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Pattern of OH Radical Reaction with 6- and 9-Substituted Purines. Effect of Substituents on the Rates and Activation Parameters of the Unimolecular Transformation Reactions of Two Isomeric OH Adducts

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The reactions of the OH radical with purines substituted at C-6 and N-9 were studied by using pulse radiolysis with optical and conductance detection. The electrophilic OH reacts with the purines by addition ($k = 1.3 \times 10^8$ to 8.4×10^9 M^{-1} s^{-1}) via a polar transition state ($\rho^+ = -0.9$) to give isomeric radicals by attachment to C-4 and C-8 and, possibly, to other positions. The adduct to C-4 (A-4-OH) undergoes a unimolecular dehydration reaction to give a radical with oxidizing properties. Substituents at C-6 exert a strong influence on the rate of this dehydration ($\rho^+ = -3$). The adduct to C-8 (A-8-OH) also undergoes a unimolecular transformation reaction which is assigned to opening of the imidazole ring. Substituents at C-6 have only a small influence on the rate of the ring-opening reaction ($\rho^+ = -0.3$). A ribose substituent at N(9) decreases the rates of both dehydration and ring opening. The transformation reactions of A-4-OH and A-8-OH can be distinguished not only by their different response to substituents at C-6 but also by their activation parameters: E_A and ΔS^* values for dehydration of A-4-OH are typically ≈9 kcal mol^{-1} and −6 eu, respectively, whereas they are ≈6 kcal mol^{-1} and −19 eu for ring opening of A-8-OH.

Introduction

The reactions of the OH radical with purines and pyrimidines have been intensively studied during the past ≈3 decades,[2] as a result of the interest in radiation-induced cell death. Whereas the reaction pattern of the pyrimidines with OH has been fully analyzable in terms of addition of OH to the C-5/C-6 double bond with a pronounced preference for C-5,[3,4] the purines have so far escaped a complete analysis of their reactions with OH. However, by use of peralkylated adenines such as N^6,N^6-dimethyladenosine (6-(dimethylamino)purine-9-riboside), it has recently been possible to show that OH adds to C-4, C-5, and C-8 of the purine system and that the so-formed radicals A-4-OH, A-5-OH, and A-8-OH undergo unimolecular transformation reactions.[5] A-4-OH and

A-5-OH were found to eliminate OH^-, these dehydration reactions having equal activation parameters. In contrast, A-8-OH undergoes opening of the imidazole ring and this process is characterized—compared to the dehydration reactions—by distinctly different activation parameters, especially with respect to the activation entropies which are strongly negative (≈−20 eu).

In that work[5] it was also found that the substituent at N-9 has a strong influence on the rates of the transformation reactions of the primarily formed radicals. Such an effect is expected also on changing the substituent at C-6, e.g., on going from the N^6-methylated adenines to the naturally occurring systems such as adenine and adenosine. In the present paper the effects are described of a systematic variation of substituents at C-6 and at N-9 on the rates of the transformation reactions involving, most probably, dehydration of A-4-OH and imidazole ring opening of A-8-OH. From a spectroscopic point of view, these isomers appear to be most easily distinguishable from one another: A-4-OH by the decrease of optical density (OD) at ≈420 nm and A-8-OH by the increase of OD at 310–380 nm, whereas (with the peralkylated systems)[5] A-5-OH could be identified only by its different redox behavior compared to A-4-OH. Therefore, in studying the substituent effect, it appeared reasonable to restrict attention to the transformation reactions of A-4-OH and A-8-OH. The result is that the sensitivity of the transformation rates at 20 °C to substitution at C-6 and N-9 is different for dehydration and for ring opening. This different response induced by the substituent is caused by different contributions of enthalpic and entropic factors to the two types of reaction. This difference in response helps to distinguish the isomeric radicals from one another, as will be shown below.

Experimental Section

The purines were obtained from Aldrich, Fluka, Merck, or Sigma and were used as received. The aqueous solutions (water purified with a Millipore-Milli-Q system) typically contained 0.1–1 mM purine, and they were saturated with N_2O in order to convert e_{aq}^- into OH. The computer-controlled 3-MeV pulse radiolysis apparatus has previously been described.[6] Dosimetry was performed with N_2O-saturated 10 mM KSCN solutions for which $G(OH) = 6.0$ and $\epsilon((SCN)_2^{\bullet-})$ at 480 nm is 7600 M^{-1} cm^{-1}.[7] The irradiated solutions were thermostated to ≤0.1 °C with cells that are an integral part of a heat exchanger.

Results and Discussion

1. Production of OH Adducts. In Figure 1 are presented the absorption spectra of the transients formed on reaction of 6- and 9-substituted purines with the OH radical. The transients absorb in the range 300–≥700 nm, and the rates of increase of optical density immediately after the pulse (spectra characterized by circles) were proportional to the concentrations of the purines (in the range 0.1–1 mM), and from these dependences the second-order rate constants for reaction of OH with the purines, k_{OH}, were obtained. The rate constants, which vary from 1.3×10^8

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(2) For reviews see, e.g.: (a) Scholes, G. In *Photochemistry and Photobiology of Nucleic Acids*; Wang, S. Y., Ed.; Academic: New York, 1976; Vol. 1, p 521. (b) Box, H. C. *Radiation Effects, ESR and ENDOR Analysis*; Academic; New York, 1977. (c) Sevilla, M. D. In *Excited States in Organic Chemistry and Biochemistry*; Pullman, B., Goldblum, N., Eds; Reidel: Dordrecht, 1977; p 15. (d) Hüttermann, J.; Köhnlein, W.; Teoule, R.; Bertinchamps, A. J., Eds. *Effects of Ionizing Radiation on DNA*; Springer-Verlag: Berlin, 1978. (e) Myers, L. S. In *Free Radicals in Biology*; Pryor, W. A., ed.; Academic: New York, 1980; Vol. 4, p 95. (f) Bernhard, W. A. *Adv. Radiat. Biol.* **1981**, *9*, 199. (g) Hüttermann, J. *Ultramicroscopy* **1982**, *10*, 25. (h) Greenstock, C. L. *Isr. J. Chem.* **1984**, *24*, 1. (i) Cadet, J.; Berger, M. *Int. J. Radiat. Biol.* **1985**, *47*, 127. (k) von Sonntag, C.; Schuchmann, H.-P. *Int. J. Radiat. Biol.* **1986**, *49*, 1. (l) Close, D. M. *Magn. Reson. Rev.*, in press.

