Substituent-Induced Effects on the Stability of Benzylated Guanosines: Model Systems for the Factors Influencing the Stability of Carcinogen-Modified Nucleic Acids

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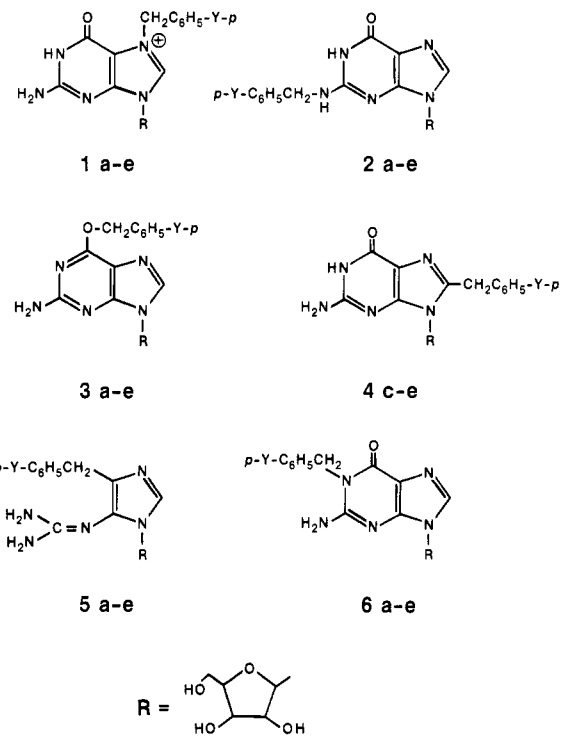
Received August 16, 1983

The preparation and properties of the series of nucleosides 7-(*p*-Y-benzyl)guanosine (**1a–e**), N^2-(*p*-Y-benzyl)guanosine (**2a,b,d**), O^6-(*p*-Y-benzyl)guanosine (**3a–e**), 8-(*p*-Y-benzyl)guanosine (**4c,d**), 1-(*p*-Y-benzyl)guanosine (**6a,b,d**), and 4-(*p*-Y-benzyl)-5-guanidino-1-β-D-ribofuranosylimidazole (**5a,b**) (Y: **a**, O_2N; **b**, Cl; **c**, H; **d**, Me; **e**, MeO) are described. Of these only the 7-substituted guanosines **1a–e** and O^6-substituted guanosines **3a–e** show instability dependent on the nature of the attached para substituent. Rates of imidazole ring opening for the 7-substituted products at pH 9.9 (40 °C) increase in the order **3e** ≃ **3d** < **3c** < **3b** < **3a**, and the relative rates are correlated with σ^n (ρ = +0.80). Rates of acid-catalyzed depurination for the 7-substituted products in 1 N HCl (50 °C) vary little but are similarly correlated with σ^n (ρ = +0.09). The O^6-substituted products **3a–e** are shown to undergo acid-catalyzed solvolysis with cleavage of the O^6-aralkyl ether linkage, and rates for this reaction increase in the order **3a** < **3b** < **3c** < **3d** < **3e**. For derivatives **3b–e** the relative rates are correlated with σ^+ (ρ = −6.1). While guanosine is the major product derived from the solvolysis of **3b–e** at pH 3.5 (40 °C), other nucleosides are produced in the solvolysis of **3c–e** under these conditions, and their yields vary as a function of the resonance electron-donating character of the para substituent attached to the parent O^6-substituted nucleoside. These model studies provide insight into factors governing the range of stabilities exhibited by carcinogen–DNA adducts.

Numerous lines of evidence point to carcinogen–DNA interactions as the key event in the initiation of the carcinogenic process. Of the four DNA bases, guanine residues are known to be the most extensively modified by the majority of chemical carcinogens, and the sites of reaction on these residues with carcinogens of one type or another have been shown to be the 7-, 1-, N^2-, 3-, O^6-, 5-, and 8-positions.[1-5] Both the distribution of carcinogen over these sites and the stability of certain of the resulting products vary as a function of the structure and reactivity of the electrophile.[6-10] Since there is enormous structural diversity among chemical carcinogens and since for most of these the ultimately reactive electrophilic form requires metabolic generation, the task of studying the mechanistic details by which all carcinogens react with nucleic acids is extremely challenging. For many carcinogens, the structure of the ultimate carcinogenic form is not known, and where it is known, synthetic routes to sufficient amounts of these derivatives for detailed chemical studies are not always available. Similarly, yields of DNA-modified products are generally quite low, making studies of the various product stabilities equally difficult. As a result, recourse to model studies involving nucleoside reactions with more accessible directly active alkylating or aralkylating electrophiles is required, and with the appropriate choice of model systems, accurate predictions about the outcome of a wide variety of DNA–carcinogen interactions may be made.

Chart I



In this regard our model studies of nucleoside reactions with benzylic electrophiles have proved particularly informative. Through changes in reaction medium[11] or benzyl leaving group[11,12] we have been able to alter sites of benzylation on adenosine and guanosine and thereby gain insight into the factors responsible for the divergent

(1) Lawley, P. D. *Prog. Nucleic Acid Res. Mol. Biol.* **1966**, *5*, 89–131.
(2) Singer, B. *Prog. Nucleic Acid Res. Mol. Biol.* **1975**, *15*, 219–332.
(3) Lawley, P. D. *ACS Monogr.* **1976**, *No. 173*, 83–244.
(4) Miller, E. C.; Miller, J. A. "Accomplishments in Cancer Research 1980"; Fortner, J. G., Rhoads, J. E., Eds.; Lippincott: Philadelphia, 1981; pp 63–98.
(5) Dipple, A.; Moschel, R. C.; Hudgins, W. R. *Drug. Metab. Rev.* **1982**, *13*, 249–268.
(6) Lawley, P. D.; Brookes, P. *Biochem. J.* **1963**, *89*, 127–138.
(7) Singer, B. *Biochemistry* **1972**, *11*, 3939–3947.
(8) Hecht, S. M.; Adams, B. L.; Kozarich, J. W. *J. Org. Chem.* **1976**, *41*, 2303–2311.
(9) Brookes, P.; Dipple, A.; Lawley, P. D. *J. Chem. Soc.* **1968**, 2026–2028.
(10) Moschel, R. C.; Hudgins, W. R.; Dipple, A. *J. Am. Chem. Soc.* **1981**, *103*, 5489–5494.
(11) Moschel, R. C.; Hudgins, W. R.; Dipple, A. *J. Org. Chem.* **1979**, *44*, 3324–3328.
(12) Moschel, R. C.; Hudgins, W. R.; Dipple, A. *J. Org. Chem.* **1980**, *45*, 533–535.

0022-3263/84/1949-0363$01.50/0

Table I. Ultraviolet Absorption Data

compd	formula[a]	acid (0.1 HCl) λ_{max}, nm	acid (0.1 HCl) $10^{-4}\epsilon$	neutral[b] (pH 6.9) λ_{max}, nm	neutral[b] (pH 6.9) $10^{-4}\epsilon$	alkaline (0.1 N NaOH) λ_{max}, nm	alkaline (0.1 N NaOH) $10^{-4}\epsilon$
7-(*p*-nitrobenzyl)guanosine (**1a**)	$C_{17}H_{19}N_6O_7Br\cdot H_2O$	262	1.97	263	1.71	266[d]	1.86
		280 (sh)[c]	1.53	285	1.48		
7-(*p*-chlorobenzyl)guanosine (**1b**)	$C_{17}H_{19}N_5O_5ClI\cdot H_2O$	257	1.17	258	0.870	266[d]	1.10
		280 (sh)	0.725	285	0.695		
7-(*p*-methylbenzyl)guanosine (**1d**)	$C_{18}H_{22}N_5O_5Br\cdot H_2O$	256	1.16	258	0.729	266[d]	0.882
		280 (sh)	0.764	285	0.670		
N^2-(*p*-nitrobenzyl)guanosine (**2a**)	$C_{17}H_{18}N_6O_7$	263	2.00	258	1.95	267 (sh)	1.93
		275 (sh)	1.91	279	1.93	274	2.02
N^2-(*p*-chlorobenzyl)guanosine (**2b**)	$C_{17}H_{18}N_5O_5Cl$	261	1.60	255	1.52	260	1.33
		281 (sh)	0.933	274 (sh)	1.08	270 (sh)	1.28
N^2-(*p*-methylbenzyl)guanosine (**2d**)	$C_{18}H_{21}N_5O_5\cdot H_2O$	259	1.44	253	1.44	257	1.28
		282 (sh)	0.867	274 (sh)	0.994	270 (sh)	1.18
O^6-(*p*-nitrobenzyl)guanosine (**3a**)	$C_{17}H_{18}N_6O_7$	248	1.11[e]	252	1.38	252	1.35
		285	1.68	282	1.80	282	1.84
O^6-(*p*-chlorobenzyl)guanosine (**3b**)	$C_{17}H_{18}N_5O_5Cl$	243	0.768[e]	247	0.986	247	0.913
		288	1.03	282	1.04	282	1.05
O^6-(*p*-methylbenzyl)guanosine (**3d**)	$C_{18}H_{21}N_5O_5$	unstable		247	0.962	248	0.962
				281	1.01	281	1.07
8-(*p*-methylbenzyl)guanosine (**4d**)	$C_{18}H_{21}N_5O_5\cdot 2H_2O$	262	1.76	255	2.02	260	1.72
		275 (sh)	1.37	272 (sh)	1.39		
1-(*p*-methylbenzyl)guanosine (**6d**)	$C_{18}H_{21}N_5O_5\cdot 2H_2O$	260	1.18	256	1.34	257	1.35
		277 (sh)	0.809	270 (sh)	0.973	270 (sh)	0.989

[a] Satisfactory analytical data (±0.3% for C, H, and N) were obtained for all compounds listed in the table. [b] 0.1 M KH_2PO_4-Na_2HPO_4 buffer. [c] An sh denotes shoulder. [d] The data is that for the anion of the imidazole ring-opened form. [e] Spectra recorded within 5 min after preparation of the acid solution.

site selectivity shown by the simple alkylating carcinogens and more complex aralkylating carcinogens in their nucleic acid interactions. More recently, our preliminary studies of how para substituents on the benzylating agent can alter sites of nucleoside substitution have led to discovery of a novel type of guanosine reaction product (i.e., 4-(*p*-Y-benzyl)-5-guanidino-1-β-D-ribofuranosylimidazoles)[10,13,14] and to the discovery of a surprising substituent-induced lability in the case of O^6-(*p*-methoxybenzyl)guanosine.[10] Hence, a more complete survey of the effects of a wider variety of para substituents on sites of benzylation of guanosine and on accompanying product stability seems desirable. Such studies require usable samples of each of the products **1–6a–e** (Chart I). We describe here the preparation and spectroscopic properties of several new guanosine products which completes the indicated series **1–6**. Of these, only the 7-(*p*-Y-benzyl)guanosines **1a–e** and O^6-(*p*-Y-benzyl)guanosines **3a–e** show notable instability dependent on the nature of the attached para substituent, and we describe the results of our observations on the kinetics and products of decomposition of these two types of guanosine derivatives.

