

Mechanisms for the Solvolytic Decompositions of Nucleoside Analogues. XII. Further Studies on the Alkaline Hydrolysis of 9-(1-Alkoxyethyl)purines

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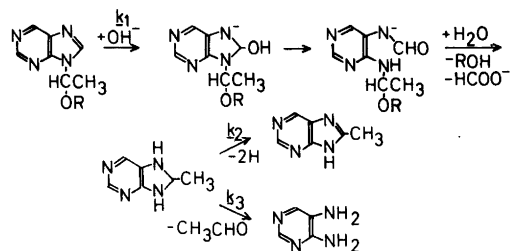
The progress of the alkaline hydrolysis of 9-(1-alkoxyethyl)purines has been studied by isotopic labelling techniques and LC analyses. The reaction has been shown to involve a rate-limiting nucleophilic attack of hydroxide ion on C8 of the purine moiety and subsequent fast departure of the alkoxy group and the attacked carbon atom as formate ion. The initial nucleophilic attack has been suggested to be susceptible to both the polar and steric nature of the alkoxy group. 7,8-Dihydro-8-methylpurine is accumulated during the alkaline cleavage of the starting material and further oxidized to 8-methylpurine by molecular oxygen. Alkaline degradation to 4,5-diaminopyrimidine competes with the oxidation.

The alkaline hydrolysis of purine nucleosides has been suggested to proceed by two concurrent pathways. Either the purine ring is displaced by hydroxide ion,¹⁻⁴ or it is attacked by hydroxide ion and subsequently opened.^{1,3,5-7} Moreover, hydroxide ion may replace carbon bonded substituents on the base moiety.^{1,7,8} At high alkalinities nucleophilic reactions at the purine ring appear to be favored.^{1,2} Usually the C8 atom is attacked with concomitant opening of the imidazole ring.^{1,2,5-9} However, with inosine nucleophilic attack on C2 leads to cleavage of the pyrimidine ring.³

It is generally suspected that nucleophilic attack of hydroxide ion on C8 is followed by a rapid rupture of the bond between C8 and N9.^{1,2,5-7} However, the data on the accumulation of the consecutive intermediates are open to various interpretations. Brown and co-workers^{5,6}

have presented chromatographic and spectroscopic evidence for the formation of considerable amounts of 5-formamido-4-glycosylamino- and 5-amino-4-glycosylaminopyrimidines during the alkaline cleavage of 9-(β -D-ribofuranosyl)purine and its 5'-monophosphate. In contrast, Garrett and Mehta² reported that 4,5,6-triaminopyrimidine is the first detectable product of the hydrolysis of adenosine. Jones *et al.*¹ were also unable to find glycosyl containing intermediates in the reaction mixtures of adenosine, but suggested tentatively that accumulation of 4,6-diamino-5-formamidopyrimidine occurred. Hydrolysis of 7-methylguanosine has been shown to yield 4- and 5-formamidopyrimidine derivatives.^{10,11}

To obtain further information of the reactions following the nucleophilic attack of hydroxide ion, we have previously reported on our studies with the 6-substituted 9-(1-ethoxyethyl)purines.⁹ No formylated intermediates could be detected by ¹H and ¹³C NMR spectroscopic measurements, the first relatively stable species being 7,8-dihydro-8-methylpurine. The latter was suggested to be formed by the loss of C8 atom and cyclization of the resulting pyrimidine derivative. The aim of the present paper is to elucidate more quantitatively these reactions by isotopic labeling studies and LC analyses. The influence of the polar nature of the departing alkoxy group on different partial reactions has been examined by replacing the ethoxy group with more and less electron-withdrawing substituents.



Scheme 1.

RESULTS AND DISCUSSION

The pathway proposed previously⁹ for the alkaline hydrolysis of 9-(1-ethoxyethyl)purine is depicted in Scheme 1. The first-order rate constant obtained by LC for the disappearance of the starting material is $(3.91 \pm 0.05) \times 10^{-4} \text{ s}^{-1}$ in 0.10 mol dm^{-3} sodium hydroxide solution at 343.2 K. Under the same conditions the rate constant for the release of the isotopically modified ethoxy group, $\text{CH}_3[^{14}\text{C}]\text{H}_2\text{O}$, is $(3.84 \pm 0.09) \times 10^{-4} \text{ s}^{-1}$. Accordingly, no intermediate containing the ethoxy group can be accumulated during the hydrolysis. A similar conclusion can also be made concerning the C8 atom of the purine ring. The release of the isotopically labeled formate ion, $\text{H}[^{14}\text{C}]\text{OO}^-$, from $[^{14}\text{C}]8$ labeled starting material exhibits the rate constant of $(3.80 \pm 0.13) \times 10^{-4} \text{ s}^{-1}$, in good agreement with the value observed for the disappearance of 9-(1-ethoxyethyl)purine.

