Selectivity in Nucleoside Alkylation and Aralkylation in Relation to Chemical Carcinogenesis

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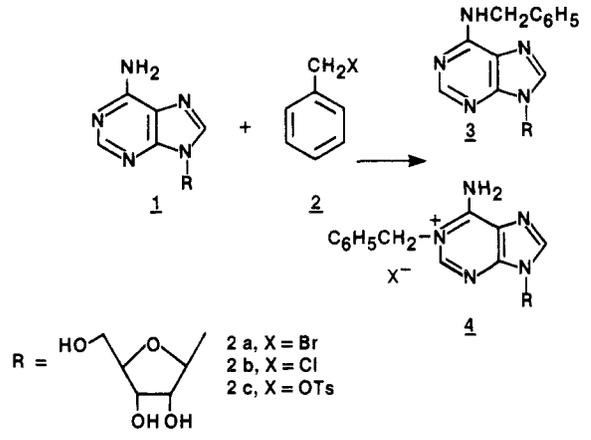
The sites of benzylation of adenosine and guanosine by different benzylating agents in various solvent mixtures were investigated in order to determine those properties of chemical reactivity which lead to reaction on the exocyclic amino and oxo groups. Modification of these sites, as opposed to ring nitrogen sites, can be associated with carcinogenic potential, and was found to be favored by changes in reaction medium or leaving group which would advance carbon leaving group bond breakage. The extents of reaction on the exocyclic amino group of guanosine were greater than for the exocyclic O^6 site but the ratio of reaction at O^6/N^2 increased with increasing leaving group hardness, suggesting that the charge localization or hardness of the reaction center determines the distribution of products over these two sites.

Over the last decade, extensive studies of the interactions of carcinogens and mutagens with DNA have considerably broadened knowledge of alkylation and aralkylation of nucleic acid constituents. In 1966, it seemed that these reactions occurred exclusively on the ring nitrogen atoms of the purine and pyrimidine bases in nucleic acids,[1] but it is now clear that reaction can also occur on the exocyclic amino groups[2] and the exocyclic oxygen atoms[3] of these bases. The distribution of substrate over these sites can vary with solvent,[2,4-7] with pH,[4,6-8] with the structure of the alkyl or aralkyl group concerned,[2-8] and, for a given alkyl group, with the nature of the leaving group.[3-6,8] However, the mechanistic basis for these variations is not clearly defined.[9] Carcinogenic potency is associated with an ability to modify the exocyclic nitrogen and oxygen atoms in nucleic acids however,[2,10] and a clearer understanding of those aspects of chemical reactivity which lead to reaction at these sites might therefore provide much needed insight into the mechanism of the carcinogenic process.

The extensive analyses of product distributions in DNA are not directly relevant to substrate control over sites of reaction because the secondary structure of DNA clearly influences product distribution.[3,11] Studies of nucleoside alkylation provide a more reliable foundation. These can be categorized according to the reaction medium. In dipolar aprotic solvents, high yields of alkylated nucleosides are generally obtained and, in the absence of base, the products generally arise only from reaction at the ring nitrogen atoms of the heterocycles. In aqueous solvents, lower yields of alkylated nucleosides are obtained and a wider spectrum of products, involving the exocyclic nitrogen and oxygen atoms as well as the ring nitrogen atoms, are obtained. Literature reports, primarily from Singer's laboratory,[3-6,8] on product distributions for purine nucleosides in aqueous solution are summarized in Table I.

The questions which we have addressed can be illustrated by consideration of these data. On the one hand, what is the essential difference in chemical reactivity toward nucleosides between dimethyl sulfate, ethyl methanesulfonate, and 7-(bromomethyl)benz[*a*]anthracene which leads the first compound to react almost exclusively on ring nitrogen atoms, the second to react both at ring nitrogens and exocyclic sites, and the third to react predominantly at exocyclic sites? On the other hand, while ethyl methanesulfonate and benzyl bromide are similar in reactivity in that both agents preferentially react at the 7-position of guanosine, what is the difference in chemical reactivity between these two agents which determines that the secondary site of reaction in the first case should be the exocyclic O^6 atom of guanosine while in the second case, it is the exocyclic N^2 atom?

