

Kinetics and Mechanism of the N₇-Hydroxyalkylation of Guanosine

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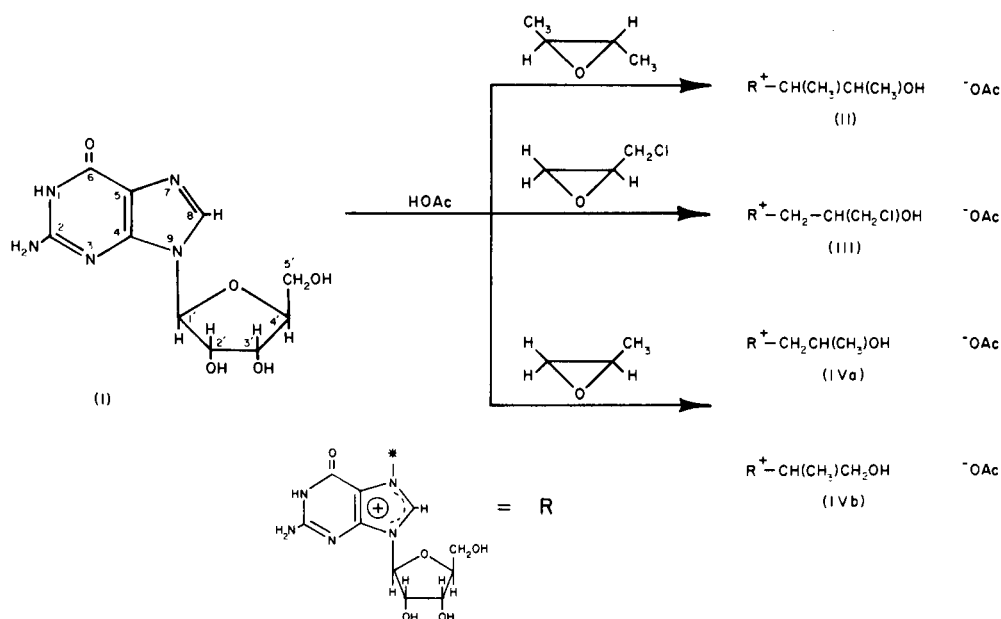
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The kinetics of the N₇-hydroxyalkylation of guanosine by the equally substituted epoxide, *trans*-2,3-epoxybutane, and the unequally substituted epoxides, 3-chloro-1,2-epoxypropane, and 1,2-epoxypropane in glacial acetic acid, have been measured by a spectrophotometric method over the range 20-40°. Activation parameters have been determined. Comparative rates calculated from the ratios of second-order rate constants indicate that 1,2-epoxypropane reacts with guanosine about three times faster than does *trans*-2,3-epoxybutane and about two times faster than 3-chloro-1,2-epoxypropane. These results coupled with the results of a previous report on the structural analysis of the products from these reactions are consistent with a "push-pull" mechanism in which N₇ of guanosine reacts preferentially at the least substituted carbon of the epoxide with simultaneous transfer of a proton from acetic acid to the oxygen of the epoxide. The lower reactivities of *trans*-2,3-epoxybutane and 3-chloro-1,2-epoxypropane in comparison to that of 1,2-epoxypropane are discussed in terms of steric factors and electronic factors which determine the stability of the requisite transition state for a "push-pull" mechanism model.

Introduction.

In a previous paper it was reported that several mono-functional epoxides reacted with guanosine in glacial acetic acid to give the corresponding 7-hydroxyalkyl-guanosinium acetates (1). These derivatives of guanosine

were prepared in the course of studies by this laboratory of the initial sites of reaction and comparative reactivities of epoxides with the nucleotides and nucleosides of DNA and RNA. It was reported that guanosine reacted pre-



ferentially at the least substituted carbon of the epoxide ring; on the basis of an analysis of the pmr spectra of epoxide-guanosine reaction products, it was concluded that *trans*-2,3-epoxybutane and 3-chloro-1,2-epoxypropane reacted with guanosine (I) to give, within the limits of detection, estimated to be about 1 part in 20, only single isomer products (II and III) respectively; however, 1,2-epoxypropane gave a mixture of 7-hydroxypropylguanosinium acetate isomers in a ratio of about 7 to 1 (IVa and IVb).