In contrast, the tritiated ethylidene group, $\text{C}[^3\text{H}]\text{CH}=\text{}$, remains attached to the base moiety during the hydrolysis. As seen from Fig. 1, about

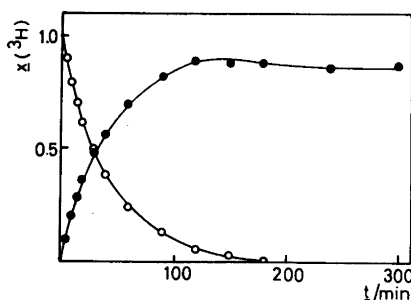


Fig. 1. Distribution of ^3H -activity between the starting material (open circles) and the nonvolatile products (filled circles) during the alkaline hydrolysis of 9-(1-ethoxy[2',- ^3H]ethyl)purine in 0.10 mol dm^{-3} aqueous sodium hydroxide at 343.2 K.

Table 1. Second-order rate constants, $k/10^{-3} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$, for the disappearance of 9-(1-alkoxyethyl)purines in aqueous sodium hydroxide solutions at 343.2 K.

[OH ⁻]/mol dm ⁻³	Oxygen bonded alkyl group		Methyl $\sigma_1 = -0.046$ $E_s = -0.00$	2-Methoxyethyl $\sigma_1 = -0.029$ $E_s = -0.77$	2-Chloroethyl $\sigma_1 = -0.010$ $E_s = -0.90$
	Isopropyl $\sigma_1 = -0.064^a$ $E_s = -0.47^b$	Ethyl $\sigma_1 = -0.055$ $E_s = -0.07$			
0.050	2.66 ± 0.07 ^c	3.67 ± 0.05	4.87 ± 0.03	4.47 ± 0.07	5.83 ± 0.07
0.10	2.70 0.05	3.91 0.05	4.93 0.16	4.73 0.07	6.16 0.03
0.15	2.88 0.04	3.89 0.05	4.94 0.05	4.79 0.07	6.38 0.05
0.20	2.90 0.09	3.93 0.06	5.13 0.04	5.33 0.10	6.40 0.10

^a Taken from Ref. 12, 13 and 14. ^b Taken from Ref. 15. ^c Means of duplicate measurements.

90 % of the initial radioactivity is present in nonvolatile products after 5 half-lives of the decomposition of the starting material. Preparative fractionation of the nonvolatile products by TLC indicated that the product mixture was composed of radiochemically inactive 4,5-diaminopyrimidine, tritiated 8-methylpurine, and a tritiated compound, which was on the basis of earlier investigations⁹ assigned as 7,8-dihydro-8-methylpurine. The slight decrease in the total radioactivity can thus be attributed to the formation of 4,5-diaminopyrimidine with release of the tritiated ethylidene group.

In summary, the studies with isotopically modified compounds lend substantial evidence for the reaction pathway suggested on the basis of spectroscopic findings,⁹ and indicate that the release of the exocyclic alkoxy group and the C8 atom of the purine moiety must occur in fast steps following the attack of hydroxide ion. The ethylidene group, however, remains attached to the accumulated intermediate.

Table 1 records the second-order rate constants obtained by LC analyses for the disappearance of several 9-(1-alkoxyethyl)purines in aqueous sodium hydroxide. The slight increasing of the rate constants with the increasing base concentration probably reflects positive salt effects on the hydrolysis rates. Comparison of the logarithmic rate constants with the substituent constants σ_1 and E_s listed in Table 1 reveals that the reactivity correlates better with the polar than the steric nature of the oxygen bonded alkyl group. The data referring to 0.10 mol dm⁻³ sodium hydroxide give the values of 5.5 ± 1.7 and -0.11 ± 0.18 for the reaction constants in eqns. (1) and (2), respectively.