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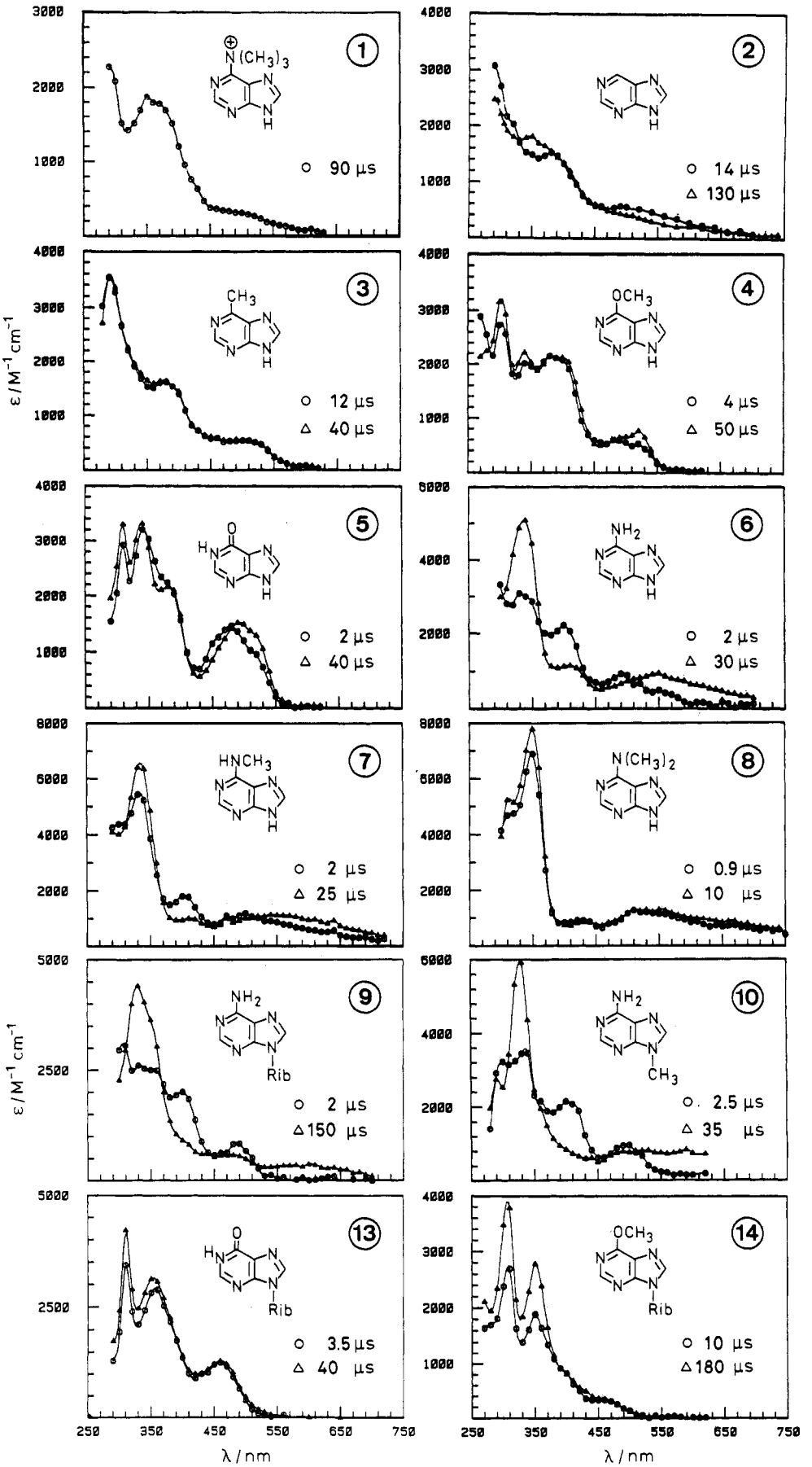
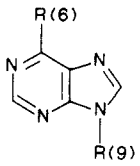


Figure 1. Absorption spectra recorded after reaction of OH with 0.5–1 mM substituted purine at pH 6–8 and 20 °C: circles, spectra measured after completion of the reaction with OH; triangles, spectra recorded after completion of the transformation reactions. The parent compounds are identified by their formula and by their number as given in Tables I–III. The ϵ values are based on G(radical) = G(OH) = 6.0.