Insert Reactions of Guanosine with Epoxides

In an effort to gain a more fundamental understanding of the reactions of epoxides with guanosine in glacial acetic acid and to obtain additional support for the assignments of IVa and IVb, the rates of several guanosine-epoxide reactions were measured and compared. Rate measurements were obtained from analysis of the absorption changes in the ultraviolet spectra associated with these reactions. We now wish to report on the kinetics and mechanism of the N_7 -hydroxyalkylation of guanosine by the equally substituted epoxide, *trans*-1,2-epoxybutane, and the unequally substituted epoxides, 3-chloro-1,2-epoxypropane and 1,2-epoxypropane in glacial acetic acid over the range 20-40°.

Results.

When guanosine reacts with epoxides in glacial acetic acid to give 7-hydroxyalkylguanosinium acetates, the associated ultraviolet spectra undergo change. Figure 1 shows typical spectra of glacial acetic acid solutions containing equal molar concentrations of guanosine and a 7-hydroxyalkylguanosinium acetate. As the N_7 -hydroxyalkylation of guanosine occurs increases in absorptions at wavelengths greater than about 280 nm are observed.

By employing a double beam instrument in which a glacial acetic acid solution of guanosine was placed in one cell and a solution containing an identical amount of guanosine plus epoxide was placed in the other cell, difference spectra were obtained for the reactions of guanosine with epoxides. Figure 2 shows scans with time of the difference spectra of guanosine reacting with *trans*-2,3-epoxybutane in glacial acetic acid.

The reactions of *trans*-2,3-epoxybutane, 3-chloro-1,2-epoxypropane and 1,2-epoxypropane with guanosine in glacial acetic acid were found to be first-order with respect to both the epoxide and guanosine. The method of ratio variation was used to establish reaction orders (2); the concentration of epoxide and guanosine were varied one at a time and the reaction rates compared by measuring ultraviolet absorption changes at 299 nm over identical reaction times up to a change in the reactants of less than 10 percent. Continuous scans at 299 nm