Results and Discussion

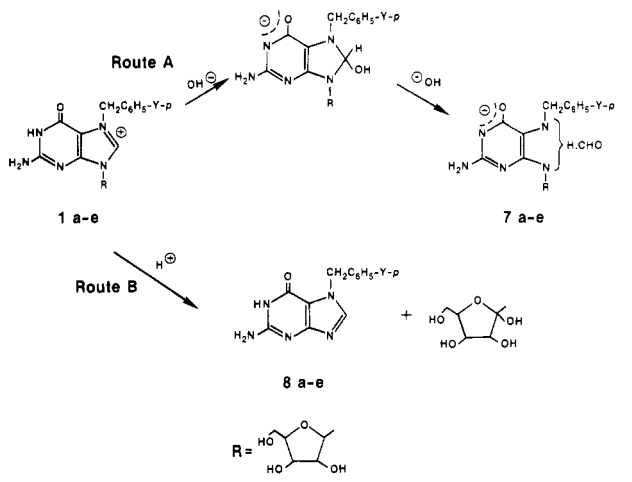
Preparation of Aralkylated Guanosines. Treatment of guanosine with *p*-nitrobenzyl bromide, *p*-chlorobenzyl iodide, or *p*-methylbenzyl bromide in *N,N*-dimethylformamide (DMF) affords the respective 7-substituted nucleosides **1a,b,d** which were purified by column chromatography (Experimental Section). Preparations of **1c**[9] and **1e**[10] were already described. Synthesis of the O^6-substituted derivatives **3b** and **3d** was carried out as described for the O^6-(*p*-methoxybenzyl) analogue (**3e**)[10] by using a modified version of the procedure presented by Gerster and Robins[15] for the preparation of O^6-benzylguanosine (**3c**). O^6-(*p*-Nitrobenzyl)guanosine (**3a**) was isolated from a large-scale reaction between guanosine and *p*-nitrobenzyl bromide in alkaline aqueous solution (Experimental Section). From the same and similar alkaline reactions with other benzylating agents the N^2-substituted products (**2a,b,d**), 8-substituted products (**4c,d**), 1-substituted products (**6a,b,d**), and 4-(*p*-Y-benzyl)-5-guanidino-1-β-D-ribofuranosylimidazole products (**5a,b**) were also isolated. Preparations of the other members of these series were presented earlier.[10,11,13,14,16] UV absorption data for the newly prepared products appear in Table I and in the Experimental Section. Since all but the *p*-nitrobenzyl chromophore are only weakly absorptive in the ultraviolet, the spectroscopic properties for the series of analogously substituted guanosines bearing substituents Y = **b–e** are very similar and consistent with the assigned structures (Table I and ref 9–16). ^1H NMR data (Experimental Section) for all compounds described here are also consistent with their assigned structures and are in agreement with data for analogues described elsewhere.[9–16]

Several aspects of the reaction of guanosine with para-substituted benzylating agents in aqueous media are worthy of mention. First, reactions in water produce a far greater variety of nucleoside products (i.e., **1–6**) than reactions in dipolar aprotic solvents (e.g., DMF) where reaction at the 7-position predominates. Under alkaline aqueous conditions yields for products **2–6** are higher than those obtained under neutral conditions although they are only modest, at best. In comparison with neutral conditions, alkaline reaction conditions cause imidazole ring opening of 7-substituted products (i.e., type **1**) and bring about more extensive reaction at the 1-position, but these same conditions also facilitate more extensive reaction at the N^2-position (i.e., products **2**), at carbon 5 (affording the 4-(*p*-benzyl)-5-guanidino-1-β-D-ribofuranozylimidazole derivatives **5**), and in some cases at carbon 8 (i.e., products of type **4**), thus providing reasonable access to modified guanosines which are not readily available otherwise.

Under identical reaction conditions, the distribution of benzylating agent over the 1-, N^2-, 5-, and 8-sites was found

(13) Moschel, R. C.; Hudgins, W. R.; Dipple, A. *Tetrahedron Lett.* **1981**, *22*, 2427–2430.
(14) Carrell, H. L.; Zacharias, D. E.; Glusker, J. P.; Moschel, R. C.; Hudgins, W. R.; Dipple, A. *Carcinogenesis* **1982**, *3*, 641–645.
(15) Gerster, J. F.; Robins, R. K. *J. Am. Chem. Soc.* **1965**, *87*, 3752–3759.
(16) Philips, K. D.; Horwitz, J. P. *J. Org. Chem.* **1975**, *40*, 1856–1858.

Scheme I



Y: a, O_2N; b, Cl; c, H; d, CH_3; e, CH_3O

to vary as a function of the para substituent on the benzylating agent. For example, the ratio of yields of N^2-/1-substituted products from the reactions of *p*-nitrobenzyl bromide or *p*-chlorobenzyl chloride with guanosine was 0.5. Values for this ratio increase to 1.4, 3.4, and 3.7[10] for reactions of benzyl, *p*-methylbenzyl, and *p*-methoxybenzyl chloride, respectively. With respect to reaction at the 5- and 8-positions of guanosine, only products **5a,b** could be reliably characterized from reactions with *p*-nitrobenzyl bromide or *p*-chlorobenzyl chloride. However, both types of products (i.e., types **4** and **5**) were produced in increasing amounts but in different proportions in reactions with benzyl, *p*-methylbenzyl, and *p*-methoxybenzyl chloride such that the ratio of 5-/8-substituted products was approximately 12, 8, and 8, respectively. Thus, even though yields are low and are based on isolated quantities, the trend established indicates that under these alkaline conditions, electron-donating para substituents on the benzylating agent favor reaction at N^2 as opposed to the 1-position, and these same substituents bring about increased reaction at both carbon 5 and 8 although reaction at carbon 5 always predominates.

Products **5** are quite basic in aqueous solution. Potentiometric titration[17] of **5c–e** at 22 °C shows that each product has two pK_a values at 9.3 and 3.7 irrespective of the nature of the substituent on the respective benzyl moiety. Hunter[18,19] reported a pK_a of 10.5 for 4(5)-guanidinoimidazole, and the titration curve presented indicates a second pK_a near 3.6.[19] It appears that the more elaborately substituted nucleosides **5** are somewhat weaker bases toward monoprotonation than the unsubstituted guanidinoimidazole. Nevertheless, the basicity of products **5** can be used to advantage in simplifying their separation from the mixture of products produced in these alkaline guanosine reactions. Since the 7-substituted products undergo imidazole ring opening under these conditions, subsequent neutralization of the reaction solution affords products **5** as the only positively charged products in the mixture. These can be selectively bound to a cation-exchange resin (e.g., Dowex 50 W-X2) and separated from the neutral nucleosides by elution with $MeOH/H_2O$ mixtures. Subsequent elution of the cation-exchange column with dilute base elutes products **5** in the neutral form, and these can be further purified by crystallization from a small volume of water or by an additional column chromatographic procedure (Experimental Section). Although products **5** are only weakly absorptive in the ultraviolet, their presence can be detected by thin-layer chromatography by using the nitroprusside–ferricyanide–hydroxide spray reagent[20] which produces an orange to brown spot with guanidine and substituted guanidines.

Table II. Observed Rate Constants for the Imidazole Ring Opening of 7-Substituted Guanosines in $MeOH/H_2O$ (3:97) at 40 °C and pH 9.9[a] and for the Depurination of 7-Substituted Guanosines in 1.0 N HCl at 50 °C

	imidazole ring opening		depurination	
7-substituted guanosine	$10k_{obsd}$, min^{-1}	$t_{1/2}$, min	10^2k_{obsd}, min^{-1}	$t_{1/2}$, min
1a	2.53	2.7	1.17	59
1b	0.949	7.3	1.24	56
1c	0.605	11.5	1.07	65
1d	0.484	14.3	0.958	72
1e	0.481	14.4	0.996	70
7-methyl	0.630	11.0	*b*	
7-ethyl	0.133	52.1	*b*	

[a] 0.05 M Na_2CO_3–0.05M $NaHCO_3$ buffer. [b] Not determined.

Alkaline Imidazole Ring Opening and Acid Depurination of 7-Substituted Guanosines. Route A in Scheme I illustrates the salient features of the mechanism of alkaline imidazole ring opening of 7-substituted guanosines **1a–e**. Initial attack by hydroxide ion at carbon 8 of the 7-substituted guanosine zwitterion affords an intermediate 8-hydroxy derivative which undergoes base-catalyzed C-8/N-7 or C-8/N-9 bond cleavage to furnish formylated and/or nonformylated pyrimidine products illustrated by generalized structure **7** (Scheme I). The possible presence or position of an *N*-formyl group in ring-opened products derived from **1a–e** has not yet been ascertained. In contrast to these multistep ring opening reactions, acid-catalyzed depurination of 7-substituted guanosines **1a–e** occurs by simple hydrolytic scission of the N-9/C-1′ bond (route B, Scheme I) to yield the 7-substituted guanines **8a–e**.