TABLE I: Rate Constants for Reaction of OH with the Purines



no.	compd	pK	R(6)	σ^+(R(6))[a]	pH	k/M^{-1} s^{-1}	notes
A: R(9) = H							
1	N^6,N^6,N^6-trimethyladeninium (chloride)	6.46[b]	$N(CH_3)_3^+$	0.41	5.5	1.3×10^8	*c*
2	purine	2.39; 8.93	H	0	6–7	3.0×10^8	from ref 28
3	6-methylpurine	2.6; 9.02	CH_3	−0.31	6.2	4.6×10^8	*d*
4	6-methoxypurine	9.23[b]	OCH_3	−0.78	6.8	2.0×10^9	*e*
5	hypoxanthine	1.98; 8.94; 12.10	OH[l]	−0.92	6–7	2.7×10^9	from ref 28
6	adenine	4.25; 9.83	NH_2	−1.30	≈7.4	4.3×10^9	from ref 27
			NH_3^+(?)		2–4.5	7.6×10^8	from ref 2a
7	N^6-methyladenine	4.2; 10.0	$NH(CH_3)$	−1.41[m]	7.8	5.4×10^9	*f*
8	N^6,N^6-dimethyladenine	3.87; 10.5	$N(CH_3)_2$	−1.70	6.4	7.1×10^9	*g*

no.	compd	pK	R(9)	pH	k/M^{-1} s^{-1}	notes
B: R(6) = NH_2						
9	adenosine	3.45; 1.25	ribose	≈7.4	3.6×10^9	from ref 27
				5–7	4.0×10^9	from ref 28
				≈2.1	1.6×10^9	from ref 28
10	9-methyladenine	3.92[b]	CH_3	7.5	5.7×10^9	*h*
C: R(6) = $N(CH_3)_2$						
11	N^6,N^6-dimethyladenosine	3.65	ribose	≈7.7	6.4×10^9	from ref 5, *h*
12	N^6,N^6-9-trimethyladenine	4.04	CH_3	7	8.4×10^9	from ref 5, *h*

no.	compd	R(6)	pH	k/M^{-1} s^{-1}	notes
D: R(9) = ribose					
13	inosin	OH	7.0	4.8×10^9	*i*
14	6-methoxypurine-9-riboside	OCH_3	6.4	2.6×10^9	*h*
15	N^6-methyladenosine	$NH(CH_3)$	7	6.0×10^9	*k*

[a] The values are from: Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley: New York, 1979. [b] This work; measured by photometric titration. [c–k] This work, measured at 20 °C. From the dependence of k(obsd) on [purine] monitored at (*c*) 350, (*d*) 380–890, (*e*) 380, (*f*) 310, (*g*) 320–330 and 510, (*h*) 400, (*i*) 380 and 460, and (*k*) 310 and 370 nm. [l] Hypoxanthine exists predominantly in the keto (amido) form. [m] Obtained by interpolation from NH_2 to $N(CH_3)_2$.

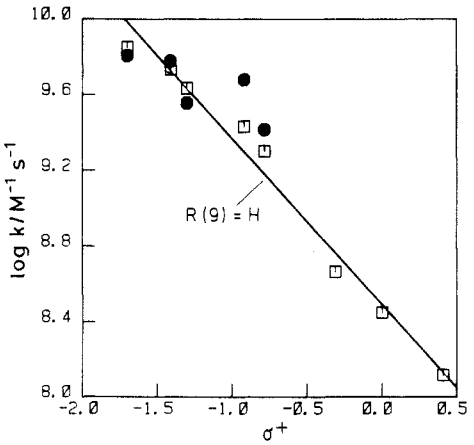


Figure 2. Dependence of log k_{OH} (from Table I) on σ^+ for a series of 6-substituted purines and purine ribosides: circles, R(9) = ribose; squares, R(9) = H. For this system, $\rho^+ = -0.9$.

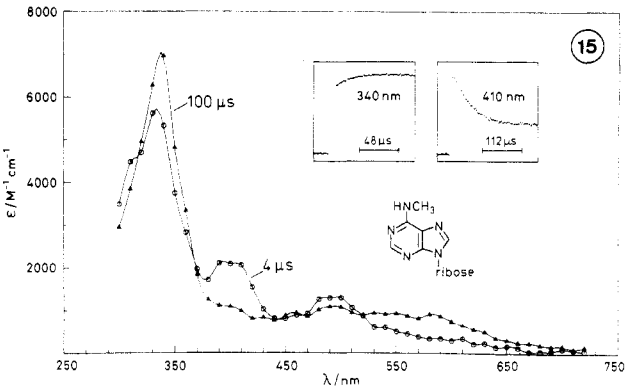


Figure 3. Changes in optical density after production of the OH adducts of N^6-methyladenosine (0.2 mM) at pH 7.2 and 20 °C. The insets show the ring-opening (at 340 nm) and the dehydration reaction (at 410 nm): circles, recorded 3–5 μs after the pulse; triangles, after 80–120 μs.

M^{-1} s^{-1} for N^6,N^6,N^6-trimethyladeninium to 8.4×10^9 M^{-1} s^{-1} for N^6,N^6,9-trimethyladenine, are presented in Table I.

It is evident that the substituents have an influence on the reactivity of the purine system with OH. Concerning R(6), the substituent at C-6, the data from Table I are sufficient to test for a Hammett relationship, and the results are shown in Figure 2. Using σ^+ values, we obtained a good correlation ($r = 0.99$) for all the substituents from the strongly electron withdrawing $N(CH_3)_3^+$ to the potent electron donor $N(CH_3)_2$ to give $\rho^+ = -0.9$. With σ_p values ρ is obtained as −1; however, the correlation coefficient is only 0.86. The negative ρ values demonstrate the electrophilic nature of OH, and they may be compared with those for reaction of OH with substituted benzenes ($\rho = -0.5$[8]) or pyridines ($\rho = -0.7$),[9] reactions that are known to proceed by addition and not by electron transfer, for which larger ρ values would be expected.[10] The fact that the ρ value for reaction of OH with purines is more negative than that for benzenes indicates that the purine system is the more sensitive one with respect to substituent-induced changes in electron density at the ring system. Concerning R(9), the substituent at N-9, its effect does not appear to be very systematic (see Figure 2).