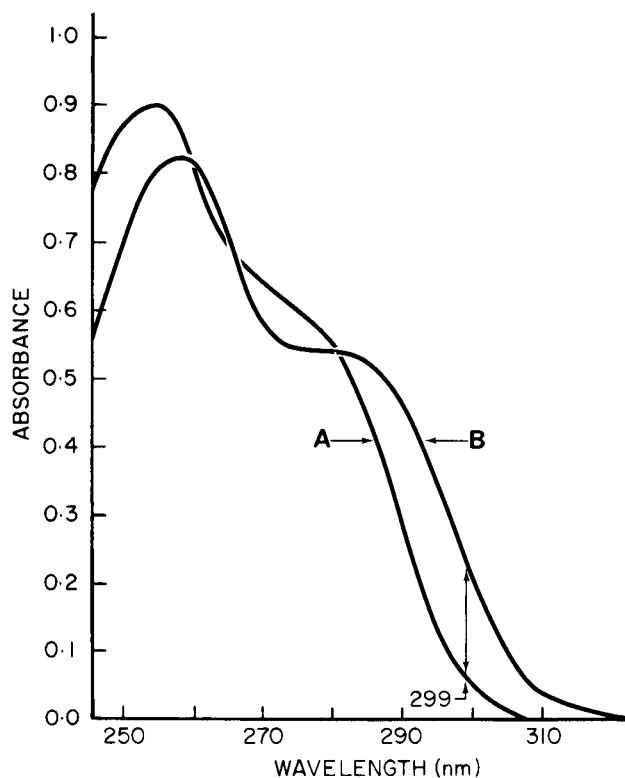


Figure 1. Comparisons of spectral scans of equal molar solutions of guanosine (A) and 7-[2-(3-hydroxybutyl)]-guanosinium acetate (B) in aqueous acetic acid. Scans were obtained on solutions prepared by the aqueous dilution of equal aliquots of a glacial acetic acid solution that initially was $3.13 \times 10^{-4} M$ guanosine and $1 M$ in *trans*-2,3-epoxybutane. Scan A was taken immediately after the reaction was initiated and Scan B was taken after the reaction was completed.

of solutions with the same amount of guanosine but varying concentrations of the epoxides, and of solutions in which the concentration of guanosine was varied and the concentration of the epoxide held constant were used to obtain rate constants.

Pseudo first-order rate constants k_{obsd} were calculated from plots of $\log(A_{\infty}^{299} - A_t^{299})$ vs time which were linear for at least 80% reaction. Second-order rate constants were obtained from the slopes of lines obtained from plotting k_{obsd} vs concentration of the epoxide (3). These lines passed through the origin, indicating that competing reactions of glacial acetic acid with the epoxides to give the corresponding hydroxyacetate esters could be neglected. Table I shows the k_{obsd} values and the calculated second-order rate constants at 30° with varying epoxide concentrations.

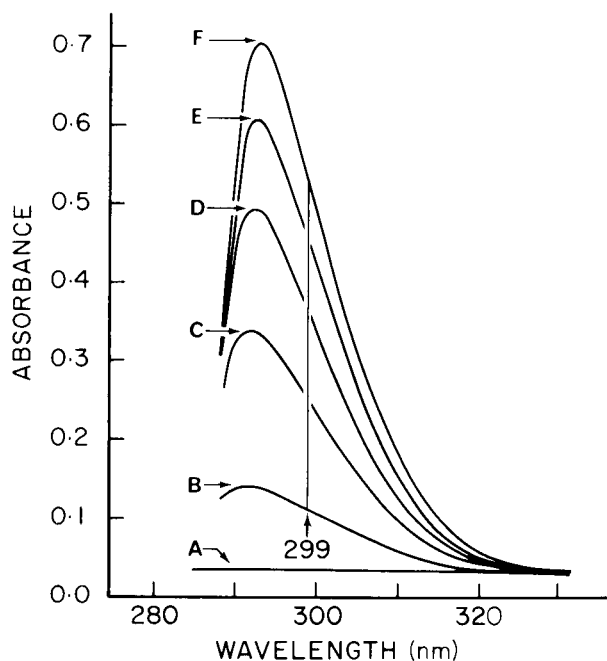


Figure 2. Difference spectral scans for the conversion of guanosine ($4.7 \times 10^{-4} M$) in glacial acetic acid to 7-[2-(3-hydroxybutyl)]guanosinium acetate by *trans*-2,3-epoxybutane at 25°. Scan A was taken at t_0 and Scans B-F were taken as the reaction progressed.

The effect of temperature on the second-order rate constants for the N₇-hydroxyalkylation of guanosine by epoxides is revealed in the data of Table II.

Activation parameters for these reactions given in Table III were derived from the slopes of the lines resulting from $\log k$ vs $1/T$ plots. These Arrhenius plots are shown in Figure 3.

Discussion.