Scheme I



We have investigated these questions through the study of a single type of aralkylation, namely benzylation. Only a limited number of synthetic markers were, therefore, required and furthermore, the chemistry of benzylating agents should fall somewhere between that of the simpler alkylating agents which react predominantly on ring nitrogen atoms and that of 7-(bromomethyl)benz[*a*]anthracene, which primarily reacts on the exocyclic amino groups in aqueous solvents[2] (Table I). The differences in chemical reactivity associated with reaction at ring nitrogen atoms as opposed to exocyclic sites have been investigated through studies of benzylation in different solvent systems, and the differences in chemical reactivity associated with a preference for either the exocyclic O^6 or N^2 positions in guanosine have been investigated through the use of benzylating agents with different leaving groups.

Results

Reactions of adenosine (**1**) or guanosine (**5**) with benzyl bromide (**2a**), benzyl chloride (**2b**) or benzyl tosylate (**2c**) were in buffered aqueous solution or buffered binary solvent mixtures (pH 6.8–7.4) at 25 °C. Since extents of reaction were low, a tritium label was incorporated either into the benzylating agent or the nucleoside. Products

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Table I. Relative Yields of Alkylation or Aralkylation Products at Sites Indicated[a]

substrate	guanosine pH	% N-7	% N^2	% O^6	adenosine pH	% N-1	% N-7	% N^6
ethyl methanesulfonate	9[b]	75		25	7[c]	50	10	25
diethyl sulfate	9[b]	97.5		2.5	7[c]	50	10	25
methyl methanesulfonate	*d*	96		4				
dimethyl sulfate	*d*	100			7[c]	80	16	
benzyl bromide[e]	7	68	29	3	7	43		57
7-(bromomethyl)benz[*a*]anthracene[f]	5.5		100		5.5			100

[a] Yields of alkylation and aralkylation products in aqueous solution as percentages of identified alkylation products. [b] Reference 4. [c] Reference 5. [d] Reference 4, pH not given. [e] Present work. [f] Reference 2.

Table II. Benzylation of Adenosine:[a] Percentage of Adenosine Benzylated at N-1 (4) and N^6 (3) by Various Benzylating Agents ($C_6H_5CH_2X$)

reaction medium[e]	X = Br[b] half-time for solvolysis,[f] min	4	3	X = Cl[c] half-time for solvolysis,[f] min	4	3	X = OTs[d] 4	3
1% DMF in H_2O	nd	0.39	0.51	837[g]	0.08	0.15	0.04	0.23
20% DMF in H_2O	123	0.27	0.10	nd	0.12	0.06	0.04	0.10
40% DMF in H_2O	155	0.26	0.04	nd	0.13	0.02	0.06	0.06
60% DMF in H_2O	190	0.25	0.01	nd	nd	nd	0.10	0.02
20% EtOH in H_2O	115	0.32	0.21	1300	0.12	0.07	0.05	0.17
40% EtOH in H_2O	350	0.39	0.10	4550	0.13	0.05	0.06	0.09
60% EtOH in H_2O	1040	0.32	0.04	nd	nd	nd	0.07	0.07

[a] Results are percentages of adenosine converted to benzylated product after approximately five reaction half-times. Conditions: All reactions were in 0.056 M $NaHCO_3$-H_2CO_3 buffer, pH 6.8–7.4, at 25 °C. [b] $[C_6H_5CH_2Br] = 3.4 \times 10^{-3}$ M (specific radioactivity, 3.02 or 0.275 Ci/mol), [adenosine] = 1.7×10^{-2} and 3.5×10^{-2} M. [c] $[C_6H_5CH_2Cl] = 3.4 \times 10^{-3}$ M, [adenosine-*G*-^3H] = 1.1×10^{-6} M (specific radioactivity, 9 Ci/mmol). [d] $[C_6H_5CH_2OTs] = 3.4 \times 10^{-3}$ M (specific radioactivity, 3.02 or 0.275 Ci/mol), [adenosine] = 1.7×10^{-2} M. [e] All solvents were prepared v/v. [f] Determined titrimetrically as described under Experimental Section. Rates for X = OTs were too rapid to be monitored by this technique. [g] R. E. Robertson and J. M. W. Scott, *J. Chem. Soc.*, 1596 (1961).

could then be separated by column chromatography and quantitated by determination of radioactivity in a liquid scintillation counter. As little as 0.01% nucleoside converted to benzylated product could then be measured.