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TABLE II: Rate Constants (at 20 °C) for the Transformations of the OH Adducts of 6- and 9-Substituted Purines[a]

no.	compd	R(6)	pH	k(transformation)/s^{-1} buildup	k(transformation)/s^{-1} decay
A: R(9) = H					
1	N^6,N^6,N^6-trimethyladeninium (chloride)	$N(CH_3)_3^+$	5.5	$\leq 10^2$ (350)	not observed
2	purine	H		1.8×10^4 (350)	2.1×10^4 (295)
3	6-methylpurine	CH_3	6.3	1×10^5 (350)	not observed
4	6-methoxypurine	OCH_3	6.0	1.0×10^5 (310–340)	$\leq 2 \times 10^3$ (460)
5	hypoxanthine	OH	6.0	1.2×10^5 (310–330)	2.3×10^4[b] (440–450)
6	adenine	NH_2	7.0	1.3×10^5 (330)	1.3×10^5 (400)
7	N^6-methyladenine	$NH(CH_3)$	8.0	1.7×10^5 (340)	2.3×10^5 (400)
8	N^6,N^6-dimethyladenine	$N(CH_3)_2$	6.6	2.3×10^5 (370)	2.6×10^6 (410)

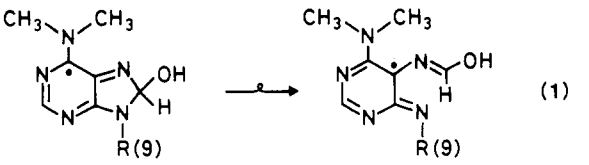
no.	compd	R(9)	pH	k(transformation)/s^{-1} buildup	k(transformation)/s^{-1} decay
B: R(6) = NH_2					
9	adenosine	ribose	7.3	2.6×10^4 (330)	1.7×10^4 (400)
10	9-methyladenine	CH_3	8.7	1.1×10^5 (330)	1.1×10^5 (400–405)
C: R(6) = $N(CH_3)_2$					
11	N^6,N^6-dimethyladenosine	ribose	7–8	9.5×10^4 (370)[c]	4.2×10^5 (420)[c]
12	N^6,N^6-9-trimethyladenine	CH_3	7–8	2.3×10^5 (370) 1.5×10^6 (620)	2.2×10^6 (430)

no.	compd	R(6)	pH	k(transformation)/s^{-1} buildup	k(transformation)/s^{-1} decay
D: R(9) = ribose					
13	inosin	OH	7–8	7×10^4 (350)	$\leq 10^3$
14	6-methoxypurine-9-riboside	OCH_3	6.0	2.6×10^4 (310) 3×10^4 (350)	$\leq 10^3$
15	N^6-methyladenosine	$NH(CH_3)$	7.2	5.4×10^4 (330–340)	2.1×10^4 (400–410)

[a] The values in parentheses denote the wavelength (in nm) of observation. [b] Error limits ±25%. [c] Values are from ref 5.

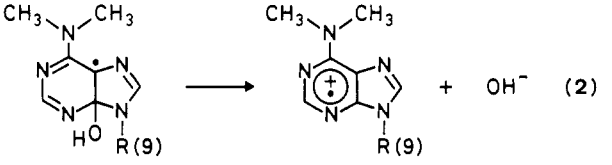
2. Unimolecular Transformation Reactions of the OH Adducts. From Figure 1 it can be seen that the initial spectra (circles) undergo time-dependent changes by which they are transformed to the resulting spectra (triangles). These changes in the optical density (shown in Figure 3 for the case of N^6-methyladenosine) are exponential, and the rates are independent of the concentration of (a) the substrate (in the range 0.2–2 mM) and of (b) the radicals initially produced (0.5–5 μM) from which it is concluded that the processes involved are unimolecular. This type of process has previously been described for adenine, adenosine, and adenosine phosphate.[11-13] The rates of the unimolecular reactions are dependent on temperature and pH.[14] The rate constants measured at 20 °C in a pH range (6–8) where there is *no* dependence on pH are presented in Table II. N^6,N^6,N^6-trimethyladeninium chloride is the only compound for which a unimolecular transformation reaction was not seen up to 10 ms after production of the OH adduct.

The spectral changes presented in Figure 1 can be divided into at least two categories. With the family of the "adenines" (defined by R(6) = NR_2 (R = H or CH_3) and R(9) = H, CH_3, or ribose), in going from the initial spectra (circles) to the final ones (triangles), there is always an increase of OD in the region 310–380 nm, a decrease in the region 390–440 nm, and again an increase at $\lambda \geq 520$ nm. With the remaining purines (R(6) = H, CH_3, OH (or O=), or OCH_3) there is an increase of OD centered around 340–350 nm, analogous to the case of the adenines, but a decrease (at 430–470 nm) is seen only in the case of hypoxanthine (compound **5**).[15] With compounds **4**, **5**, and **13** there are also increases at 500–530 nm, but with the remaining members of this class this type of change is not discernible.[16] The common feature of the changes of the spectra of the OH adducts from all the purines (except compound **1**) is thus the first-order OD increase around 350 nm. In the case of N^6,N^6-dimethyladenosine and N^6,N^6,9-trimethyladenine it has been demonstrated[5] that this type of OD increase is due to opening of the imidazole ring of A-8-OH, the radical formed by addition of OH to C-8 of the purine, as shown in eq 1.



R(9) = CH_3, ribose

It was also demonstrated[5] (by conductance experiments) that the decrease of OD in the 390–440-nm region is due to elimination of OH^- from A-4-OH (eq 2).