The enthalpies of activation are probably accurate to ± 0.1 Kcal mole⁻¹ and the entropies of activation to ± 0.1 cal mole⁻¹ deg⁻¹; the free energies of activation in Table III indicate the observed order of reactivity of the epoxides with guanosine to be 1,2-epoxypropane > 3-chloro-1,2-epoxypropane > *trans*-2,3-epoxybutane. Calculation of the expected ratios of the rates of these reactions from differences in their free energies of activation by the equation, $\log k_1/k_2 = \frac{\Delta\Delta G_a}{2.303 RT}$, predicts rather closely

the observed differences in reaction rates with temperature. Additionally, the previously reported 7 to 1 isomer ratio resulting from the reaction of 1,2-epoxypropane with

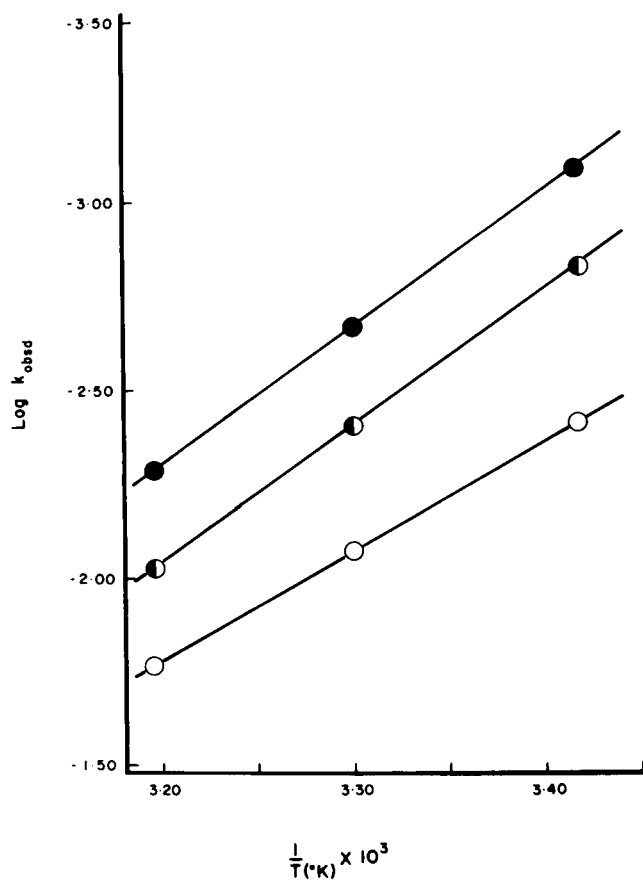


Figure 3. Arrhenius plots for the N₇-hydroxyalkylation of guanosine in glacial acetic acid by *trans*-2,3-epoxybutane (●), 3-chloro-1,2-epoxypropane (◐) and 1,2-epoxypropane (○).

guanosine is consistent with the finding that 1,2-epoxypropane reacts three times faster than *trans*-2,3-epoxybutane. Since there are two equivalent carbons in *trans*-2,3-epoxybutane, it follows that the reaction of 1,2-epoxypropane is about six times faster than the reaction at each of the carbons of *trans*-2,3-epoxybutane. Table IV shows a comparison of the observed and calculated rate ratios of these epoxides at 30.0°.

The appreciable negative entropies of activation associated with these reactions indicates the formation of transition state complexes with the loss of several degrees of freedom. The role of the acetic acid solvent in these reactions appears to be two-fold. First, the acetic acid forms a complex with epoxides which alters the reactivity of the epoxide and secondly, it donates a proton to the epoxide oxygen and furnishes a counter anion to the 7-hydroxyalkylguanosinium cation. For this reason, these

TABLE I

Effect of Epoxide Concentration of the Pseudo-First-Order Rate Constant for the N_7 -Hydroxyalkylation of Guanosine by Epoxides in Glacial Acetic Acid at 30° (a).