Benzylation of Adenosine (1). Over a range of solvent composition from 60% ethanol or 60% *N,N*-dimethylformamide (DMF) in water to 99% aqueous solution, only two benzylated adenosines, 1-benzyladenosine (4) and 6-(benzylamino)purine riboside (3), were detected as products of adenosine benzylation (Scheme I). Since it is known that 1-substituted adenosines can rearrange to N^6-substituted derivatives,[12-14] the rearrangement of 4 to 3 under these reaction conditions was investigated. In buffered aqueous solution, 20% EtOH, or 20% DMF, the rate of rearrangement of 4 to 3 could only account for less than 10% of the observed yields of 3 showing that 3 arose primarily through the direct benzylation of adenosine at the exocyclic amino group under these conditions.

The relative yields of 1- and N^6-benzyladenosines obtained with various benzylating agents in various solvent mixtures are summarized in Table II. The results are expressed as percentages of adenosine converted to benzylated product by a 3.4×10^{-3} M solution of benzylating agent. These percentages are independent of adenosine concentration over the range 1.1×10^{-6} to 3.5×10^{-2} M since results were identical when low concentrations of radioactive adenosine were used and when this was diluted with unlabeled adenosine.

It is clear that for any given benzylating agent, changes in solvent composition influence the overall yield of ring-substituted product (4) only slightly while these same changes from 60% EtOH or DMF in H_2O to 99% H_2O dramatically increase the yield of exocyclic substitution from fairly low levels to levels at which this represents the major product (Table II). In all of these experiments, most of the benzylating agent is solvolyzed. The relatively constant yields of 1-benzyladenosine (4) indicate that the rate of reaction at this ring nitrogen is enhanced in more aqueous solvents in roughly the same fashion as the solvolysis rate. In contrast, the increasing yields of 6-(benzylamino)purine ribonucleoside (3) with increasing water content of the solvent indicate that the rate of exocyclic substitution is increased by a greater factor than is the overall solvolysis rate. This suggests that, as the water content of the solvent increases, the reaction increasingly proceeds through a pathway which is facilitated by water and which leads not only to faster solvolysis but also to reaction on the exocyclic amino group of adenosine. Since the total yields of benzyladenosines tend to be higher in $EtOH/H_2O$ mixtures than in DMF/H_2O mixtures, primarily because higher yields of exocyclic substitution are obtained in the ethanolic solvent, this suggests that the electrophilic properties of the protic solvents are of importance in leading to exocyclic substitution.

The effects of the different leaving groups examined are such that the yield of ring-substituted product is always greater for the bromide (**2a**) than for the chloride (**2b**). In turn, greater yields are obtained with the chloride (**2b**) than with the tosylate (**2c**). With respect to the yields from exocyclic substitution (3), a different and more complex relationship emerges. Yields are always lower for the chloride (**2b**) than for the other two leaving groups, bromide and tosylate. However, while exocyclic substitution for the tosylate is similar to or somewhat greater than that for the bromide in 20–60% organic solvent in water, it is considerably less than that for the bromide in the most aqueous solvent. A more consistent picture emerges if the ratio of exocyclic product to ring product