Rates of imidazole ring opening for **1a–e** (route A, Scheme I) as well as 7-methyl- and 7-ethylguanosine were determined spectrophotometrically in $MeOH/H_2O$ (3:97) at 40 °C and pH 9.9. The observed rate constants and half-times for these reactions are presented in Table II. These data (Table II) indicate that the rates of imidazole ring opening for the 7-(*p*-methylbenzyl) and 7-(*p*-methoxybenzyl) derivatives **1d,e** are very similar but slower than the observed rates for 7-benzyl, 7-(*p*-chlorobenzyl), and 7-(*p*-nitrobenzyl) derivatives **1c,b,a**, respectively. Thus, rates of imidazole ring opening clearly increase as the para substituent on the 7-benzyl group is made more electron withdrawing. Interestingly, the data of Table II confirm that the rates of ring opening for 7-methyl- and 7-benzylguanosine are very similar[9] while imidazole ring opening for 7-ethylguanosine is 4.7 times slower than that for the methyl analogue.

Rates of acid-catalyzed depurination of **1a–e** (route B, Scheme I) were determined in 1.0 N HCl at 50 °C. The observed rate constants (k_{obsd}) and half-times for these reactions are also presented in Table II. These data indicate a slight increase in depurination rate brought about by electron-withdrawing para substituents, but in the main

(17) Albert, A.; Serjeant, E. P. "Ionization Constants of Acids and Bases"; Methuen: London, 1962.
(18) Hunter, G. *Biochem. J.* **1936**, *30*, 1183–1188.
(19) Hunter, G.; McFarlane, W. D. *Biochem. J.* **1936**, *30*, 1189–1201.
(20) Zweig, G.; Sherma, J., Eds. "Handbook of Chromatography"; CRC Press: Cleveland, OH, 1972, Vol. 2, p 118.

Table III. Observed Rate Constants ($\times 10^2$, min^{-1}) for the Decomposition of O^6-Substituted Guanosines 3b-d in MeOH/H_2O (5:95) at 40 °C as a Function of pH

O^6-substituted guanosine	pH											
	5.65	5.11	4.15	3.22	2.61	2.05	1.70	1.40	1.10	0.70	0.30	0.10
3d	0.105	0.322	1.86	15.4	41.6							
3c				0.162	0.482	1.15	2.07	2.10	2.99	4.72	7.79	22.4
3b					0.231	0.519	0.861	1.12	1.24	1.84	3.86	9.20

all 7-substituted guanosine derivatives **1a–e** undergo depurination at very similar rates. Interestingly, others have shown that rates of acid-catalyzed depurination of 7-methyl- and 7-ethylguanosine are also very similar.[6,7] Thus, hydrolytic cleavage of the base–carbohydrate bond in 7-substituted guanosines is far less sensitive to the nature of the alkyl or aralkyl group attached to the 7-position than the corresponding rates for the alkaline imidazole ring-opening reaction (Table II).

Hammett plots for both the imidazole ring opening and depurination reactions of **1a–e** are shown in Figure 1. The best correlations were obtained for plots of log rate vs. σ^n, the substituent constant derived from reaction series where direct resonance interaction between the substituent and reaction center is minimized by intervening methylene groups.[21] The ρ value for the imidazole ring opening of **1a–e** is 0.80 but only 0.09 for the depurination reaction. This latter value reflects the low sensitivity of this reaction to changes in the para substituent on the benzyl moiety which is reasonable since the benzyl group and reaction center (i.e., C-1′) are widely separated (route B, Scheme I). On the other hand, the sensitivity of the ring-opening reaction (route A, Scheme I) to electron-withdrawing para substituents is more apparent (Figure 1), reflecting the greater proximity of the para substituted benzyl group to the reaction center (i.e., C-8) in this case. The positive sign for the ρ value associated with this ring-opening reaction is characteristic of reaction series whose transition states involve creation of negative charge or a diminution of positive charge. Since attack by hydroxide ion at C-8 of the zwitterionic **1a–e** converts the positively charged imidazole portion of the substituted purine to a neutral 8-hydroxy derivative, it seems reasonable that this first step in the route A sequence (Scheme I) would be most sensitive to the nature of the substituent on the benzyl group and would be accelerated by substituents that withdraw electrons through a polar inductive effect. A similar polar effect may be operative in the imidazole ring opening of

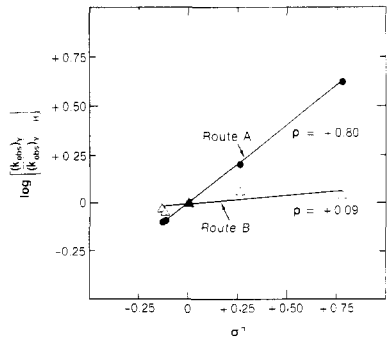


Figure 1. Effect of substituents on imidazole ring opening of **1a–e** in MeOH/H_2O (3:97) at 40 °C and pH 9.9 (●) and on acid depurination of **1a–e** in 1.0 N HCl at 50 °C (△). Values of σ^n are from ref 21.

7-methyl- and 7-ethylguanosine since the ethyl derivative reacts more slowly than the methyl analogue, but the magnitude of the rate difference (Table II) is more substantial than anticipated from the known polarity differences between a methyl and ethyl group.[21] It may be that stabilization by hyperconjugation in addition to inductive electron donation is operative for the 7-ethyl derivative. In any event, it is not possible at present to satisfactorily correlate the rates of ring opening of both the 7-alkyl and 7-aralkyl derivatives **1a–e** with a common measure of substituent polarity[21] although it is clear that electron donation or withdrawal has a marked effect on the rate at which 7-substituted guanosines undergo ring opening.

These same alkyl or aralkyl groups exert a similar influence on the rate at which esters of the analogously substituted acetic acids undergo alkaline hydrolysis. In Figure 2 we illustrate this relationship in a log–log plot of the rate of saponification of the ethyl esters of propionic, acetic, and *p*-(methoxyphenyl)-, *p*-(methylphenyl)-, phenyl-, *p*-(chlorophenyl)-, and *p*-(nitrophenyl)acetic acid in EtOH/H_2O (88:12) at 30 °C[22] against our data for the imidazole ring opening of 7-ethyl- and 7-methylguanosine and **1a–e** (Table II). Although there is some scatter in the plot (Figure 2), the slope is 1, and the actual rate constants for any reaction are correlated by the same line to within a factor of 2. While the number of reaction rates so correlated is far from exhaustive, we believe the relationship (Figure 2) provides a guide to estimating how any group attached to the 7-position of guanine nucleosides will influence the rate at which imidazole ring opening will occur if data are available for the same or a similar group's influence on the relative rate of its carboxylic acid ester hydrolysis in alkali. A large amount of data of this latter type is available for comparison.[21-24]

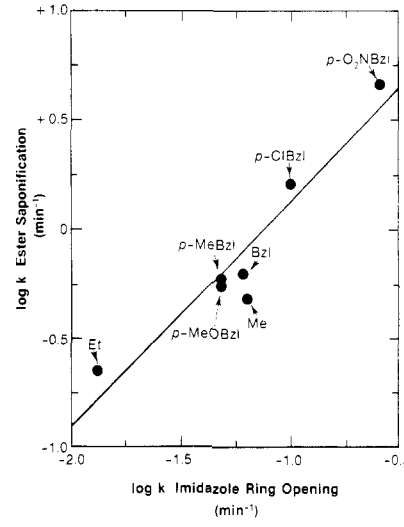


Figure 2. Correlation of ester saponification rates[22] and rates of imidazole ring opening of 7-substituted guanosines.

(21) Hine, J. "Structural Effects on Equilibria in Organic Chemistry"; Wiley: New York, 1975.

(22) Kindler, K. *Justus Liebigs Ann. Chem.* **1927**, *452*, 90–120.

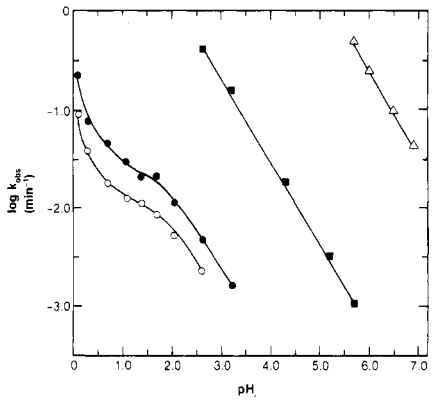


Figure 3. Plot of log k_{obsd} vs. pH for the disappearance of **3e** (△), **3d** (■), **3c** (●), and **3b** (○) in MeOH/H_2O (5:95) at 40 °C.

Acid-Catalyzed Solvolysis of O^6-Substituted Guanosines 3a–e. Rates of disappearance of **3a–e** in MeOH/aqueous buffer (5:95) at 40 °C were determined spectrophotometrically. For derivatives **3b–e** the absorbance at 280 nm decreased with time, indicating cleavage of the O^6-aralkyl ether linkage, and values of k_{obsd} for these reactions were calculated. The magnitude of k_{obsd} was found to be pH dependent for each compound, and values of k_{obsd} as a function of pH are presented in Table III. Plots of log k_{obsd} vs. pH for **3b–d** appear in Figure 3, and, for comparison, our previously reported data for the disappearance of **3e** below pH 7[10] are included. Figure 3 illustrates that the rates of disappearance of **3b–e** show markedly different sensitivities to hydrogen ion concentration. For example, readily measurable rates of disappearance of **3b** are obtained only below pH 3 while similar rates of disappearance of **3c** and **3d** are measurable in the vicinity of pH 3.5 and 5.5, respectively. The *p*-methylbenzyl analogue **3e** decomposes rapidly at all pH values below pH 7. Clearly, electron-donating para substituents greatly enhance the acid lability of the O^6-benzyl carbon bond.

The slope of the log k_{obsd} vs. pH plot for **3d** and **3e** is −1, and a linear region of similar slope is suggested by the data for **3b** and **3c** (Figure 3). However, the log k_{obsd} vs. pH plot for these latter derivatives shows an inflection below pH 3 and a clear indication of a plateau between pH 1–2. The appearance of a plateau in such plots is expected at pH values in the vicinity of the pK_a of a basic reactant when the conjugate acid form is the only kinetically significant species.[25] Estimates of the pK_a of O^6-(*p*-chlorobenzyl)- and O^6-benzylguanosine may be derived from such plots (Figure 3),[25] and these suggest values of 1.7 and 1.9, respectively. These values are near the values reported by Singer[7] for O^6-methyl- (pK_a = 2.4) and O^6-ethylguanosine (pK_a = 2.5). It is reasonable that pK_a values for **3b** and **3c** might be somewhat lower than those for the simpler alkyl analogues, and, in addition, the higher temperatures of our kinetic measurements would be expected to further weaken their basicity.[17] If the singly protonated form of **3b** and **3c** were the only reactive form of these nucleosides, then the plateau in the log k_{obsd} vs. pH plot for these derivatives (Figure 3) would be expected to extend to the lower limit of the pH range we have examined. However, since the magnitude of k_{obsd} for both analogues shows a significant increase at pH <1, it may be that an additional contribution to their rate of disappearance results from a doubly protonated species with a pK_a less than 0.