R(9) = CH_3, ribose

Conductance experiments have also been performed with the adenines **6–10** and **15**, which are all not fully alkylated at N^6 and N^9. In agreement with earlier studies,[17] conductance changes were *not* seen on the time scales in which the optical changes occur.

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(14) The pH dependence will be described separately (Vieira, A.J.S.C.; Steenken, S., manuscript in preparation).
(15) With (unsubstituted) purine the OD decrease at ≈295 and 500–600 nm, and the increase at ≈350 nm is assigned to the same reaction, namely, the ring opening of A-8-OH (see eq 1).
(16) The nondetectability, under the experimental conditions, of first-order changes may be due to their rates being $\leq 10^3$ or $> 10^6$ s^{-1} and/or to $\Delta\epsilon$ (initial/final radical) ≤ 500 M^{-1} cm^{-1}.
(17) On adenine and adenosine, cf.: Asmus, K.-D.; Deeble, D. J.; Garner, A.; Idriss Ali, K. M.; Scholes, G. *Br. J. Cancer* **1978**, *37* (Suppl. III), 46. Willson, R. L.; Wardman, P.; Asmus, K.-D. *Nature (London)* **1974**, *252*, 323.

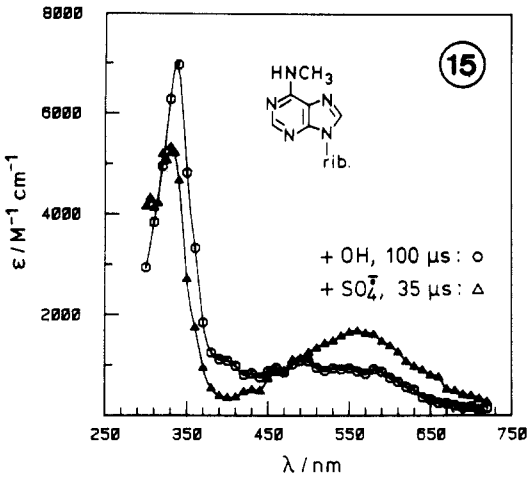
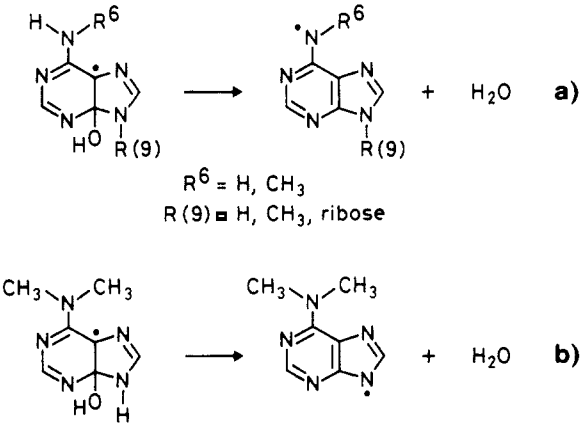


Figure 4. Comparison of the spectra observed on reaction at pH 8 of $SO_4^{\bullet-}$ (triangles, recorded 25–45 μs after the pulse) and OH (circles, 80–120 μs after the pulse) with 0.4 mM N^6-methyladenosine. The spectrum from OH is that after completion of the transformation reactions. The spectrum from $SO_4^{\bullet-}$ was measured with a solution containing 5 mM $K_2S_2O_8$ and 0.1 M *tert*-butyl alcohol. The ε values are based on $G(OH) = 6.0$ and $G(SO_4^{\bullet-}) = 3.0$.

This indicates that if there is OH^- elimination, it is followed (or accompanied) by rapid deprotonation of the incipient radical cation;[18,19] i.e., a (nonconducting) water molecule is the product of the dehydration reaction which is suggested to lead to a radical centered at N-6 or N-9 (cf. reaction 3).



This mechanism is supported by the following two types of experiment: (a) Reaction of the adenines with the oxidizing radical $SO_4^{\bullet-}$ (produced by 248-nm laser photolysis of $S_2O_8^{2-}$ or by its reaction with e_{aq}^-) always led to spectra that closely resemble those measured on reaction of the substrates with OH at times *after* the transformation reactions. An example for this is given in Figure 4, which refers to N^6-methyladenosine. (b) The second piece of evidence in support of scheme 3 is the observation that with all the adenines and with hypoxanthine the transformation of the initially produced OH adducts into their products is accompanied by a redox inversion:[20,21] the initial OH adducts have reducing properties (with respect to, e.g., tetranitromethane), and they are inefficient in oxidizing reductants such as *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD). However, as a result

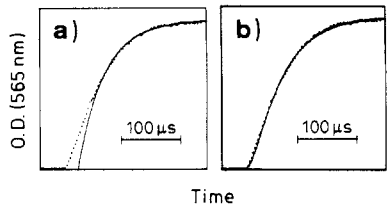
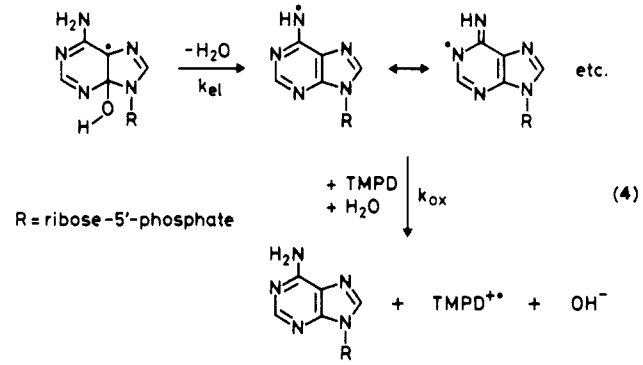


Figure 5. Kinetics of the formation of $TMPD^{\bullet+}$ on reaction of OH with 1 mM adenosine 5'-phosphate in the presence of 20 μM TMPD at pH 8.3. (a) The curve is from a computer fit assuming a single-exponential buildup. (b) The curve is from a computer fit assuming a consecutive reaction with the rate constants given in the text.[29]

of a transformation reaction, oxidizing properties (with respect to TMPD) are acquired. The rate of this redox inversion was found to be identical with the rate of the OD decrease in the ≈430-nm region.[21] An example for this is shown in Figure 5, which refers to adenosine 5'-phosphate. The oxidizing properties of the product radical are understandable in terms of a large unpaired spin density on the heteroatoms, e.g.