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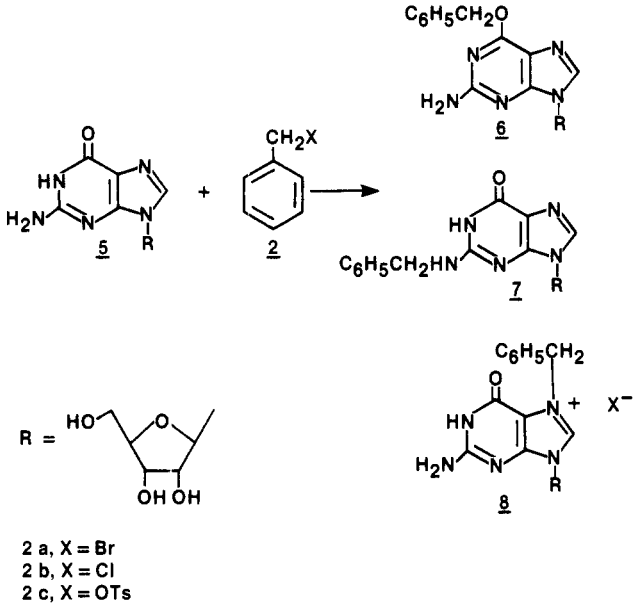
Table III. Benzylation of Guanosine:[a] Percentage of Guanosine Benzylated at N-7 (8), N^2 (7), and O^6 (6) by Various Benzylating Agents ($C_6H_5CH_2X$)

	X = Br[b]			X = Cl[c]			X = OTs[d]		
reaction medium[e]	8	7	6	8	7	6	8	7	6
1% DMF in H_2O	1.61 (0.64)	0.68 (0.27)	0.07 (0.03)[b]	0.72	0.33	0.05	0.09 (0.43)	0.12 (0.57)	0.07 (0.33)[d]
20% EtOH in H_2O	1.03	0.24	trace	1.41	0.22	0.05	0.50	0.45	0.30
40% EtOH in H_2O	1.36	0.09	trace	2.09	0.12	0.04	0.80	0.31	0.27
60% EtOH in H_2O	1.39	0.02	trace	nd	nd	nd	1.09	0.24	0.23

[a] Results are percentages of guanosine converted to benzylated product after approximately five reaction half-times. Conditions: All reactions were in 0.056 M $NaHCO_3$-H_2CO_3 buffer, pH 6.8-7.4, at 25 °C. [b] In all but the most aqueous reaction, $[C_6H_5CH_2Br] = 3.4 \times 10^{-3}$ M (specific radioactivity, 3.02 or 0.275 Ci/mol), [guanosine] = 8.4×10^{-3} M. In the most aqueous solvent, $[C_6H_5CH_2Br] = 8.5 \times 10^{-3}$ M and [guanosine-5′-^3H] = 5×10^{-7} M (specific radioactivity 21 Ci/mmol). The figures in parentheses represent an attempt to compare yields in this reaction with those in the other benzyl bromide reactions. The actual yields have been divided by the ratio of the two bromide concentrations used. [c] $[C_6H_5CH_2Cl] = 1.7 \times 10^{-2}$ M, [guanosine-5′-^3H] = 5×10^{-7} M (specific radioactivity, 21 Ci/mmol). [d] [Guanosine-5′-^3H] = 5×10^{-7} M (specific radioactivity, 21 Ci/mmol). In all but the most aqueous solvent $[C_6H_5CH_2OTs] = 1.6 \times 10^{-2}$ M. In the most aqueous solvent, $[C_6H_5CH_2OTs] = 3.4 \times 10^{-3}$ M. For this reaction the figures in parentheses were derived from the actual yields divided by the ratio of the two tosylate concentrations. [e] All solvents were prepared v/v.

Scheme II



2 a, X = Br
2 b, X = Cl
2 c, X = OTs

3/4 is examined. Under all conditions (Table II), this ratio is greater for the tosylate than for the chloride and bromide with these latter two leaving groups behaving fairly similarly in this respect.

Benzylation of Guanosine (5). In guanosine a choice of exocyclic sites of reaction, the amino group attached to carbon-2 and the oxygen attached to carbon-6, i.e. the N^2 and O^6 position, is available (Scheme II). Attempts were made to monitor reaction at both of these sites for benzyl bromide, chloride, and tosylate. Because extents of reaction varied with these different agents and the limited extents of reaction at the O^6 position made product quantitation difficult, different concentrations of reactants were chosen for some of these studies as indicated in the footnote to Table III.