<i>trans</i> -2,3-Epoxybutane		3-Chloro-1,2-Epoxypropane		1,2-Epoxypropane	
[E] (d)	$k_{\text{obsd}}(b)$ $\text{min}^{-1} \times 10^3$	[E] (d)	$k_{\text{obsd}}(b)$ $\text{min}^{-1} \times 10^3$	[E] (d)	$k_{\text{obsd}}(b)$ $\text{min}^{-1} \times 10^3$
	$k(c)$ $M^{-1} \text{min}^{-1} \times 10^3$		$k(c)$ $M^{-1} \text{min}^{-1} \times 10^3$		$k(c)$ $M^{-1} \text{min}^{-1} \times 10^3$
0.222	2.49	0.255	1.04	0.286	2.11
0.444	2.65	0.510	1.96	0.573	4.36
0.666	2.50	0.765	3.05	0.858	6.39
0.888	2.49	1.020	3.99	1.145	8.54
					7.38
					7.61
					7.44
					7.49

(a) All runs were performed with a guanosine concentration of $3.13 \times 10^{-4} M$. (b) Pseudo-first-order rate constants were calculated from plots of $\log(A_{\infty}^{299} - A_t^{299})$ vs t . (c) Second-order rate constants were calculated from $k_{\text{obsd}}/[E]_{\text{epoxide}}$. (d) Concentration of epoxide, M .

reactions might be expected to follow third-order kinetics. However, the participation of the solvent does not show up directly as a reactant in the rate equation because of its large excess.

The kinetic results of the N_7 -hydroxyalkylation of guanosine by epoxides in glacial acetic acid as well as the stereochemistry and product distributions reported in a previous study (1) are consistent with an acid solvent-catalyzed "push-pull" mechanism for the opening of epoxide rings (5). However, in these reactions the solvent is also a participant. On the basis of this type of mechanism, the opening of the epoxide ring involves the nearly concerted attack of a nucleophile (push) and the carbon-oxygen bond breaking (pull) as indicated in Scheme I. Accordingly, in the reactions with unequally substituted epoxides with guanosine, the factors which play a role in determining product ratios and reaction rates are the nucleophilicity and bulkiness of the guanosine nucleophile, the substituents of the epoxide and the polarity of the acetic acid medium. Based on this model both the nucleophilicity of N_7 of guanosine and the bulkiness of the environs of N_7 would influence the push, while the substituents of the epoxide and the polarity of the media would influence the pull. Electron releasing substituents on the epoxide ring and increasing solvent polarity would be expected to stabilize the developing charge on the ring carbon and favor attack of N_7 of guanosine on the more substituted carbon, whereas electron withdrawing substituents would tend to favor the attack of N_7 on the least substituted carbon. Increasing the bulkiness of the substituents on an epoxide ring carbon would be expected to disfavor attack at that carbon.

In Scheme I the attack of N_7 of guanosine at the least substituted carbon of the epoxide, the bond making step, the formation of the N_7 -carbon bond, is slightly ahead of the bond-breaking step, the breaking of the carbon-oxygen bond; in the attack of N_7 of guanosine at the more hindered carbon of the epoxide, these steps are reversed. In this scheme, the role of the protic solvent is to form a complex with the epoxide oxygen and to furnish a counter anion for the cationic product. Presumably, the stronger the interaction of the solvent with the epoxide oxygen, the easier the bond-breaking step. The ability of the carbons of the epoxide ring to carry a positive charge would influence the bond-breaking step. The methyl substituent on the carbon of 1,2-epoxypropane makes this carbon more susceptible to reaction by N_7 of guanosines than the carbon bearing the chloromethyl substituent of 3-chloro-1,2-epoxypropane. The epoxide oxygen of 3-chloro-1,2-epoxypropane would be expected to associate by hydrogen bonding with the glacial acetic acid less strongly than would the epoxide oxygens of 1,2-epoxypropane and *trans*-2,3-epoxybutane. However, the epoxide

TABLE II

Effect of Temperature on the Second-Order Rate Constants for the N₇-Hydroxyalkylation of Guanosine by Epoxides in Glacial Acetic Acid (a).