Table IV. Rate Constants for the Acid-Catalyzed Solvolysis of O^6-Substituted Guanosines 3b–e in MeOH/H_2O (5:95) at 40 °C

O^6-substituted guanosine	k_2, min^{-1}	k_{H^+}, M^{-1} min^{-1}
3e	2.27×10^{3} [a]	2.27×10^{5} [b]
3d	3.14 [a]	3.14×10^{2}
3c	3.15×10^{-2}	2.50
3b	1.79×10^{-2}	0.90

[a] Calculated assuming $K_a = 1 \times 10^{-2}$. [b] Data from ref 10.

O^6-(*p*-Nitrobenzyl)guanosine (**3a**) is the most stable of the five O^6-aralkylated derivatives described here. At 40 °C, changes in the UV absorption spectrum for solutions of **3a** were conveniently measurable only in 1 N HCl or more acidic solutions, and under these conditions, the observed spectral changes differed from those observed for **3b–e**. For example, in 1 N HCl, the absorptivity at 280 nm increased with time and reached a maximum after approximately 7 h of incubation. The resulting spectrum lacked the relative maximum at 248 nm characteristic of the acid spectrum for **3a** (Table I) and instead showed a single maximum at 285 nm. The alkaline spectrum for this same material showed a shoulder at approximately 250 nm and a single maximum at 283 nm although the total absorptivity was significantly reduced relative to that under acid conditions. These spectral properties resemble those reported for O^6-substituted guanines.[26] If the material derived from **3a** is allowed to remain in 1 N HCl, a subsequent decrease in absorptivity at 285 nm ensues, and estimates of the half-time for this second stage of the decomposition are on the order of 40 h. These data are consistent with a preliminary hydrolytic loss of ribose from **3a** to liberate O^6-(*p*-nitrobenzyl)guanine which subsequently undergoes very slow cleavage of the O^6-*p*-nitrobenzyl ether linkage. This mechanism for the decomposition of **3a** differs from that observed with **3b–e** since with these latter derivatives, the rates of O^6-aralkyl ether cleavage always exceed rates of deribosylation (see below).

The log k_{obsd} vs. pH data for **3b** and **3c** over the range of pH 1–3.5 (Figure 3) are consistent with a decomposition mechanism involving preliminary monoprotonation of neutral O^6-substituted guanosine followed by rate-limiting cleavage of the O^6-aralkyl ether linkage as indicated by eq 1 and 2, where K_a is the equilibrium constant for dissociation of the conjugate acid form of the nucleoside and k_2 is a first-order rate constant for its conversion to products. On the basis of such a sequence, the complete expression for k_{obsd} in terms of k_2 and K_a is given by eq 3. Reasonably accurate values of k_2 for the reactions of **3b** and **3c** may be calculated from the respective k_{obsd} data (Table III and Figure 3) and the apparent pK_a for these derivatives obtainable from Figure 3[25] (see above). Al-

$$O^6H^+ \overset{K_a}{\rightleftharpoons} O^6 + H^+ \quad (1)$$

$$O^6H^+ \xrightarrow{k_2} \text{products} \quad (2)$$

$$k_{obsd} = k_2/(1 + K_a/[H^+]) \quad (3)$$

(23) Jaffé, H. H. *Chem. Rev.* **1953**, *53*, 191–261.
(24) Taft, R. W. "Steric Effects in Organic Chemistry"; Newman, M. S., Ed.; Wiley: New York, 1956; Chapter 13, pp 556–675.
(25) Bruice, T. C.; Benkovic, S. "Bioorganic Mechanisms"; W. A. Benjamin: New York, 1966; Vol. 1.
(26) Singer, B. "Handbook of Biochemistry and Molecular Biology", 3rd. ed.; Fasman, G. D., Ed.; CRC Press: Cleveland, OH, 1975; Vol. 1, pp 409–449.

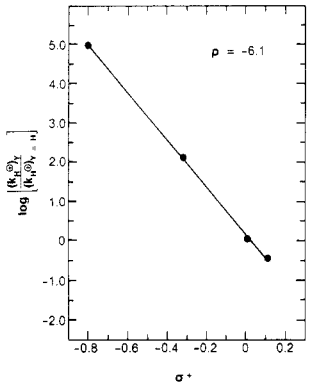


Figure 4. Effect of substituents on k_{H^+} for **3b–e** in MeOH/H_2O (5:95) at 40 °C. Values of σ^+ are from ref 21.

though the mechanism for the reactions of **3d** and **3e** is no doubt the same as that for **3b** and **3c**, the former nucleosides decompose far too rapidly below pH 3 to permit acquisition of a more extensive log k_{obsd} vs. pH profile at lower pH. Consequently, estimates for these apparent pK_a values cannot be obtained graphically. However, if a linear relation between σ^n and pK_a is assumed, then from the apparent pK_a values for O^6-(*p*-chlorobenzyl)- and O^6-benzylguanosine, an apparent pK_a of approximately 2 may be calculated for both **3d** and **3e**. With this value, estimates of k_2 for these two derivatives may be calculated by using eq 3. A tabular summary of such k_2 values appears in Table IV, and these emphasize the profound accelerating effect of electron-donating para substituents on the rate at which the singly protonated forms of these nucleosides decompose to products.

We previously expressed k_{obsd} for the decomposition of **3e** as $k_{obsd} = k_{H^+}[H^+] + k_0$ where k_{H^+} is an observed second order rate constant for its acid catalyzed decomposition and k_0 is the first-order rate constant for the spontaneous or pH-independent disappearance.[10] An accurate value for k_0 was readily determinable for **3e** over the pH range from 7.5 to 13, but, as indicated in Figure 3, analogous rate constants for the reactions of **3b–d** would no doubt be very small compared with that for **3e** over a similar pH range at 40 °C. For example, even at pH 6.9, we failed to detect any change in the UV absorption spectrum for **3a–d** over a 28-h period while the half-time for disappearance of **3e** was 15 min under these conditions. Thus, for **3b–d** below pH 6, expressions for k_{obsd} analogous to that reported for **3e**[10] would clearly be dominated by the $k_{H^+}[H^+]$ term. By equating this term with the right-hand side of eq 3, it is apparent that $k_{H^+} = k_2/(K_a + [H^+])$. Obviously, k_{H^+} is a "constant" only at hydrogen ion concentrations much lower in magnitude than K_a. When this is true, k_{H^+} is a measure of the k_2/K_a ratio, and this can be evaluated from the ratio of $k_{obsd}/[H^+]$ for these reactions over the range in pH where the log k_{obsd} vs. pH plot is fairly linear. This method was used to calculate k_{H^+} for **3e**[10] and is suitable for determination of the k_{H^+} value for the decomposition of **3d**. The k_2/K_a ratio for **3b** and **3c** is obtainable from the data of Table IV and the respective apparent K_a values for these nucleosides. The various k_{H^+} values for the decomposition of **3b–e** are included in Table IV. These provide a more convenient comparison of the substituent-induced sensitivity of these reactions to hydrogen ion concentration, and they allow ready calculation of the relative rates of decomposition for this series of nucleosides at acid pH values well above their apparent pK_a's.

A Hammett plot for this k_{H^+} data is presented in Figure 4. As is apparent, the data are well correlated with σ^+ values for the substituents which measure their ability to stabilize developing positive charge through direct resonance interaction with the benzylic carbon.[21] The ρ value calculated from this plot is −6.1 (Figure 4). The same treatment of the k_2 data (Table IV) shows a similar correlation (ρ = −5.8 for all substituents **b–e**) although in such plots the data for the O^6-(*p*-chlorobenzyl) derivative lies slightly above the correlation line established by the reactions of the O^6-benzyl, *p*-methylbenzyl, and *p*-methoxybenzyl derivatives (ρ = −6.1 for these three derivatives). Numerous studies of the solvolyses of other benzyl derivatives have revealed similar rate deviations with electron-withdrawing para substituents, and the mechanistic implications of these deviations have been widely debated.[23,27-35] Suffice it to say here that either ρ value for our data (i.e., −5.8 or −6.1) suggests a transition state for these reactions where positive charge development at the benzylic carbon atom is quite advanced, and these values are as large or larger than any so far reported for solvolysis of other benzylating agents bearing more traditional "leaving groups".

Table V. Percentage Yield of Guanosine and Products of Types 1, 2, 4, and 6 from the Decomposition of 3b-d in MeOH/H_2O (5:95) at 40 °C and pH 3.5[a]

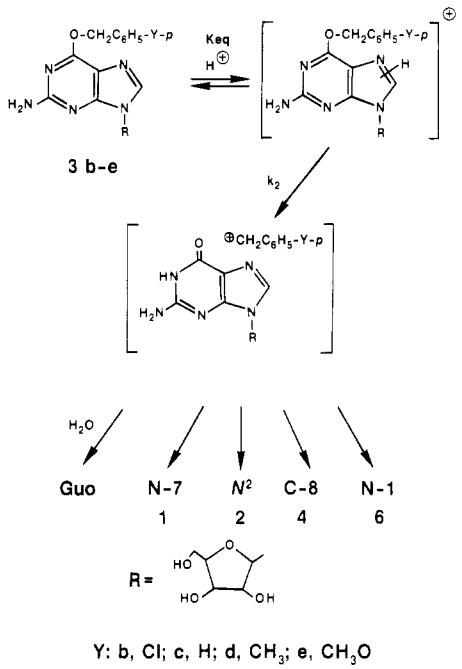
O^6-substituted guanosine	% yield				
	guanosine	1	2	4	6
3e	76.8	1.1	21.5	0.4	0.2
3d	89.4	2.9	3.9	2.6	1.2
3c	97.9	0.4	1.2	0.5	*b*
3b	100				

[a] 0.1 M $NH_4^+HCO_2^-$ buffer. [b] Not detected.