In these studies, three benzylated guanosines, 7-benzylguanosine (8), *O*^{6}-benzylguanosine (6), and the hitherto undescribed *N*^{2}-benzylguanosine (7), were detected. The structure of 7 was confirmed, following its isolation from a large-scale guanosine–benzyl bromide reaction in ethanol/water (1:4), by elemental analysis, mass spectra, NMR spectra, and hydrolysis to the known *N*^{2}-benzylguanine.

It was found that, as the reaction medium is made increasingly aqueous and reaction, therefore, becomes faster, reaction at the ring nitrogen (N-7) tends to decrease somewhat while total reaction on exocyclic sites (N^2 and O^6) increases (Table III). It is clear that the more aqueous solvents specifically promote reaction on the exocyclic amino group, as observed in the adenosine studies. There may be a similar relationship for reaction at the exocyclic oxygen (O^6) but, in this case, the effect is far less dramatic. In the studies reported herein, 1-benzylguanosine was not detectable in the more aqueous solvents while traces of this product were observed in the 40% and 60% organic solvent mixtures. Since Sullivan and Wong[15] have reported extensive methylation on O^6 accompanied by extensive methylation on N-1 in reactions with diazomethane in methanol/ether mixtures, it is conceivable that O^6-benzylation could arise through one route, perhaps involving a guanosine anion, in the more organic solvents and through a different route (where it is not accompanied by alkylation at N-1) in the more aqueous solvents. Such a phenomenon would be consistent with the data in Table III.

The effects of the different leaving groups on the distribution of benzyl groups over exocyclic vs. ring nitrogen receptor sites are similar to those observed in reactions with adenosine in that the ratio of exocyclic to ring reaction is again greatest for the tosylate, with the ratios for chloride and bromide being somewhat similar. However, when the relative distribution of benzyl over the two exocyclic sites is examined, it is clear that the O^6/N^2 product ratio is always greater for the tosylate than for the chloride, and that this ratio is always greater for the chloride than for the bromide (Table III).

Discussion

The primary objective of these studies was to obtain a clearer understanding of those aspects of chemical reactivity which determine sites of reaction on nucleic acid constituents. One point which clearly emerges is that reactions on ring nitrogen atoms and on exocyclic sites are distinguishable in terms of their relative responses to changes in solvent composition. Thus, while the extent of reaction on ring nitrogen either decreases or remains relatively constant with increasing ionizing power of the solvent, reaction on the exocyclic amino groups increases notably and reaction on the exocyclic oxygen of guanosine also increases (Tables II and III). Since the nucleophilicities of ethanol and water are similar,[16-18] the increases

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in reaction rate and in exocyclic substitution/ring substitution ratios, accompanying increases in the water content of ethanol/water mixtures, can be attributed to the electrophilic pull (anionic solvation)[19] of water on the leaving group. It is clear that product ratios are not simply a function of reaction rate because, although the rates are faster in DMF/water mixtures than in ethanol/water mixtures, exocyclic substitution is greater in the latter system.

Exocyclic substitution is favored by protic solvents with electrophilic properties which can stabilize some degree of bond breakage in the substrate. However, benzyl carbonium ions are presumably not involved in these reactions because the ratio of the two types of exocyclic product in guanosine, O^6/N^2, i.e. **6/7**, varies with the nature of the leaving group, suggesting that the leaving groups are involved in the transition states. This report constitutes the first description of the reaction of any alkylating or aralkylating agent with both exocyclic sites in guanosine and it permits, therefore, the factors which influence reaction at these two sites to be examined. Reaction at the N^2 site is clearly more sensitive to the changes in solvent composition studied herein than is reaction on the O^6 site. However, reaction on this latter site is very sensitive to changes in the leaving group of the substrate. The ratio **6/7** increases in the leaving group sequence $Br^- < Cl^- < OTs^-$, i.e., in a sequence of decreasing polarizability of the atom carrying the developing negative charge or increasing leaving group "hardness".[20] This is also the sequence of increasing carbonium ion character in the transition states for solvolysis of the corresponding arylmethyl derivatives,[21] suggesting that a preference for reaction at O^6 may be associated with an increased charge on the carbon atom at the reaction center.