<i>trans</i> -2,3-Epoxybutane		3-Chloro-1,2-Epoxypropane		1,2-Epoxypropane	
Temp. (b) °C	k (c) M ⁻¹ min ⁻¹ × 10 ³	Temp. (b) °C	k (c) M ⁻¹ min ⁻¹ × 10 ³	Temp. (b) °C	k (c) M ⁻¹ min ⁻¹ × 10 ³
20.0	1.10	20.0	1.59	20.0	3.59
30.0	2.53	30.0	3.96	30.0	7.50
40.0	6.15	40.0	8.94	40.0	15.7

(a) All runs were performed with a guanosine concentration of 3.13×10^{-4} M. (b) Temperature maintained to within $\pm 0.1^\circ$. (c) The second-order rate constants was obtained as the slope of the line on plotting k_{obsd} vs [Epoxide].

TABLE III

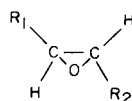
Activation Parameters for the N₇-Hydroxyalkylation of Guanosine by Epoxides in Glacial Acetic Acid

Epoxide	ΔH_a (a) kcal mole ⁻¹	ΔS_a (b) cal mole ⁻¹ deg ⁻¹	ΔG_a (c) kcal mole ⁻¹
<i>trans</i> -2,3-Epoxybutane	15.7	-18.3	21.3
3-Chloro-1,2-Epoxypropane	15.8	-17.2	21.0
1,2-Epoxypropane	13.4	-23.6	20.6

(a) Calculated from slope of $\log k$ vs $1/T$. (b) Calculated from activation energies and second-order rate constants. (c) Calculated at 30.0° .

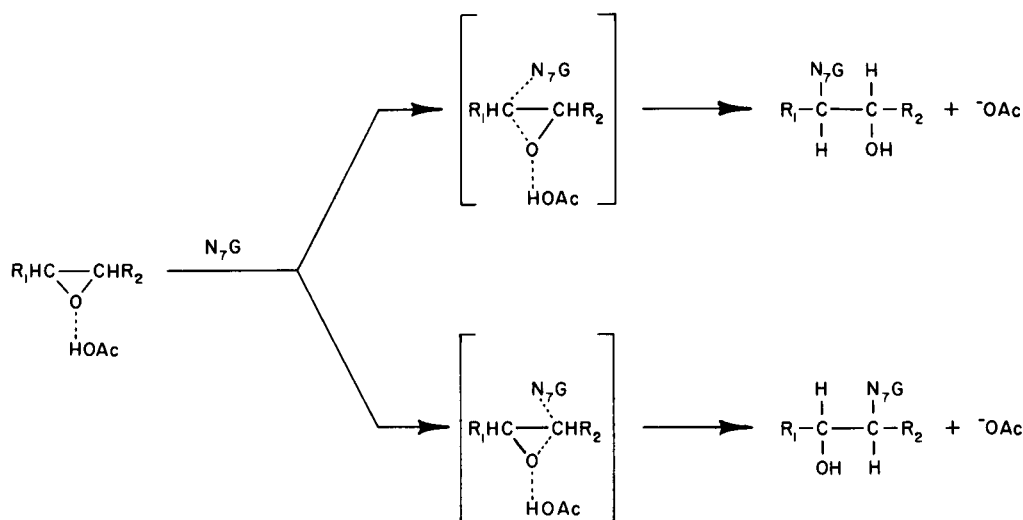
TABLE IV

Observed vs Calculated Ratios of Rate Constants for the N₇-Hydroxyalkylation of Guanosine by Epoxides at 30°



Epoxide	R ₁	R ₂	k (a) M ⁻¹ min ⁻¹ × 10 ³	(k/k ₁) (b)	(k/k ₁) _{cal} (c)
<i>trans</i> -2,3-Epoxybutane	CH ₃	CH ₃	2.53	0.34	0.31
3-Chloro-1,2-Epoxypropane	H	CH ₂ Cl	3.96	0.53	0.53
1,2-Epoxypropane	H	CH ₃	7.50	1.00	1.00

(a) From Table II at 30° . (b) Calculated with respect to 1,2-epoxypropane, k_1 . (c) Calculated from $\log k_1/k_2 = \Delta\Delta G_a/2.303RT$ using ΔG_a values from Table III.