Products of the Acid-Catalyzed Decomposition of 3b–e. We have already demonstrated that the decomposition of O^6-(*p*-methoxybenzyl)guanosine produces guanosine and a variety of other *p*-methoxybenzylated nucleosides (e.g., **1e**, **2e**, and **4–6e**) under both acidic and alkaline aqueous conditions.[10] Similar studies with **3b–d** were necessarily confined to acidic solutions since, as noted above, the rates of decomposition of these latter nucleosides under neutral or alkaline conditions are too slow to be conveniently measured. At pH 3.5, 40 °C, reasonable rates of disappearance for all O^6 derivatives other than **3a,b** were anticipated (Figure 3), and under these conditions only the singly protonated form of the respective starting nucleoside would undergo reaction in all cases. Thus, following incubation of solutions of **3c–e** for four to five half-times or for 2 weeks in the case of **3b**, the resulting mixtures were chromatographically fractionated (Experimental Section), and the product distributions were examined. The percentage yield of guanosine and other identified products resulting from the solvolysis of **3b–e** are reported in Table V. The identity of each of these products was confirmed by comparison of its column

(27) Kochi, J. K.; Hammond, G. S. *J. Am. Chem. Soc.* **1953**, *75*, 3445–3451.
(28) Okamoto, Y.; Brown, H. C. *J. Org. Chem.* **1957**, *22*, 485–494.
(29) Swain, C. G.; Langsdorf, W. P. *J. Am. Chem. Soc.* **1951**, *73*, 2813–2819.
(30) Shiner, V. J. *ACS Monogr.* **1970**, *No. 167*, 90–159.
(31) Streitwieser, A.; Hammond, H. A.; Jagow, R. H.; Williams, R. M.; Jesaitis, R. G.; Chang, C. J.; Wolf, R. *J. Am. Chem. Soc.* **1970**, *92*, 5141–5150.
(32) Koshy, K. M.; Robertson, R. E.; Strachan, W. M. J. *Can. J. Chem.* **1973**, *51*, 2958–2962.
(33) Harris, J. M.; Mount, D. L.; Smith, M. R.; Neal, W. C.; Dukes, M. D.; Raber, D. J. *J. Am. Chem. Soc.* **1978**, *100*, 8147–8156.
(34) Young, P. R.; Jencks, W. P. *J. Am. Chem. Soc.* **1979**, *101*, 3288–3294.
(35) Harris, J. M.; Shafer, S. G.; Moffatt, J. R.; Becker, A. R. *J. Am. Chem. Soc.* **1979**, *101*, 3295–3300.

Scheme II



Y: b, Cl; c, H; d, CH_3; e, CH_3O

chromatographic and UV spectroscopic properties with those of the appropriate reference compound isolated from the larger scale reactions. The data of Table V indicate that although guanosine is the major product of these decomposition reactions in all cases, its yield decreases as the para substituent on the benzene ring of the starting nucleoside is made resonance electron donating, and a concomitant increase in formation of other benzylated nucleoside products is observed. For the case of **3e**, N^2-(*p*-methoxybenzyl)guanosine was clearly the predominant product while for the reaction of **3c** and **3d**, the respective benzyl group was more evenly distributed over the 7-, N^2-, and 8-positions. These product distributions are similar to those observed in the forward reaction of a series of para-substituted nitrosobenzylureas with guanosine, and the details of these studies will be published later.

Scheme II summarizes our conclusions regarding the mechanism for these O^6-substituted guanosine solvolyses. Under acidic conditions, protonation of the neutral nucleoside leads to formation of its conjugate acid from which undergoes rate-determining dissociation to neutral guanosine and the respective cationic para-substituted benzylating agent. The ease of formation of this latter intermediate is clearly substituent dependent (Table IV), being markedly facilitated by electron-donating para substituents. Additionally, the propensity of the resulting benzylating species for reaction with water (to liberate guanosine) or with a "leaving group" (to form products of types **1**, **2**, **4**, and **6**) is also dependent on the nature of the para substituent, and it appears that greater resonance stabilization of the respective benzyl cation enhances both its ability to reassociate and to discriminate among the various available sites on guanosine. For example, in the solvolysis of **3b**, the *p*-chloro substituent would be expected to destabilize the incipient *p*-chlorobenzyl cation, resulting in low rates of its formation from **3b** and more extensive capture of the aralkyl group by water than by a leaving group. Changes in the para substituent from *p*-H through *p*-methoxy bring about faster solvolysis rates for **3c–e** and increasing extents of internal return accompanied by a marked increase in site selectivity.

The properties of the O^6-substituted guanosines examined here define a range of stability for the O^6-aralkyl ether linkage where, at one extreme (i.e., for **3a**), it is of greater stability than the glycosidic bond, and, at the other extreme, it is rapidly hydrolyzed even at neutral pH (i.e., as for **3e**). This spans the range of stability that is of interest for guanine residues substituted at O^6 by carcinogens. Since our studies indicate that increasingly negative σ^+ values are associated with increasing instability of the O^6-substituted derivatives in an aqueous environment, O^6-substituted guanine nucleosides derived from polycyclic aromatic hydrocarbon carcinogens can be expected to exhibit similar instability. For example, Streitweiser et al.[31] have compiled σ^+ values for a number of polycyclic aromatic hydrocarbon residues. Of these, σ^+ values for 1-pyrenyl, 1-anthracenyl, and 1-phenanthryl are −1.0, −0.56, and −0.33, respectively.[31] These values suggest that O^6-substituted guanine nucleosides derived from reaction of the "bay region" dihydrodiol epoxides of benzo[*a*]pyrene, benz[*a*]anthracene, or chrysene should be as unstable as **3d** in neutral or acidic aqueous solution or even more unstable given that the hydrocarbon would be attached through a secondary benzylic carbon rather than a primary carbon. The presence of methyl groups on such hydrocarbon residues, e.g., that derived from 7,12-dimethylbenz[*a*]anthracene, might be expected to impart greater stabilization to a positively charged hydrocarbon intermediate and thus lead to greater instability. Although reactions of hydrocarbon derivatives at the O^6-position of guanine nucleosides have been detected in alkaline and/or largely nonaqueous solutions,[36,37] our data suggest that similar products would not be found under aqueous physiological conditions. Similar predictions may be made about the instability of O^6-substituted products derived from reactive derivatives of safrole[38] or estragole[39] since these latter agents, like the hydrocarbons, should ionize readily and stabilize developing positive charge. With both these types of carcinogens, only the amino groups of the DNA bases have so far been shown to be the major sites of reaction.[38-50]

Since depurination of the 7-substituted guanosines occurs at similar rates irrespective of the nature of the 7-substituent, the fate of such an adduct in a biological system would be largely determined by the substituent's effect on the rate of imidazole ring opening. Our data and

(36) Lyle, T. A.; Royer, R. E.; Daub, G. H.; Vander Jagt, D. L. *Chem.-Biol. Interact.* **1980**, *29*, 197–207.
(37) Osborne, M. R.; Jacobs, S.; Harvey, R. G.; Brookes, P. *Carcinogenesis* **1981**, *2*, 553–558.
(38) Phillips, D. H.; Miller, J. A.; Miller, E. C.; Adams, B. *Cancer Res.* **1981**, *41*, 2664–2671.
(39) Phillips, D. H.; Miller, J. A.; Miller, E. C.; Adams, B. *Cancer Res.* **1981**, *41*, 176–186.
(40) Dipple, A.; Brookes, P.; Mackintosh, D. S.; Rayman, M. P. *Biochemistry* **1971**, *10*, 4323–4330.
(41) Jeffrey, A. M.; Blobstein, S. H.; Weinstein, I. B.; Beland, F. A.; Harvey, R. G.; Kasai, H.; Nakanishi, K. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 2311–2315.
(42) Jeffrey, A. M.; Grezeskowiak, K.; Weinstein, I. B.; Nakanishi, K.; Roller, P.; Harvey, R. G. *Science (Washington, D.C.)* **1979**, *206*, 1309–1311.
(43) Jeffrey, A. M.; Jennette, K. W.; Blobstein, S. H.; Weinstein, I. B.; Beland, F. A.; Harvey, R. G.; Kasai, H.; Miura, I.; Nakanishi, K. *J. Am. Chem. Soc.* **1976** *98*, 5714–5715.
(44) Koreeda, M.; Moore, P. D.; Yagi, H.; Yeh, H. J. C.; Jerina, D. M. *J. Am. Chem. Soc.* **1976**, *98*, 6720–6722.
(45) Meehan, T.; Straub, K.; Calvin, M. *Nature (London)* **1977**, *269*, 725–727.
(46) Osborne, M. R.; Beland, F. A.; Harvey, R. G.; Brookes, P. *Int. J. Cancer* **1976**, *18*, 362–368.
(47) Rayman, M. P.; Dipple, A. *Biochemistry* **1973**, *12*, 1202–1207.
(48) Rayman, M. P.; Dipple, A. *Biochemistry* **1973**, *12*, 1538–1542.
(49) Shapiro, R.; Shiuey, S. J. *J. Org. Chem.* **1976**, *41*, 1597–1600.
(50) Royer, R. E.; Lyle, T. A.; Moy, G. G.; Daub, G. H.; Vander Jagt, D. L. *J. Org. Chem.* **1979**, *44*, 3202–3207.

those of others[6-8] demonstrate that this latter reaction is quite sensitive to the overall electron-withdrawing capacity of the group attached to the 7-position which rationalizes recent findings that imidazole ring-opened products are formed in the nucleic acid reactions of the potent carcinogen aflatoxin B_1[4,51,52] and those of certain cytostatic drugs.[53]

Experimental Section

Ultraviolet absorption spectra were recorded on a Cary 17 spectrophotometer. Kinetic measurements were carried out on a Gilford Model 250 spectrophotometer equipped with a temperature-controlled cell compartment maintained at 40 ± 0.3 °C. ^{1}H NMR spectra were recorded on a Varian XL-100 or Nicolet NT-300 instrument operated in the Fourier transform mode at 100 and 300 MHz, respectively. Samples were dissolved in dimethyl-d_6 sulfoxide with 0.5% tetramethylsilane as an internal standard. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

Guanosine was obtained from PL Biochemicals, Inc., Milwaukee, WI. 2-Amino-6-chloropurine riboside, *p*-methylbenzyl and *p*-chlorobenzyl chloride, and *p*-methylbenzyl and *p*-nitrobenzyl bromide were products of Aldrich Chemical Co., Inc., Milwaukee, WI. *p*-Chlorobenzyl iodide was prepared from the chloride by treatment with sodium iodide in acetone, and the resulting iodide was crystallized from methanol. The method of Lawley and Brookes[6] was used to prepare 7-methyl- and 7-ethylguanosine.

