9-ethyladenine or 1-cyclohexyluracil have been found in the range 1–22 000 in cyclohexane at 10 °C. Although studies in chloroform have shown that competition with solvent is important, the order of specificity is respected: 1-cyclohexylcytosine > 9-ethyladenine > 1-cyclohexyluracil for association with butyric acid. Since several 1:1 complexes can be made between nucleic acid bases and butyric acid, the constants reported in these studies were the sum of the association constants corresponding to the different 1:1 complexes. Sites of H-bonding interactions cannot be altogether available during the recognition of nucleic acids by proteins and enzymes. It is therefore important to know the association constants of the different 1:1 complexes. The present paper is concerned with an investigation of the interactions of the four nucleic acid bases with butyric acid used as a model for the side chain of glutamic or aspartic acid. Among the number of techniques used to study hydrogen-bond formation, proton magnetic resonance and infrared measurements have been the most