Scheme 1

carbons of *trans*-2,3-epoxybutane are more sterically crowded than the methylene carbons of 1,2-epoxypropane and 3-chloro-1,2-epoxypropane. The complex interplay of steric and electronic factors are evident in these reactions. Whereas, 1,2-epoxypropane, the most reactive of these epoxides at 30°, has the lowest enthalpy of activation, it has the most negative entropy of activation.

The activation parameters in Table III indicate that the reactions of guanosine with *trans*-2,3-epoxybutane and 3-chloro-1,2-epoxypropane require higher enthalpies of activation than does the reaction of 1,2-epoxypropane. This is consistent with the concept that reactions requiring more energetic collisions are less influenced by the orientation requirements of a transition state than are reactions requiring less energetic collisions. Apparently, *trans*-1,2-epoxybutane has a higher enthalpy of activation than does 1,2-epoxypropane because of the steric interaction between guanosine and the methyl and hydrogen substituent on the carbon of the epoxide ring; this would prevent bond formation between N₇ and the epoxide carbon in the transition state complex. The higher enthalpy of activation of 3-chloro-1,2-epoxypropane than for 1,2-epoxypropane is attributed to effects which cause a less strong interaction between this epoxide and glacial acetic acid.

The findings that 1,2-epoxypropane reacts with guanosine about three times faster than does *trans*-2,3-epoxybutane and about two times faster than 3-chloro-1,2-epoxypropane is evidence against a fully protonated epoxide intermediate in these reactions. If a fully protonated epoxide intermediate were involved in these reactions, *trans*-2,3-epoxybutane would be expected to react faster than 1,2-epoxypropane and the reaction of

1,2-epoxypropane would be expected to be much faster than the reaction of 3-chloro-1,2-epoxypropane with guanosine. It has been reported that the acid-catalyzed hydrolysis of *trans*-2,3-epoxybutane is more than twice as fast as that of 1,2-epoxypropane and that the reaction of the latter is more than seventy times faster than the reaction of 3-chloro-1,2-epoxypropane (5).

In summary, the mechanism for the N₇-hydroxyalkylation of guanosine by epoxides in glacial acetic acid may be visualized as follows: the N₇ of guanosine approaches a ring carbon of the glacial acetic acid epoxide complex, bond formation begins; simultaneously, that epoxide carbon loses electrons to the oxygen of the epoxide, and bond breaking begins; as the electron density on the epoxide oxygen increases, a stronger interaction between the epoxide oxygen and the acidic hydrogen of the complexed acetic acid occurs. The reaction is complete when the N₇ of guanosine has approached sufficiently close for covalent bond formation with the carbon of the epoxide. The approach of guanosine is determined by the steric bulk and nucleophilicity of N₇, the steric and electronic properties of the substituent on the epoxide ring and the activation of the epoxides by complexation with acetic acid. The reaction of N₇ at the least substituted carbons of 1,2-epoxypropane and 3-chloro-1,2-epoxypropane is explained in terms of steric interactions. The slower rate of the reaction of the latter with guanosine compared to that of 1,2-epoxypropane is explained in terms of the initial electron densities on the epoxide oxygens; the electron density on the epoxide oxygen of 1,2-epoxypropane would be greater than on the oxygen of 3-chloro-1,2-epoxypropane because of the differences in electron donating abilities of the methyl and chloromethyl groups.

The higher the electron density on the epoxide ring oxygen, the stronger is the interaction with the glacial acetic acid and the more reactive the epoxide; the more strongly complexed the epoxide, the more ordered the transition state complex for the reaction. The formation of transition states involving less strongly complexed epoxides would require higher enthalpies of activation; these transition states would require less order and have less negative entropies of activation.