7-(*p*-Y-Benzyl)guanosines 1a,b,d. To a suspension of 0.6 g of guanosine in 30 mL of *N,N*-dimethylformamide (DMF) was added 2 g of either *p*-methylbenzyl bromide, *p*-nitrobenzyl bromide, or *p*-chlorobenzyl iodide, and the mixtures were stirred at room temperature for 24 h after homogeneous solution was obtained. Crude product (0.4–0.6 g) was precipitated by the addition of a large excess of ether with stirring. Repeated ether precipitation of the recovered solids dissolved in MeOH was required to remove residual DMF. Portions (0.1–0.2 g) of the resulting solids were dissolved in a minimum volume of Me_2CO–MeOH (4:1) and treated with 2 equiv of concentrated NH_4OH solution. A precipitate formed on cooling the solutions, and this was filtered, dried, and suspended in 30 mL of MeOH with stirring until no further dissolution of solid was apparent. The suspension was filtered, and the filtrate was loaded on a 2.8 × 71 cm Sephadex LH-20 column and eluted with methanol (flow rate 1 mL/min). UV absorption was continuously monitored at 254 nm, and fractions (10 mL) were collected. Under these chromatographic conditions 7-(*p*-nitrobenzyl)guanosine hydrobromide (**1a**) elutes in fractions 28–33, 7-(*p*-chlorobenzyl)guanosine hydroiodide (**1b**) elutes in fractions 27–30, and 7-(*p*-methylbenzyl)guanosine hydrobromide (**1d**) elutes in fractions 24–27. UV absorption data for **1a,b,d** appear in Table I. **1a**: NMR δ 3.70 (m, 2, H-5′), 4.05 (m, 1, H-4′), 4.18 (m, 1, H-3′), 4.45 (m, 1, H-2′), 5.13 (t, 1, OH-5′, exchanges with D_2O), 5.33 (d, 1, OH-3′, exchanges with D_2O), 5.71 (d, 1, OH-2′, exchanges with D_2O), 5.80 (s, 2, $ArCH_2$), 5.89 (d, 1, H-1′), 7.35 (br s, 2, NH_2, exchange with D_2O), 8.01 (q, 4, Ar), 9.64 (s, 1, H-8), 11.72 (br s, 1, 1-NH, exchanges with D_2O). **1b**: NMR δ 3.69 (m, 2, H-5′), 4.04 (m, 1, H-4′), 4.16 (m, 1, H-3′), 4.43 (m, 1, H-2′), 5.13 (t, 1, OH-5′, exchanges with D_2O), 5.33 (d, 1, OH-3′, exchanges with D_2O), 5.63 (s, 2, $ArCH_2$), 5.69 (d, 1, OH-2′, exchanges with D_2O), 5.88 (d, 1, H-1′), 7.29 (br s, 2, NH_2, exchange with D_2O), 7.51 (q, 4, Ar), 9.59 (s, 1, H-8), 11.69 (br s, 1, 1-NH, exchanges with D_2O). **1d**: NMR δ 2.30 (s, 3, $ArCH_3$), 3.70 (m, 2, H-5′), 4.04 (m, 1, H-4′), 4.17 (m, 1, H-3′), 4.46 (m, 1, H-2′), 5.18 (t, 1, OH-5′, exchanges with D_2O), 5.33 (d, 1, OH-3′, exchanges with D_2O), 5.62 (s, 2, $ArCH_2$), 5.70 (d, 1, OH-2′, exchanges with D_2O), 5.89 (d, 1, H-1′), 7.35 (q, 4, Ar), 7.38 (br s, 2, NH_2, exchange with D_2O), 9.67 (s, 1, H-8), 11.96 (br s, 1, 1-NH, exchanges with D_2O).

O^6-(*p*-Y-Benzyl)guanosines 3b,d. These products were prepared through reactions of either sodium *p*-chlorobenzylate or sodium *p*-methylbenzylate (0.8 g) with 2-amino-6-chloropurine riboside (0.25 g) in 5 mL of the respective alcohol at 100 °C for 25 min essentially as described for the preparation of **3c** by Gerster and Robins.[15] Crude products were recovered by pouring the reaction solutions into a large excess of ether. The recovered precipitates were dissolved in 40 mL of MeOH/H_2O/NH_4OH (5:5:0.3) and were loaded on a 2.8 × 71 cm Sephadex LH-20 column. The column was eluted with the same solvent (flow rate 1 mL/min), and fractions (10 mL) were collected. UV absorption was continuously monitored at 254 nm. O^6-(*p*-Chlorobenzyl)guanosine (**3b**) eluted in fractions 118–146, and O^6-(*p*-methylbenzyl)guanosine (**3d**) eluted in fractions 97–113. The pooled fractions were evaporated to dryness, and the recovered solids were crystallized from MeOH to afford **3b** and **3d** in yields of 45% and 64%, respectively. UV data for these products appear in Table I. **3b**: NMR δ 3.59 (m, 2, H-5′), 3.90 (m, 1, H-4′), 4.12 (m, 1, H-3′), 4.49 (m, 1, H-2′), 5.11 (t, 1, OH-5′, exchanges with D_2O), 5.15 (d, 1, OH-3′, exchanges with D_2O), 5.41 (d, 1, OH-2′, exchanges with D_2O), 5.54 (s, 2, $ArCH_2$), 5.82 (d, 1, H-1′), 6.50 (s, 2, NH_2, exchange with D_2O), 7.53 (q, 4, Ar), 8.15 (s, 1, H-8). **3d**: NMR δ 2.30 (s, 3, $ArCH_3$), 3.60 (m, 2, H-5′), 3.91 (m, 1, H-4′), 4.12 (m, 1, H-3′), 4.48 (m, 1, H-2′), 5.09 (t, 1, OH-5′, exchanges with D_2O), 5.13 (d, 1, OH-3′, exchanges with D_2O) 5.40 (d, 1, OH-2′, exchanges with D_2O), 5.47 (s, 2, $ArCH_2$), 5.81 (d, 1, H-1′), 6.49 (s, 2, NH_2, exchange with D_2O), 7.32 (q, 4, Ar), 8.12 (s, 1, H-8).

Aralkylation of Guanosine in Alkaline Aqueous Solution. General Method for the Preparation and Isolation of the Product Nucleosides. Guanosine (5 g) in 200 mL of H_2O containing 9 g of Na_2CO_3 was treated with a 4-fold molar excess of the appropriately substituted aralkyl halide at 50–60 °C with vigorous stirring. Reactions of *p*-nitrobenzyl bromide and *p*-chlorobenzyl chloride were carried out for 72 h while *p*-methylbenzyl chloride reactions were for 24 h. After cooling to room temperature, the alkaline suspensions were twice extracted with an equal volume of $CHCl_3$ to remove unreacted aralkyl halide, *p*-Y-benzyl alcohol, and the O^6-substituted guanosine derivatives **3a,b,d**. The pH of the remaining aqueous phase was adjusted to 5.5 with HCl and stirring until effervescence ceased. The resulting suspensions were filtered, and the filtrates were evaporated to dryness. The dry residue was extracted with 100 mL of MeOH, the suspension was filtered, and the filtrate was again evaporated to dryness. The residue was dissolved in a minimum volume of warm MeOH/H_2O (1:1), the pH was readjusted to neutrality if necessary, and the solution was poured through a 1 × 3 in. column of Dowex 50W-X2 (Bio-Rad Laboratories) resin (NH_4^+ form) to remove the positively charged 4-(*p*-Y-benzyl)-5-guanidino-1-β-D-ribofuranosylimidazole derivatives **5a,b,d**. Elution of the Dowex column with MeOH/H_2O was continued until no UV-absorbing products were detectable in the eluent, and the pooled eluent was evaporated to dryness. Elution of the Dowex column was then carried out with MeOH/H_2O/NH_4OH (5:5:0.3) to elute derivatives **5** in the neutral form. Additional column chromatography on Sephadex LH-20 with MeOH/H_2O/NH_4OH (5:5:0.3) afforded chromatographically homogeneous **5a** (5 mg) and **5b** (38 mg). **5a**: UV λ_{max} (pH 1) 275 nm (contributed by the *p*-nitrobenzyl moiety); NMR δ 3.52 (m, 2, H-5′), 3.76 (s, 2, $ArCH_2$), 3.76 (m, 1, H-4′), 4.04 (m, 1, H-3′), 4.24 (m, 1, H-2′), 5.35 (d, 1, H-1′), 5.80 (br s, 7, 2 NH_2 + 3 OH), 7.58 (s, 1, H-2), 7.80 (q, 4, Ar). **5b**: UV λ_{max} (pH 1) 253, 260, 267, 275 (contributed by the *p*-chlorobenzyl moiety); NMR δ 3.52 (m, 2, H-5′), 3.58 (s, 2, $ArCH_2$), 3.76 (m, 1, H-4′), 4.03 (m, 1, H-3′), 4.22 (m, 1, H-2), 5.35 (d, 1, H-1′), 5.35 (br s, 7, 2 NH_2 + 3 OH), 7.22 (q, 4, Ar), 7.48 (s, 1, H-2).