The rate constant k_{ox} was measured to be 5×10^9 M^{-1} s^{-1} by varying [TMPD] in the range 2–5 μM. It follows that in the presence of ≥20 μM TMPD the dehydration step (with the rate constant $k_{el} = 1.9 \times 10^4$ s^{-1}) is rate-determining. The yield and rate of dehydration can thus be measured by this redox scavenger method. The values obtained are 50% of oxidizing radicals (relative to the initial yield of OH) and $k_{el} = 2.0 \times 10^4$ s^{-1}, in very good agreement with the directly measured number. It may therefore be concluded that the identification of the OD decrease in the ≈440-nm region in terms of a dehydration reaction that leads to oxidizing, heteroatom-centered radicals is correct.

Concerning the OD changes observed at >520 nm, a general assignment to particular transformation reactions of radical isomers is very difficult, due to the very uncharacteristic and broad absorption bands (see Figure 1). Therefore, in the following attention will be restricted to the OD changes in the ≈350- and ≈440-nm ranges, as outlined above.

3. The Effect of Substituents at C-6 and N-9 on the Transformation Reactions. (a) The Dehydration Reaction. In Table II are collected the rate constants for the unimolecular transformations of the OH adducts of a series of purines carrying different substituents at C-6 and N-9. Concerning the OD decrease at 400–440 nm (assigned to dehydration of A-4-OH; cf. eq 2 and 3), the rate constants increase strongly with increasing electron-donating power of the substituent at C-6 and they decrease if the substituent at N-9 is electron-withdrawing. Changing R(6), the substituent at C-6, from CH_3O to $(CH_3)_2N$ leads to an increase in the rate constant from $\leq 2 \times 10^3$ to 2.6×10^6 s^{-1} (for R(9) = H). A similar increase is seen for R(9) = ribose, if R(6) is changed from NH_2 to the more electron-donating $(CH_3)_2N$. The substituent at N-9, R(9), also has a pronounced influence on the rate of the OD decrease at 400–440 nm: replacement of H or CH_3 by the more electron-withdrawing ribose substituent[22] leads to a decrease in the dehydration rate constants

(18) In the case of the N^6-methyladenosine radical cation (produced by reaction with $SO_4^{\bullet-}$ generated by 248-nm laser photolysis of $S_2O_8^{2-}$) the rate constant for deprotonation from N^6 was measured at pH 5 by conductance to be $\geq 1 \times 10^7$ s^{-1}.

(19) Rapid deprotonation from N^6 of the radical cation has also been found to take place in single crystals at 4 K; cf.: Kar, L.; Bernhard, W. A. *Radiat. Res.* **1983**, *93*, 232. Close, D. M.; Nelson, W. H.; Sagstuen, E. Presented at the 69th Canadian Chemical Conference, University of Saskatchewan, 1986; paper PH-C2-6.

(20) For a review relating to redox inversion, see: Steenken, S. *J. Chem. Soc., Faraday Trans. 1* **1987**, *83*, 113.

(21) This phenomenon has previously been observed; cf. ref 13d.

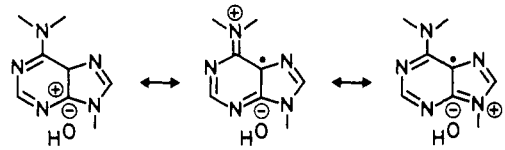
(22) The Taft σ* value for ribose is estimated to be greater than or equal to that for $HOCH_2$ (=0.555).

TABLE III: Activation Parameters for the Transformation Reactions of the OH Adducts of 6- and 9-Substituted Purines[a]

no.	compd	type of OD change	assignt[b]	k(obsd)/s^{-1} at 20 °C[c]	E_A/ kcal mol^{-1}	$\Delta S^{\ddagger}$/ cal mol^{-1} K^{-1}	log A/s^{-1}
2	purine	decay (295)[f]	ro	2.1×10^4	7.8	−14.6	9.8
		buildup (350)	ro	1.8×10^4	7.8	−14.1	10
6	adenine	buildup (330)	ro	1.3×10^5	7.4	−11.8	10.7
		decay (400)	dehyd	1.3×10^5	9.0	−6.5	11.8
7	N^6-methyladenine	buildup (340)	ro	1.7×10^5	5.1	−20	8.9
		decay (400)	dehyd	2.3×10^5	8.0	−9.1	11.3
8	N^6,N^6-dimethyladenine	buildup (370)	ro	2.3×10^5	4.4	−21	8.5
		decay (410)	dehyd	2.6×10^6	6.6	−9.2	11.3
9	adenosine	buildup (330)	ro	2.6×10^4	7.5	−15	10
		decay (400)	dehyd	1.7×10^4	11.1	−3.3	12.5
10	9-methyladenine	buildup (330)	ro	1.1×10^5	8.3	−9.3	11.3
		decay (400–405)	dehyd	1.1×10^5	8.9	−7.3	11.6
11	N^6,N^6-dimethyladenosine[d]	buildup (370)	ro	9.5×10^4	4.3	−22	8.3
		decay (420)	dehyd	4.2×10^5	9.0	−4.2	12.3
12	N^6,N^6,9-trimethyladenine	buildup (370)	ro	2.3×10^5	4.2	−21	8.5
		decay (430)	dehyd	2.2×10^6	8.9	−1.5	12.9
13	hypoxanthine	buildup (330)	ro	1.2×10^5	3–4	≈−25	7.5
		decay (450)	dehyd	2.3×10^4	*e*	*e*	*e*
15	N^6-methyladenosine	buildup (330–340)	ro	5.4×10^4	3.5	−26.6	7.4
		decay (400–410)	dehyd	2.1×10^4	9.9	−6.7	11.8