The determination of product distributions and kinetics of additional guanosine-epoxide reactions run in solvents of varying polarity and over a wide temperature range would serve to provide a more fundamental understanding of these reactions. It is likely that the previously described pmr method for determining product distributions, (1) and modification of the spectrophotometric methods described in this report will be useful in future investigations of these reactions. Studies of the reactions of other variously substituted epoxides, as well as other types of alkylating agents with the nucleotides and nucleosides of DNA and RNA and other biologically important compounds containing purine and imino-type nitrogens, are needed to assess the scope of N-alkylation reactions in carboxylic acids.

EXPERIMENTAL

The guanosine and epoxides were obtained from commercial sources. On the basis of elemental and pmr analysis, the guanosine appeared to consist of approximately two moles of water per mole of anhydrous guanosine. The purity of the guanosine was considered in the preparation of the guanosine solutions; the epoxides were freshly distilled prior to their use in kinetic runs. Analyzed standards of II, III and (IVa and IVb) which were prepared in a previous study (1) were used to prepare solutions for spectral comparison to those resulting from complete reaction of the guanosine with epoxides.

General Procedure for Kinetic Measurements.

Stock solutions of guanosine ($3.13 \times 10^{-3} M$) in glacial acetic acid and of the epoxides ($2.000 M$) in glacial acetic acid were prepared. The guanosine stock solution was prepared by dissolving 0.1000 g. of guanosine dihydrate in sufficient glacial acetic acid to give a volume of 100 ml. To each of a series of 25 ml. volumetric flasks was added 2.50 ml. of guanosine stock solution. One of the 25 ml. volumetric flasks containing 2.50 ml. of guanosine stock solution was diluted with glacial acetic acid to give a $3.13 \times 10^{-4} M$ guanosine standard. To the other flasks varying amounts of epoxide stock solutions were added and diluted to 25 ml. with glacial acetic acid. Stock solutions and glacial acetic acid were kept in a temperature bath maintained at $\pm 0.1^\circ$. Absorbance

measurements were taken on aliquots of the guanosine-epoxide solutions as the reactions occurred. Infinity absorbance measurements were taken after ten half-life periods and compared to the absorbance measurements obtained on standard solutions of the 7-hydroxyalkylguanosinium acetates. The first-order dependence of the guanosine in these reactions was determined by varying the guanosine concentrations and maintaining the epoxide concentrations.

Absorbance changes were measured with a Beckman Acta V spectrophotometer. The N₇-hydroxyalkylation reactions of guanosine by epoxides were carried out in a constant temperature bath maintained at $\pm 0.1^\circ$. Reactions were followed spectrophotometrically by measuring the differences in absorption at 299 nm between guanosine reference solutions and solutions containing guanosine and epoxide. Data were treated in the standard manner for pseudo-first order reactions using the graphic form of the equation

$$\ln(A_\infty^{299} - A_t^{299}) / (A_\infty^{299} - A_0^{299}) = kt$$

where k is the first-order rate constant in reciprocal time units and A_0^{299} , A_∞^{299} and A_t^{299} are differences in absorption between the guanosine reference standards and the reaction solutions at times zero, infinity and time "t", respectively. Second-order rate constants were obtained as the slopes of the lines obtained from plotting the first-order rate constants vs epoxide concentrations (3). Enthalpies of activation were derived from the slopes of plotting $\log k$ vs $1/T$. Entropies of activation were calculated

from the equation, $k = \frac{RT}{N_h} e^{\Delta S/R} e^{-\Delta H_a/RT}$, where k is the

second-order rate constant at temperature T , ΔH_a the enthalpy of activation, ΔS_a the entropy of activation, $R/N = 1.3805 \times 10^{-16}$ erg deg⁻¹ and $h = 6.624 \times 10^{-27}$ erg sec. Free energies of activation were calculated from the enthalpies and entropies of activation. Ratios of rate constants were calculated from $\log k_1/k_2 = \Delta\Delta G_a/2.303RT$.

Acknowledgements.

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