The residue obtained from the Dowex column elution with MeOH/H_2O (1:1) (see above) was redissolved in a minimum volume of MeOH/H_2O (3:7) and loaded on a 2.8 × 71 cm Sephadex LH-20 column. The column was eluted with the same solvent at 1 mL/min, and fractions (10 mL) were collected. UV absorption was continuously monitored at 254 nm. Unreacted guanosine and imidazole ring-opened 7-substituted derivatives elute in the first 50–60 fractions while products of types **2**, **4**, and **6** elute together generally between fractions 80 and 120. The N^2- and 8-substituted derivatives (**2** and **4**) are readily resolved from the 1-substituted isomer (**6**) by rechromatography of the mixture of compounds on

(51) Essigmann, J. M.; Croy, R. G.; Bennett, R. A.; Wogan, G. N. *Drug. Metab. Rev.* **1982**, *13*, 581–602.
(52) Hertzog, P. J.; Smith, J. R. L.; Garner, R. C. *Carcinogenesis* **1982**, *3*, 723–725.
(53) Chetsanga, C. J.; Polidori, G.; Mainwaring, M. *Cancer Res.* **1982**, *42*, 2616–2621.

a Sephadex LH-20 column with MeOH/H_2O/NH_4OH (3:7:0.3) as the solvent. The position of elution of the 1-substituted product remains essentially unchanged from that observed under neutral solvent conditions while the N^2- and 8-substituted compounds elute together but much earlier than the 1-substituted isomer. The N^2- and 8-substituted products can be resolved from one another on a 0.78 × 30 cm Aminex A-5 column (NH_4^+ form) eluted with 0.05 M $NH_4^+HCO_2^-$ (pH 4.5) in DMF/H_2O (1:10) at 40 °C. The 8-substituted product elutes ahead of the N^2-substituted isomer under these chromatographic conditions.

By use of these isolation methods, the reaction of *p*-nitrobenzyl bromide with guanosine afforded 0.055 g of N^2-(*p*-nitrobenzyl)-guanosine (**2a**) [UV, Table I; NMR δ 3.55 (m, 2, H-5′), 3.86 (m, 1, H-4′), 4.05 (m, 1, H-3′), 4.42 (m, 1, H-2′), 4.68 (d, 2, $ArCH_2$, changes to a singlet on addition of D_2O), 4.95 (t, 1, OH-5′, exchanges with D_2O), 5.16 (d, 1, OH-3′, exchanges with D_2O), 5.38 (d, 1, OH-2′, exchanges with D_2O), 5.70 (d, 1, H-1′), 7.18 (t, 1, N^2H, exchanges with D_2O), 7.95 (q, 4, Ar), 7.97 (s, 1, H-8), 10.95 (br s, 1, 1-NH, exchanges with D_2O)] and 0.105 g of the 1-substituted derivative **6a**: NMR δ 3.58 (m, 2, H-5′), 3.90 (m, 1, H-4′), 4.10 (m, 1, H-3′), 4.56 (m, 1, H-2′), 5.04 (t, 1, OH-5′, exchanges with D_2O), 5.17 (d, 1, OH-3′, exchanges with D_2O), 5.44 (d, 1, OH-2′, exchanges with D_2O), 5.44 (s, 2, $ArCH_2$), 5.77 (d, 1, H-1′), 7.17 (s, 2, NH_2, exchange with D_2O), 7.82 (q, 4, Ar), 8.04 (s, 1, H-8). A sample (0.02 g) of O^6-(*p*-nitrobenzyl)guanosine (**3a**) was recovered from the $CHCl_3$ extract of the alkaline reaction solution (see above). After the extract was evaporated to dryness, the residue was treated with 40 mL of MeOH/H_2O (1:1), filtered, and chromatographed on Sephadex LH-20 as described above for the purification of **3b** and **3d**. The *p*-nitrobenzyl analogue (**3a**) eluted in fractions 98–116.

3a: UV, Table I; NMR δ 3.60 (m, 2, H-5′), 3.92 (m, 1, H-4′), 4.14 (m, 1, H-3′), 4.52 (m, 1, H-2′), 5.12 (t, 1, OH-5′, exchanges with D_2O), 5.18 (d, 1, OH-3′, exchanges with D_2O), 5.44 (d, 1, OH-2′, exchanges with D_2O), 5.70 (s, 2, $ArCH_2$), 5.84 (d, 1, H-1′), 6.56 (s, 2, NH_2, exchange with D_2O), 8.04 (q, 4, Ar), 8.20 (s, 1, H-8).

From the reaction of *p*-chlorobenzyl chloride with guanosine, samples of N^2-(*p*-chlorobenzyl)- and 1-(*p*-chlorobenzyl)guanosine (0.075 and 0.155 g, respectively) were isolated by the above chromatographic procedures. **2b**: UV, Table I; NMR δ 3.55 (m, 2, H-5′), 3.88 (m, 1, H-4′), 4.10 (m, 1, H-3′), 4.50 (m, 1, H-2′), 4.52 (d, 2, $ArCH_2$, changes to a singlet on addition of D_2O), 4.93 (t, 1, OH-5′, exchanges with D_2O), 5.14 (d, 1, OH-3′, exchanges with D_2O), 5.36 (d, 1, OH-2′, exchanges with D_2O), 5.72 (d, 1, H-1′), 6.99 (t, 1, N^2H, exchanges with D_2O), 7.42 (m, 4, Ar), 7.96 (s, 1, H-8), 10.75 (br s, 1-NH, exchanges with D_2O). **6b**: NMR δ 3.57 (m, 2, H-5′), 3.87 (m, 1, H-4′), 4.09 (m, 1, H-3′), 4.42 (m, 1, H-2′), 5.03 (t, 1, OH-5′, exchanges with D_2O), 5.16 (d, 1, OH-3′, exchanges with D_2O), 5.22 (s, 2, $ArCH_2$), 5.44 (d, 1, OH-2′, exchanges with D_2O), 7.07 (s, 2, NH_2, exchange with D_2O), 7.29 (q, 4, Ar), 7.99 (s, 1, H-8). Sufficient amounts of 8-substituted guanosines for spectroscopic analysis could not be isolated from the alkaline guanosine reactions with either *p*-nitrobenzyl bromide or *p*-chlorobenzyl chloride. However, a trace amount (0.004 g) of 8-benzylguanosine (**4c**) was isolated from the reaction of benzyl chloride with guanosine in Na_2CO_3 solution along with the N^2-(0.20 g) and 1-benzylguanosine (0.14 g) whose spectroscopic properties have already been described. **4c**: NMR δ 3.59 (m, 2, H-5′), 3.86 (m, 1, H-4′), 4.08 (m, 1, H-3′), 4.15 (s, 2, $ArCH_2$), 4.67 (m, 1, H-2′), 5.10 (d, 1, OH-3′, exchanges with D_2O), 5.18 (t, 1, OH-5′, exchanges with D_2O), 5.28 (d, 1, OH-2′, exchanges with D_2O), 5.70 (d, 1, H-1′), 6.34 (s, 2, NH_2, exchange with D_2O), 7.26 (m, 5, Ar), 10.20 (br s, 1, 1-NH, exchanges with D_2O).

The reaction of guanosine with *p*-methylbenzyl chloride in Na_2CO_3 afforded 0.203 g of **2d**, 0.060 g of **6d**, and 0.025 g of **4d**. UV data for these materials appear in Table I. **2d**: NMR δ 2.28 (s, 3, $ArCH_3$), 3.55 (m, 2, H-5′), 3.88 (m, 1, H-4′), 4.11 (m, 1, H-3′), 4.46 (m, 1, H-2′), 4.46 (d, 2, $ArCH_2$, changes to a singlet on addition of D_2O), 4.95 (t, 1, OH-5′, exchanges with D_2O), 5.16 (d, 1, OH-3′, exchanges with D_2O), 5.38 (d, 1, OH-2′, exchanges with D_2O), 5.72 (d, 1, H-1′), 6.89 (t, 1, N^2H, exchanges with D_2O), 7.23 (q, 4, Ar), 7.95 (s, 1, H-8), 10.62 (br s, 1, 1-NH, exchanges with D_2O). **4d**: NMR δ 2.26 (s, 3, $ArCH_3$), 3.59 (m, 2, H-5′), 3.83 (m, 1, H-4′), 4.10 (m, 1, H-3′), 4.10 (s, 2, $ArCH_2$), 4.67 (m, 2, H-2′), 4.97 (d, 1, OH-3′, exchanges with D_2O), 5.10 (t, 1, OH-5′, exchanges with D_2O), 5.17 (d, 1, OH-2′, exchanges with D_2O), 5.67 (d, 1, H-1′), 6.22 (s, 2, NH_2, exchange with D_2O), 7.10 (s, 4, Ar), 10.40 (br s, 1, 1-NH, exchanges with D_2O). **6d**: NMR δ 2.24 (s, 3, $ArCH_3$), 3.56 (m, 2, H-5′), 3.87 (m, 1, H-4′), 4.10 (m, 1, H-3′), 4.43 (m, 1, H-2′), 5.02 (t, 1, OH-5′, exchanges with D_2O), 5.13 (d, 1, OH-3′, exchanges with D_2O), 5.20 (s, 2, $ArCH_2$), 5.41 (d, 1, OH-2′, exchanges with D_2O), 5.74 (d, 1, H-1′), 7.01 (s, 2, NH_2, exchange with D_2O), 7.12 (s, 4, Ar), 8.00 (s, 1, H-8).

Kinetics for the Imidazole Ring Opening of 7-Substituted Guanosines and for the Acid-Catalyzed Depurination of 1a–e. Aliquots of individual stock solutions of **1a–e** as well as 7-methyl- and 7-ethylguanosine (1 mg/mL) in MeOH/H_2O (3:7) were diluted (1:10) in 0.05 M $NaHCO_3$–0.05 M Na_2CO_3 buffer (pH 9.9, 40 °C). Rates of imidazole ring opening were determined spectrophotometrically by monitoring the time-dependent increase in absorbance of these solutions at 266 nm. Observed first-order rate constants (k_{obsd}) were determined from the slopes of ln ($OD_\infty - OD_t$) vs. time plots and are the average of a least duplicate determinations. For the acid-catalyzed depurination of **1a–e**, individual stock solutions (1 mg/mL) in 1.0 N HCl were incubated at 50 °C. At various times, aliquots were removed and diluted (1:20) with 0.15 M phosphate buffer (pH 7.0) containing 25% EtOH. The optical density for the resulting solutions was determined at 260 nm, and values of k_{obsd} were determined from plots of ln ($OD_t - OD_\infty$) vs. time for duplicate determinations.