Previous separate rationalizations of O^6 and N^2 alkylation of guanine residues[2,9,22-27] can now be unified. In light of the data presented here, the most plausible rationalization of both alkylation and aralkylation of nucleosides which emerges is that reaction with exocyclic sites (as opposed to ring nitrogen sites) is favored by changes in substrate structure or reaction medium which tend to advance carbon leaving group bond breakage (i.e. increase S_N1 character in the reaction). Furthermore, when incipient charge is localized on the reaction center (i.e. when the center is "hard"[20]) reaction with exocyclic oxygen (e.g. O^6) is favored. Delocalization of charge, creating a "softer" center, directs reaction to the exocyclic amino groups.

In conclusion, it is interesting to examine these observations with respect to those properties of chemical reactivity which are associated with carcinogenic activity. Since potent carcinogens are found among agents which modify the O^6 site on guanine residues[10] and also among those that modify the N^2 site on guanine residues,[2,11,28-30] then a limited dependence on, or susceptibility to, nucleophilicity would appear to be enough to render a reactive chemical potentially carcinogenic. In terms of the mutation theory of chemical carcinogenesis, this might suggest that those genomic sites which are necessarily modified during the initiation of the carcinogenic process are surrounded (protected) by some highly nucleophilic centers, such that only those reactive chemicals which are not particularly sensitive to nucleophilicity can diffuse past these centers and damage these key genomic sites.

Experimental Section

Adenosine, guanosine, N^6-benzyladenosine, benzyl chloride, benzyl bromide, [5′-^{3}H]guanosine (specific radioactivity: 21 Ci/mmol) and [G-^{3}H]adenosine (specific radioactivity: 9 Ci/mmol) were obtained commercially. [^{3}H]Benzyl bromide (specific radioactivity: 3.02 or 0.275 Ci/mol was synthesized by bromination of [^{3}H]toluene by Dr. G. M. Muschik of this laboratory. [^{3}H]-Benzyl tosylate and unlabeled tosylate were prepared from either labeled or unlabeled benzyl bromide and silver *p*-toluenesulfonate by the method of Emmons and Ferris.[31] Both 1-benzyladenosine and 7-benzylguanosine were prepared by the method of Brookes et al.[13] 1-Benzylguanosine was prepared from the sodium salt of guanosine and 1 equiv of benzyl bromide in DMF and the product was isolated chromatographically. The isolated product was identical with that described by Philips and Horwitz.[32] O^6-Benzylguanosine was prepared by the method of Gerster and Robins.[33] N^2-Benzylguanine was prepared from 2-chloro-6-hydroxypurine and benzylamine by the method of Shapiro et al.[34]

Mass spectra were obtained on a Finnegan 3300 mass spectrometer equipped with a Finnegan 6000 MS data system. Ultraviolet spectra were recorded on a Cary 17 UV spectrophotometer. Nuclear magnetic resonance spectra were measured on a Varian XL-100 spectrometer using $(CD_3)_2SO$ (0.5% Me_4Si) as solvent. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

Solvolysis rates for **2a** and **2b** in various solvents (Table II) were determined by titration (0.01 N NaOH; phenolphthalein endpoint) of the acid liberated in 10-mL aliquots of 5 mM solutions as a function of time. Rate constants and half-times were determined from semilog plots of $[H^+]_\infty - [H^+]_t$ vs. time. For duplicate determinations, values of $t_{1/2}$ agreed to within ±10%. Under these conditions changes in solvolysis rates in the presence of nucleoside were not detected.