$$\lg(k/\text{dm}^3 \text{ mol}^{-1} \text{ s}^{-1}) = \rho_1 \sigma_1 + \text{constant} \quad (1)$$

$$\lg(k/\text{dm}^3 \text{ mol}^{-1} \text{ s}^{-1}) = \rho_s E_s + \text{constant} \quad (2)$$

Accordingly, the major reason for the reactivity differences appears to be the polar effect of the oxygen bonded alkyl group on the electron density of the purine ring and hence on the ease of the nucleophilic attack of hydroxide ion. However, the best structure-reactivity correlation is obtained, when both the polar and steric properties are taken into account. The least-squares fitting by eqn. (3) gives the values of 8.4 ± 0.3 and 0.22 ± 0.02 for ρ_1 and ρ_s , respectively.

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$$\lg(k/\text{dm}^3 \text{ mol}^{-1} \text{ s}^{-1}) = \rho_1 \sigma_1 + \rho_s E_s + \text{constant} \quad (3)$$

In other words, the alkoxy group appears to retard sterically the nucleophilic attack of hydroxide ion, but this effect is less important than the polar influence.

The decomposition of all the compounds studied was observed to occur with formation of the same UV-absorbing products. Two of them could be identified as 4,5-diaminopyrimidine and 8-methylpurine by comparing the retention times and UV-spectra with those obtained for authentic samples. The third one, appearing as an intermediate, was tentatively assigned as 7,8-dihydro-8-methylpurine. The absorption maximum of this compound was at 300 nm, in consistence with the fact that 7,8-dihydropurines absorb at higher wavelengths than their parent purines.¹⁶ In addition to the compounds mentioned above some purine was formed, but its mole fraction remained less than 0.03 in each kinetic run. Fig. 2 shows an example of the time-dependent concentrations of the products formed during the alkaline cleavage of 9-(1-methoxyethyl)purine.

In each kinetic run the ratio of the mole fractions of 4,5-diaminopyrimidine and 8-methylpurine remained fairly constant with time. The value of the ratio was almost independent of the compound studied, but was decreased slightly with the decreasing base concentration. Accordingly, it seems reasonable to assume that both of these products are formed *via* the same accumulated intermediate, 7,8-dihydro-8-methylpurine.

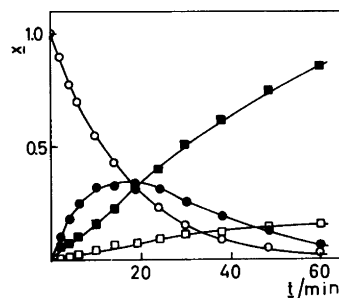


Fig. 2. Time-dependent product distribution for the alkaline hydrolysis of 9-(1-methoxyethyl)purine in 0.20 mol dm⁻³ aqueous sodium hydroxide at 343.2 K. Open circles refer to the starting material, filled circles to the accumulated intermediate, open squares to 4,5-diaminopyrimidine, and filled squares to 8-methylpurine.

Purging the reaction solution with nitrogen markedly retarded the decomposition of this intermediate by diminishing the rate of formation of 8-methylpurine. At the same time the formation of 4,5-diaminopyrimidine was slightly accelerated and it became the main product. The latter rate-enhancement probably results from the fact that the standing concentration of the intermediate is increased due to the retardation of its oxidation. The hydrolysis rate of the starting material remained unaffected. The preceding findings strongly suggest that 7,8-dihydro-8-methylpurine reacts further by two concurrent routes, the major reaction pathway in oxygen saturated solutions being oxidation to 8-methylpurine with molecular oxygen. Base-catalyzed degradation to 4,5-diaminopyrimidine appears to compete with this pathway.

If it is assumed that the concentration of molecular oxygen in the reaction mixture remains constant during the kinetic run, *i.e.* equilibrium with the gas phase prevails, kinetics of two consecutive first-order reactions can be applied to the formation and disappearance of the intermediate. It should be noted that the substrate concentration is always negligible compared to the concentration of hydroxide ion. The first-order rate constants for the formation step equals with the rate constants for the decomposition of the starting material, since 7,8-dihydro-8-methylpurine is the first detectable intermediate. The pseudo first-order rate constants obtained for the disappearance of the accumulated intermediate are listed in Table 2. As expected, they are independent of the structure of the starting material, but are slightly decreased with the decreasing concentration of hydroxide ion. These constants can further be bisected to the pseudo first-order rate constants for the formation of

8-methylpurine and 4,5-diaminopyrimidine. As seen from Table 2, the rate for the formation of 8-methylpurine is almost independent of the base concentration, in consistence with the assumption that this partial reaction involves oxidation with molecular oxygen. The concentration of the latter can be expected to be relatively independent of the alkalinity. For comparison, the oxidations of a great number of 7,8-dihydropurines to purines have been shown to be insensitive to the pH of the reaction solution.¹⁶ In contrast, the degradation to 4,5-diaminopyrimidine seems to be base-catalyzed, since the observed first-order rate constants are linearly related to the concentration of hydroxide ion. The mechanism for the latter reaction cannot be deduced on the basis of the available data.