Kinetics for the Hydrolysis of O^6-(*p*-Y-Benzyl)guanosines 3a–e and the Resulting Product Distributions at pH 3.5. Rates of disappearance of products **3** (1×10^{-4} M) in MeOH/aqueous buffer (5:95) at the indicated pH (Table III and Figure 3) were determined from the time-dependent decrease in absorbance of these solutions at 280 nm. Values for k_{obsd} were determined from the slopes of ln ($OD_t - OD_\infty$) vs. time plots for at least duplicate determinations. Product distributions were measured after solutions of **3c–e** (1.2×10^{-3} M) were allowed to incubate in MeOH/aqueous buffer (5:95, pH 3.5, 40 °C) for 4–5 half-times. Suspensions of **3b** were incubated for 2 weeks with continuous stirring. Product separations were carried out by loading aliquots of these resulting solutions (2 mL) on a 0.78 × 30 cm Aminex A-5 column which was initially eluted with 1 M $NH_4^+HCO_2^-$ (pH 4.5, 40 °C) at 0.5 mL/min. Ultraviolet absorption was continuously monitored at 254 nm, and fractions (1.0 mL) were collected. Guanosine eluted in fractions 25–32. After 100 mL of this solvent has passed through the column, elution was carried out with a variety of subtly different soliutions of $NH_4^+HCO_2^-$ (in the range of 1–1.5 M) in DMF/H_2O mixtures (in the range of 1:9 to 15:85) at pH 4.5–4.7 in order to properly resolve all congeneric adducts of types 1–6 from one another. Conditions were developed for each set of marker compounds to separate the neutral adducts of types **4**, **6**, **2**, and **3** in this order from those which are positively charged at pH <7 (i.e., products of types **1** and **5**) and remain bound to the column. When the last of the neutral adducts was eluted, the chromatography solvent was changed to 1 M $NH_4^+HCO_2^-$ in DMF/H_2O (3:7, pH 6, 40 °C). Under these conditions products **5** elute ahead of products **1** except for the case of the parent derivatives **5c** and **1c** which are not resolved under these conditions. For separation of these latter derivatives, the column is eluted with 0.4 M $NH_4^+HCO_2^-$ in CH_3CN/H_2O (4:6, pH 7, 40 °C). Product **1c** elutes ahead of **5c** under these conditions.

These chromatographic systems were employed to separate potential products obtained from the acid-catalyzed decomposition of **3b–d** at pH 3.5. Fractions containing the various products were pooled, and the concentration of each was determined spectrophotometrically.

Acknowledgment. We are indebted to Drs. David Wilbur and Bruce Hilton for obtaining the NMR spectra. Research was sponsored by the National Cancer Institute, DHHS, under Contract No. NO1-CO-23909 with Litton Bionetics, Inc. The contents of this publication do not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Registry No. **1a**, 88158-04-7; **1b**, 88158-05-8; **1c**, 71171-61-4; **1d**, 88158-06-9; **1e**, 88158-07-0; **2a**, 88158-08-1; **2b**, 88158-09-2; **2d**,

79396-24-0; **2e**, 78907-25-2; **3a**, 88158-10-5; **3b**, 88158-11-6; **3c**, 4552-61-8; **3d**, 79384-30-8; **3e**, 78907-22-9; **4c**, 88158-12-7; **4d**, 88158-13-8; **5a**, 88158-14-9; **5b**, 88158-15-0; **6a**, 88158-16-1; **6b**, 88158-17-2; **6d**, 88158-18-3; guanosine, 118-00-3; *p*-methylbenzyl bromide, 104-81-4; *p*-nitrobenzyl bromide, 100-11-8; *p*-chlorobenzyl iodide, 35424-56-7; sodium *p*-chlorobenzylate, 60812-71-7; sodium *p*-methylbenzylate, 71190-75-5; 2-amino-6-chloropurine riboside, 2004-07-1; *p*-chlorobenzyl chloride, 104-83-6; *p*-methylbenzyl chloride, 104-82-5; 7-methylguanosine, 20244-86-4; 7-ethylguanosine, 71369-24-9.

Interaction between Triple Bonds in 1,8-Diethynylnaphthalenes

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Received August 3, 1983

The He(I) photelectron spectra of the alkyl-substituted 1,8-diethynylnaphthalenes **2–5** are reported. The comparison between experimental data and the results of MINDO/3 calculations indicates that the energy split between the molecular orbitals derived from the linear combinations of the acetylenic in plane π orbitals is essentially a function of their spatial separation.

1,8-Diethynylnaphthalene (**1**,[1] Chart I) and its alkyl derivatives **2–5**[2] are interesting model compounds to study the mutual interaction of two acetylenic moieties. As has been shown by X-ray studies,[3,4] the triple bonds of these compounds are fixed by the naphthalene frame and the chain at a distance of about 3 Å. Our recent studies on 1-ethynylnaphthalene, 1,5-diethynylnaphthalene, and **1**[5] demonstrated that He(I) photoelectron (PE) spectroscopy is ideally suited to probe the interaction in **1–5**.

Model Considerations

Before discussing the PE spectra, we will use the concepts of through-space and through-bond interaction[6] to analyze the effects prevailing in **1–5**.

Consider two acetylenic units arranged parallel to each other as shown in the middle of Figure 1. The overlap between the π clouds will cause a different interaction for the "in plane" (π_i) and "out of plane" (π_o) orbitals since the in plane overlap is of $2p_\sigma$–$2p_\sigma$ type and thus considerably larger at distances around 3 Å than the out of plane $2p_\pi$–$2p_\pi$ overlap. The corresponding interaction diagram is shown in Figure 1.

The energy levels of the bonding ($\epsilon(\pi_i^+)$) and antibonding ($\epsilon(\pi_i^-)$) linear combinations of two acetylenic π systems have been obtained by MINDO/3[7] calculations. In Figure 2 these orbital energies as well as their difference, Δ, are plotted against the distance between the two triple bonds. Besides the through-space interaction of the two acetylenic moieties in **1–5**, the through-bond interaction with the σ frame must be taken into account. The influence of the naphthalene fragment has been estimated by comparison of the PE spectra of **1** and 1,5-diethynylnaphthalene.[5] In the following discussion we assume this influence to be similar in all compounds. The influence of the bridges, however, is different. Consider Figure 3. On the left side of this figure is indicated the highest occupied σ orbital of the isolated tetramethylene bridge

Chart I

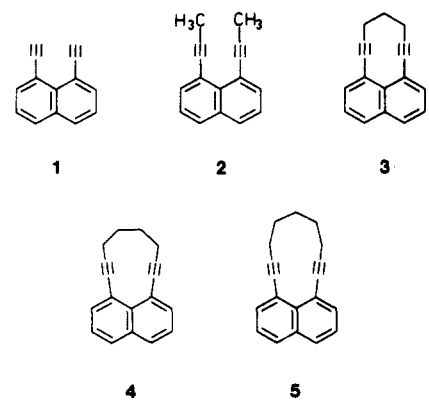

Table I. Ionization Energies of the Compounds 1-5[a]

peak no.	compd 1	2	3	4	5
1	7.88	7.48	7.53	7.50	7.48
2	8.99	8.6	8.5	8.6	8.5
3	9.31	8.7	8.7	8.7	8.6
4	9.49	8.9	8.8	8.9	8.8
5	10.27	9.8	9.78	9.8	9.7
6	10.82	10.0	10.4	9.9	9.8
7	11.15	10.7	10.7	10.6	10.6

[a] All values are in electron volts.

Table II. Orbital Energies (eV) of the Six Highest Occupied Orbitals of 1-5 Using the MINDO/3 Method[a]

irreducible representation	compd 1	2	3	4	5
$3a_2$ (π)	−7.94	−7.83	−7.83	−7.83	−7.83
$4b_2$ (π)	−8.89	−8.89	−8.87	−8.86	−8.86
$3b_2$ (π)	−9.37	−9.10	−9.03	−9.09	−9.10
$12b_1$ (π_i^-)	−9.33	−9.05	−8.51	−8.91	−8.99
$12a_1$ (π_i^+)	−10.44	−9.91	−10.19	−9.83	−9.82
$2a_2$ (π)	−10.63	10.27	−10.40	−10.21	−10.22

[a] The irreducible representations refer to compound **1** (C_{2v}).

(1) Mitchell, R. H.; Sondheimer, F. *Tetrahedron* **1968**, *24*, 1973. Staab, H. A.; Ipaktschi, J.; Nissen, A. *Chem. Ber.* **1971**, *104*, 1182.
(2) Nissen, A.; Staab, H. A. *Chem. Ber.* **1971**, *104*, 1191.
(3) Jungk, A. E. *Chem. Ber.* **1972**, *105*, 1595.
(4) Irngartinger, H.; Jungk, A. E. *Chem. Ber.* **1977**, *110*, 749.
(5) Gleiter, R.; Schäfer, W.; Eckert-Maksič, M. *Chem. Ber.* **1981**, *114*, 2309.
(6) Hoffmann, R.; Imamura, A.; Hehre, W. J. *J. Am. Chem. Soc.* **1968**, *90*, 1499. Hoffmann, R. *Acc. Chem. Res.* **1971**, *4*, 1. Gleiter, R. *Angew. Chem.* **1974**, *86*, 770; *Angew. Chem., Int. Ed. Engl.* **1974** *13*, 696. Heilbronner, E.; Schmelzer, A. *Helv. Chim. Acta* **1975**, *58*, 936. Paddon-Row, M. N. *Acc. Chem. Res.* **1982**, *15*, 245. Martin, H. D.; Mayer, B. *Angew. Chem.* **1983**, *95*, 281; *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 283.
(7) Bingham, R. C.; Dewar, M. J. S.; Lo, D. H. *J. Am. Chem. Soc.* **1975**, *97*, 1285. Bischof, P. *J. Am. Chem. Soc.* **1976**, *98*, 6844.
(8) Koopmans, T. *Physica (Amsterdam)* **1934**, *1*, 104.

0022-3263/84/1949-0372$01.50/0