N^2-Benzylguanosine (7). A suspension of 4 g of guanosine (14 mmol) in 2 L of $EtOH/H_2O$ (2:8) was warmed to 45 °C until all guanosine had dissolved. Sodium bicarbonate (6.2 g, 74 mmol) and benzyl bromide (12.6 g, 74 mmol) were added and the mixture was stirred at 40–45 °C for 1 week. The resulting homogeneous solution was evaporated to dryness under reduced pressure and the resulting solid residue was triturated with warm MeOH (100 mL). The MeOH triturant was filtered, concentrated to dryness and treated with 10 mL of hot MeOH. This final triturant was diluted with 20 mL of H_2O and filtered, and the total 30 mL of solution was loaded on a 2.8 × 71 cm Sephadex LH-20 column. The column was eluted with $MeOH/H_2O$ (3:7) at a flow rate of 1 mL/min. UV absorption was monitored at 254 nm and 10-mL fractions were collected. 7-Benzylguanosine eluted in fractions 33–39, unreacted guanosine eluted in fractions 40–49, and N^2-benzylguanosine eluted in fractions 71–90. These latter fractions were pooled and evaporated to dryness to afford 42 mg of

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amorphous white solid. Two crystallizations from H_2O afforded 16 mg of chromatographically homogeneous material: UV λ_{max} (pH 1) 259 (ϵ 14 200), 282 nm (sh) (ϵ 8190); UV λ_{max} (pH 6.9) 253 (ϵ 14 100), 274 nm (sh) (ϵ 9400); UV λ_{max} (pH 13) 257 (ϵ 12 500), 270 nm (sh) (ϵ 11 200); MS *m/e* 373 (M^+), 241 ($B + 1^+$), 91 ($C_7H_7^+$); NMR δ 4.50 (m, 3, H-2′ + $C_6H_5CH_2$, changes shape on addition of D_2O), 5.72 (d, 1, H-1′), 6.92 (t, 1, $C_6H_5CH_2NH$, J = 6 Hz, disappears on addition of D_2O), 7.36 (br s, 5, $C_6H_5CH_2$), 7.96 (s, 1, H-8), 10.66 (br s, 1, 1-H, disappears on addition of D_2O). Anal. Calcd for $C_{17}H_{19}N_5O_5 \cdot {}^1/_2H_2O$: C, 53.40; H, 5.27; N, 18.32. Found: C, 53.40; H, 5.22; N, 18.14.

Hydrolysis of this material in 1 N HCl at 65–70 °C for 12 h afforded a single UV-absorbing component which was chromatographically and spectroscopically indistinguishable from N^2-benzylguanine.[34]

Benzylation of Adenosine. Reactions of adenosine (0.12 or 0.25 g, 0.4 or 0.8 mmol for the hemihydrate, respectively) and [^{3}H]benzyl bromide (**2a**) or [^{3}H]benzyl tosylate (**2c**) were carried out in 25 mL of reaction solvent (Table II) containing 0.12 g (1.4 mmol) of $NaHCO_3$. The solutions were saturated with gaseous CO_2 to arrive at a final pH in the range 6.8–7.4. Following temperature equilibration (15 min) **2a** or **2c** (0.084 mmol in 0.25 mL of dry DMF) was added and the resulting solutions were stirred continuously during the reaction incubation. When reactions were complete (~5 half-times for **2a** and **2b** or 24 h for **2c**) an aliquot (0.1 mL) of reaction solution was withdrawn and mixed with an equal volume of marker solution (5 mM in both **3** and **4**). The sample was loaded on a 0.72 × 18 cm Aminex A-6 column (ammonium ion form). The column was initially eluted with 0.1 M ammonium formate (pH 4.5) in MeOH/H_2O (3:7) at 40 °C (flow rate 0.3 mL/min; operating pressure 90 psi). Column effluent was continuously monitored at 254 nm. Fractions (1.0 mL) were collected and mixed with 10 mL of PCS (Amersham/Searle) for scintillation counting. [^{3}H]Benzyl alcohol eluted in fractions 15,16; unmodified adenosine (**1**) in fractions 20–23; N^6-benzyladenosine (**3**) eluted in fractions 26–40. When 48 mL of initial buffer had passed through the column, elution was carried out at 60 °C using 1.0 M ammonium formate (pH 4.5) in MeOH/H_2O (3:7). 1-Benzyladenosine (**4**) eluted in fractions 75–77.