In summary, the preceding discussions indicate that the only accumulated intermediate in the alkaline hydrolysis of 9-(1-alkoxyethyl)purines is 7,8-dihydro-8-methylpurine formed by the cleavage of the C8 atom as formate ion and subsequent recyclization of the resulting pyrimidine derivative with loss of the alkoxy group. The question of the appearance of similar intermediates during the hydrolysis of purine nucleosides remains open.

EXPERIMENTAL

Materials. 9-(1-Alkoxyethyl)purines were obtained by treating purine with appropriate alkyl 1-chloroethyl ethers in DMF.¹⁷ Alkyl 1-chloroethyl ethers were prepared by conducting dry hydrogen chloride into the corresponding vinyl ethers and purified by distillation under reduced pressure. Ethyl, methyl, and 2-chloroethyl vinyl ethers were commercial products. Isopropyl and 2-methoxyethyl vinyl ethers were

Table 2. Pseudo first-order rate constants, k_d , for the disappearance of the intermediate accumulated during the alkaline hydrolysis of 9-(1-alkoxyethyl)purines, and the pseudo first-order rate constants, k_2 and k_3 , for the subsequent formation of 8-methylpurine and 4,5-diaminopyrimidine at 343.2 K.

$[\text{OH}^-]/\text{mol dm}^{-3}$	$k_d/10^{-4} \text{ s}^{-1}$	$k_2/10^{-4} \text{ s}^{-1}$	$k_3/10^{-4} \text{ s}^{-1}$
0.050	7.7 ± 0.6^a	7.3	0.4
0.10	8.8 0.9	8.1	0.7
0.15	9.7 0.8	8.6	1.1
0.20	12 1	10	1.7

^a Means of four kinetic runs.

Table 3. Results of elemental analyses and ^1H and ^{13}C NMR chemical shifts^a for 9-(1-alkoxyethyl)purines.

	C %	H %	$\delta(2)$	$\delta(4)$	$\delta(5)$	$\delta(6)$	$\delta(8)$	$\delta(1')$	$\delta(2')$	$\delta(\text{R})$
1	58.39 (58.24) ^b	6.66 (6.84)	^1H : 8.97 ^{13}C : 152.8 the data given in Ref. 13	151.1	134.2	89.13 142.8	88.35 148.8	96.20 78.6	d1.79 23.2	m3.63, d1.27, d1.03 70.7, 22.9, 21.3
2	56.34 (56.24)	6.15 (6.29)								
3	54.09 (53.92)	5.62 (5.66)	^1H : 8.96 ^{13}C : 152.9	151.3	134.2	89.13 142.8	88.27 148.9	95.97 82.6	d1.85 22.2	s3.30 56.7
4	57.36 (57.16)	6.65 (6.72)	^1H : 8.90 ^{13}C : 152.7	151.3	134.2	89.07 143.0	88.30 148.6	96.10 81.6	d1.83 22.4	m 3.4-3.6, s3.37 59.0, 68.6, 71.4
5	47.73 (47.69)	4.81 (4.89)	^1H : 8.93 ^{13}C : 153.0	151.3	134.1	89.11 142.7	88.33 149.0	96.13 81.0	d1.89 22.2	m 3.5-3.9 42.4, 69.3

^a In CDCl_3 as ppm from TMS. ^b Calculated values in parentheses.

synthesized from ethyl vinyl ether and the appropriate alcohols by mercury(II) acetate catalyzed vinyl transesterification.¹⁸

9-(1-Alkoxyethyl)purines, obtained as described above, were separated from their 7-isomers, formed in small quantities, by eluting the crude product with methanol through a strong cation exchange resin (Dowex 50 WX2, mesh 100-200) loaded with magnesium(II) ions.¹⁹ The homogeneity of the products was checked by LC on a LiChrosorb RP-18 (10 μm , 250 mm) column using the mixture (80:20) of acetonitrile and acetic acid buffer (0.02 mol dm^{-3} , buffer ratio 1:1) as eluant. Table 3 records the ^1H and ^{13}C NMR chemical shifts and the results of the elemental analyses for the compounds prepared.