[a] Obtained by optical measurements at 0–40 °C, pH 6–8. [b] ro = ring opening, dehyd = dehydration. [c] The values are from Table II. [d] The values are from ref 5. [e] Not accurately determinable due to admixture of radical–radical decay. [f] The values in parentheses denote the wavelength (in nm) of observation.

by a factor of 6–11. (Compare adenine, N^6-methyladenine, and N^6,N^6-dimethyladenine with their ribosides.) For the family of compounds R(9) = H the data in Table II are sufficient to test for a Hammett relation. This is shown in Figure 6. If one uses σ^+ values, the points for CH_3O to $(CH_3)_2N$ lie well on a straight line, the slope of which corresponds to $\rho^+ = -3.0$. This indicates that the transition state for the reaction is stabilized by delocalization of positive charge to the substituents at C-6 (+M effect).[23] The rate-retarding effect of substitution by ribose at N-9 is the result of transition-state destabilization by electron withdrawal (−I effect). The direction of these substituent effects is in support of the identification of the reaction as a dehydration involving OH^- elimination in the rate-determining step in the course of which a positive charge is developed which has to be accommodated on the purine backbone (cf. scheme 5).



etc. (5)

Due to the (partial) positive charge on N^6 and N-9, deprotonation from these sites should be rapid. As a result, the separate steps in the ultimate formation of a water molecule should not be distinguishable, in agreement with experiment (time resolution 50 ns).

A Hammett plot can also be constructed for the systems R(9) = ribose (Figure 6). Within the experimental error limits for the three points, the slope ($\rho^+ \approx -3.7$) is similar to that for the case of R(9) = H.

(b) The Ring-Opening Reaction. From the data in Table II is evident that substituents at C-6 have only a small effect on the rate constant for the OD buildup in the 310–370-nm region (assigned to ring opening of A-8-OH; cf. eq 1). Figure 6 shows the Hammett plots: for R(9) = H, ρ^+ is only −0.3, and for R(9) = ribose the dependence is the same. These numbers suggest that in the transition state of the ring-opening reaction only a small degree of positive charge is developed at the purine, in contrast to the case of the dehydration reaction. The largely different ρ^+ values for the two different optical changes (OD increase at ≈350 nm and decrease at ≈430 nm) are of course strong support for the different chemical nature of the radicals responsible for the reactions.

(23) On the basis of the ρ^+ values, the transition state for elimination of OH is similar to that of its addition.

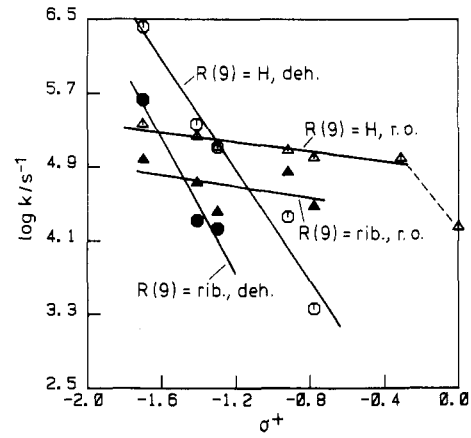


Figure 6. Hammett plots for the effect of variation of R(6) on the dehydration (circles) and ring-opening reactions (triangles) at 20 °C: open symbols, R(9) = H; filled symbols, R(9) = ribose. In calculating the ρ^+ for the ring-opening reaction for R(9) = H, the point for purine has been disregarded.

It is interesting that the Hammett lines for the dehydration and for the ring-opening reactions for the case of the bases (R(9) = H) intersect at the position of adenine (R(6) = NH_2); i.e., with adenine the room-temperature rates for ring opening and for dehydration are accidentally the same. This makes understandable the fact that the unimolecular transformations of the OH adducts of adenine have so far been interpreted in terms of reaction of only *one* isomer: as a ring opening by van Hemmen[11] on the basis of product analysis results, whereas O'Neill[13] suggested a dehydration in order to explain the oxidizing properties of the resultant radicals.

With the ribosides there is more scatter about the Hammett lines (Figure 6). As it happens, also for adenosine the rate constants for dehydration and ring opening are nearly the same, thereby pretending the presence of only one isomeric OH adduct.