For reactions involving [G-^{3}H]adenosine, a 10-μL aliquot of an aqueous stock solution of labeled nucleoside (1.1×10^{-4} M) was added to 1 mL of buffered reaction solution (Table II). A 10-μL aliquot of 0.35 M benzyl chloride (**2b**) in DMF or EtOH was added and the solutions were incubated at 25 °C. Product analyses by column chromatography were carried out as above.

Benzylation of Guanosine. Reactions involving [5′-^{3}H]-guanosine were prepared by adding a 10-μL aliquot of a 5×10^{-5} M solution of labeled guanosine to 1 mL of buffered reaction solution (Table III). A 10-μL aliquot of an appropriately concentrated solution of unlabeled **2a**, **2b**, or **2c** in either EtOH or DMF was added to arrive at the final concentrations of benzylating agents cited (Table III).

Guanosine and [^{3}H]benzyl bromide reactions in aqueous ethanol were carried out in 25 mL of buffered solutions like those for adenosine (see above).

When reactions were complete, aliquots were removed and were mixed with marker solutions containing 1-benzylguanosine, **6**, **7**, and **8**. These solutions were loaded on a 0.72 × 30 cm Aminex A-5 column (ammonium ion form). The column was initially eluted with 1 M ammonium formate in DMF/H_2O (1:9) (pH 4.2) at 40 °C (flow rate 0.5 mL/min; operating pressure 250 psi). Column effluent was monitored at 254 nm and fractions (1.0 mL) were collected for scintillation counting. Unmodified guanosine eluted in fractions 15–17; 1-benzylguanosine eluted in fractions 33–38; N^2-benzylguanosine eluted in fractions 45–53; O^6-benzylguanosine eluted in fractions 58–67. When 75 mL of solvent had passed through the column, the eluting buffer was changed to 1 M ammonium formate in DMF/H_2O (3:7), pH 7, 50 °C. 7-Benzylguanosine eluted in fractions 100–105.

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Registry No. **1**, 58-61-7; **2a**, 100-39-0; **2b**, 100-44-7; **2c**, 1024-41-5; **3**, 4294-16-0; **4** (X = Br), 20757-58-8; **4** (X = Cl), 71171-55-6; **4** (X = OTs), 71171-57-8; **5**, 118-00-3; **6**, 4552-61-8; **7**, 71171-58-9; **8** (X = Br), 71171-59-0; **8** (X = Cl), 71171-60-3; **8** (X = OTs), 71171-62-5.

Base-Catalyzed Dehydrogenation of 2,2′,4,4′,6,6′-Hexanitrobibenzyl by Quinones†

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The dehydrogenation of 2,2′,4,4′,6,6′-hexanitrobibenzyl by quinones takes place only in basic medium, particularly in hexamethylphosphoramide alone, or in dimethylformamide in the presence of a suitable base. A study of the reaction mechanism indicates that hydrogen is transferred heterolytically and that the abstraction of H^- occurs only after removal, or partial removal, of H^+. The yield of 2,2′,4,4′,6,6′-hexanitrostilbene was highest with 2,3-dichloro-5,6-dicyanobenzoquinone and generally decreased with declining quinone redox potential.

In a study of the dehydrogenation of tetralin, acenaphthene, and bibenzyl by quinones in aromatic solvents, Braude, Brook, and Linstead[1] found 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) to be the most effective hydrogen-transfer reagent. Dehydrogenated product was obtained from bibenzyl in rather low yield (22%), however, in contrast to tetralin (70%) and acenaphthene (51%). It has been reported[2] that 4,4′-dimethoxystilbene is formed in 85% yield from the bibenzyl and DDQ in dioxane. The dehydrogenation of hydroaromatic compounds appears to proceed, at least in some cases, via hydride ion abstraction and is catalyzed by proton donors. Less is known about the dehydrogenation of bibenzyl compounds, which may

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(1) E. A. Braude, H. G. Brook, and R. P. Linstead, *J. Chem. Soc.*, 3569 (1954).

(2) H. O. House, "Modern Synthetic Reactions", 2nd ed., W. A. Benjamin, Menlo Park, CA, 1972, p 42.