9-(1-Ethoxyethyl)purine labeled with ^{14}C at C1 of the ethoxy group, was prepared as described above. The isotopically modified ethyl vinyl ether, employed as starting material, was prepared from ethyl vinyl ether and [^{14}C]ethanol by mercury(II) acetate catalyzed vinyl transesterification.¹⁸

9-(1-Ethoxyethyl)purine labeled with ^{14}C at C8 of the purine moiety was prepared as described above. [^{14}C]Purine employed as starting material was synthesized by refluxing 4,5-diaminopyrimidine in [^{14}C]formic acid,²⁰ and cyclizing the formamido derivative formed in boiling formamide.²¹

9-(1-Ethoxyethyl)purine tritiated at C2 of the ethylidene group was obtained as described above, using isotopically modified ethyl 1-chloroethyl ether. The latter was prepared by conducting tritiated hydrogen chloride in ethyl vinyl ether and distilling the product under reduced pressure.

Kinetic studies with isotopically modified compounds. The release of the [^{14}C]ethoxy group during the alkaline hydrolysis of 9-(1-ethoxyethyl)purine was examined as follows. Aliquots (5 cm^3) were withdrawn from the reaction solution at suitable intervals, cooled rapidly to 0 $^\circ\text{C}$, and extracted with dichloromethane (5 cm^3). The organic and neutralized aqueous phases were evaporated to dryness under reduced pressure, and the residues were transferred with water (4 cm^3) to the scintillation vials containing lumagel (10 cm^3). The cpm values observed were corrected to a constant quenching by the method of external standardization.

The release of the ^{14}C 8 atom during the alkaline hydrolysis of 9-(1-ethoxyethyl)purine was followed by passing the aliquots (5 cm^3), withdrawn at suitable intervals, through a strong cation exchange resin (Dowex 50 WX2, mesh 100-200, H^+ -form). The unreacted starting

material and all the products containing a nitrogen base moiety were retained in the resin. The radioactivity of the eluates was determined as described above. The release of the ^{14}C atom was also followed by passing the aliquots through a strong anion exchange resin (Dowex 1 \times 2, mesh 100–200, Cl $^-$ -form). [^{14}C]Formate ions were now retained in the resin and the amount of the other radioactive compounds in the eluates was determined by liquid scintillation counting.

The release of the tritiated ethylidene group during the alkaline hydrolysis of 9-(1-ethoxyethyl)purine was followed by the method described above for the release of the [^3H]ethylidene group.

LC analyses of the reaction mixtures. The hydrolyses were carried out in stoppered bottles immersed in a water bath, the temperature of which was kept constant within 0.05 K. The reactions were started by adding the substrate in the pre-thermostated reaction solutions to give the initial substrate concentration of 1.0×10^{-3} mol dm $^{-3}$. The reaction vessels were shaken mechanically and opened at suitable time intervals to maintain the concentration of the molecular oxygen constant. Samples of 0.5 cm 3 were withdrawn, cooled and neutralized with anhydrous acetic acid. The compositions of the aliquots were analyzed by reversed phase LC (Varian Aerograph 5020) using a commercial Hibar column (250–4 mm) packed with LiChrosorb RP-18 (10 μm) and a variable wavelength UV detector (Varian UV-100). Elution with an acetic acid buffer (0.02 mol dm $^{-3}$, buffer ratio 1:1) containing 20% (v/v) acetonitrile gave a good separation for purine, 8-methylpurine and starting materials. The assignment of the peaks were performed by comparing the retention times and UV-spectra with those for the authentic samples. The concentrations of 4,5-diaminopyrimidine and the accumulated intermediate were determined analogously using a mixture of acetonitrile and acetic acid buffer (80:20 as v/v) as eluent. Solutions of known concentrations were employed in calibration of the peaks of purine, 8-methylpurine, 4,5-diaminopyrimidine and the starting materials. The peak for the observed intermediate was calibrated on the basis of the assumption that the total concentration of all the compounds detected remained constant during the hydrolysis.

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