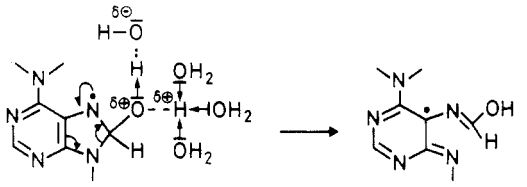
4. The Activation Parameters for the Transformation Reactions. In Table III are collected the activation parameters of the ring-opening (ro) and dehydration (dehyd) reactions of A-8-OH and A-4-OH, respectively, for some representative purines. The values were obtained from Arrhenius plots ($r \geq 0.99$) of the rate data measured between 0 and 40 °C. It is evident that, for each compound presented, the activation parameters for the buildup and the decay are quite different, which further proves that the two phenomena are caused by two different reactions. Concerning the ring-opening reaction, the activation energies are rather small

TABLE IV: Comparison of Some Rate Constants and Activation Parameters[a] for Dehydration and Ring Opening

	R(9) = H						R(9) = ribose					
	dehydration			ring opening			dehydration			ring opening		
R(6)	k/s^{-1}	$\Delta H^{\ddagger}$	$\Delta S^{\ddagger}$	k/s^{-1}	$\Delta H^{\ddagger}$	$\Delta S^{\ddagger}$	k/s^{-1}	$\Delta H^{\ddagger}$	$\Delta S^{\ddagger}$	k/s^{-1}	$\Delta H^{\ddagger}$	$\Delta S^{\ddagger}$
NH_2	1.3×10^5	8.4	−6.5	1.3×10^5	6.8	−11.8	1.7×10^4	10.5	−3.3	2.6×10^4	6.9	−15.0
$N(CH_3)_2$	2.6×10^6	6.0	−9.2	2.3×10^5	3.8	−21.0	4.2×10^5	8.4	−4.2	9.5×10^4	3.7	−22.0

[a] The enthalpies are in kcal mol^{-1}; the entropies are in cal mol^{-1} K^{-1}. Rate constants refer to 20 °C. The error in the values is ≈±10% (±15% for $\Delta S^{\ddagger}$).

(average 5.7 ± 2.0 kcal mol^{-1}), but the entropies are quite negative (−18.5 ± 6.5 eu). In comparison, the dehydration reaction is characterized by higher activation energies (9.1 ± 1.4 kcal mol^{-1}) and by considerably more positive entropies (−5.5 ± 2.5 eu). This latter value is interesting in so far as it indicates that in the necessarily ionic transition state of the OH^- elimination (scheme 5) immobilization of water molecules by hydration of the incipient ions is not an important effect. (Immobilization of four water molecules by hydration of a proton leads to an entropy loss of 25 eu.[24]) In contrast, the strongly negative entropy values for the ring-opening reaction are difficult to understand *without* invoking immobilization of water molecules. A mechanism that offers such a possibility (and that also takes into account the fact that electron-donating substituents at C-6 and N-9 increase the rate of the reaction) involves protonation by H_2O of the hemiortho-amide-type OH group at C-8, as shown below:[25]



Such a mechanism is analogous to the hydrolysis of nonradical nitrogen-containing tetrahedral intermediates.[26]

An interesting phenomenon is the existence of pronounced compensatory effects of the substituents at C-6: A substituent that lowers the activation energy, thereby increasing the isothermal reaction rate, also lowers the activation entropy, thereby decreasing the reaction rate. Examples are the couples adenine/N^6,N^6-dimethyladenine and the corresponding nucleosides (compounds **9** and **11**). The data for these couples, taken from Table III, are collected in Table IV for easier comparison. It is evident that compensatory effects are more pronounced on changing the substituent at C-6 than that at N-9. Furthermore, changing R(6) has a more strongly compensatory influence on the activation parameters for the ring-opening reaction than on those for the dehydration reaction. A more pronounced compensatory effect of a substituent on a particular reaction leads to a smaller slope of an isothermal Hammett plot for that reaction. This phenomenon may be responsible for the smaller ρ^+ value observed for the ring-opening reaction. This serves to indicate that care should be exercised in drawing mechanistic conclusions from ρ^+ values.

5. Summary and Conclusions. It has been demonstrated that the rates of the addition reaction of the OH radical to purines increase with increasing electron-donating power of substituents on the purine system. The Hammett ρ^+ value (−0.9) is more negative than that (−0.5) for OH addition to substituted benzenes.

The addition of OH to purines results in (at least) two different types of adduct. One is suggested to be that produced by addition at C-4. This adduct undergoes a unimolecular heterolysis reaction that involves elimination of OH^- or water to give radicals with oxidizing character. Substituents at C-6 or N-9 strongly influence the rate of this reaction ($\rho^+ = -3$ for R(9) = H). The other type of adduct is formed by addition at C-8. This radical undergoes opening of the imidazole ring, with a rate that is influenced by substituents at C-6 only to a small extent ($\rho^+ = -0.3$ for R(9) = H). The two types of reaction differ also by their activation parameters. This fact provides a second method of distinguishing between the isomers.

The Hammett lines for the room-temperature dehydration and for the ring-opening rates intersect accidentally at the positions of adenine and of adenosine, respectively, giving the wrong impression that there is in each case only one type of transformation reaction.

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Registry No. N^6,N^6,N^6-trimethyladeninium (chloride), 13020-83-2; purine, 120-73-0; 6-methylpurine, 2004-03-7; 6-methoxypurine, 1074-89-1; hypoxanthine, 68-94-0; adenine, 73-24-5; N^6-methyladenine, 443-72-1; N^6,N^6-dimethyladenine, 938-55-6; adenosine, 58-61-7; 9-methyladenine, 700-00-5; N^6,N^6-dimethyladenosine, 2620-62-4; N^6,N^6-9-trimethyladenine, 3013-82-9; inosin, 58-63-9; 6-methoxypurine-9-riboside, 5746-29-2; N^6-methyladenosine, 1867-73-8; OH radical, 3352-57-6.

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(25) The transition state may also be cyclic involving several molecules in proton-transfer processes.

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(28) Farhataziz; Ross, A. B. *Natl. Stand. Ref. Data Ser.* (*U.S., Natl. Bur. Stand.*) **1977**, 59.

(29) An even better fit is obtained if it is taken into account that A-4-OH is able to oxidize TMPD slowly ($k = 2.3 \times 10^7$ M^{-1} s^{-1} (Vieira, A.J.S.C.; Steenken, S., manuscript in preparation).