Reactions of Benzenediazonium Ions with Guanine and Its Derivatives

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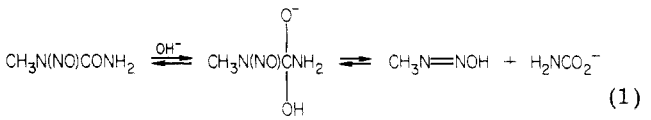
Guanine reacts with several benzenediazonium ions rapidly in aqueous solution at pH 10.5 to form 8-(arylazo)guanines in good yield. The reaction of guanine with 4-bromobenzenediazonium ion forms 8-[(4-bromophenyl)azo]guanine about 50-fold more rapidly than the reaction of adenine with this ion to yield 6-[3-(4-bromophenyl)-2-triazen-1-yl]purine under these experimental conditions. Guanosine reacts much more slowly than guanine with the benzenediazonium ions in aqueous solution at pH 8.5 or 10.5 to give 8-arylguanosines. The structures of these products were established by their spectroscopic properties and by their quantitative conversion to 8-arylguanines. 5'-Guanylic acid also reacts quite slowly with the benzenediazonium ions in aquous solution at pH 10.5. Only the compounds with strong electron-withdrawing groups yield N-2 triazenes at ambient temperature. No 8-aryl or 8-arylazo compounds are formed with 5'-guanylic acid at this temperature. However, 4-bromo- and 4-sulfobenzenediazonium ions react with 5'-guanylic acid at higher temperatures to yield the 8-aryl-5'-guanylic acids in low yield. The structures of these products were proven by hydrolysis to 8-arylguanines. The 8-arylguanosines and the 8-aryl-5'-guanylic acids are formed via free-radical phenylation reactions. The factors governing the reactivity of the adenines and the guanines are discussed.

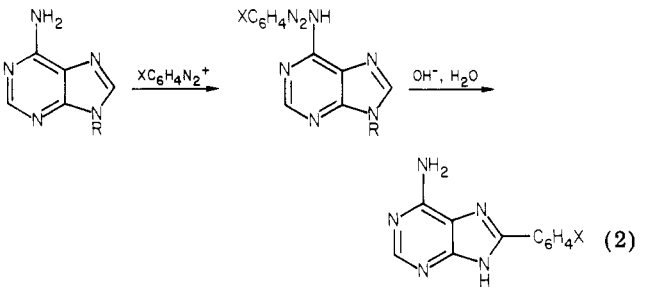
Introduction

The base-catalyzed decomposition of *N*-methyl-*N*-nitrosourea and other alkylnitrosoureas proceeds in neutral or basic aqueous solution to yield an alkyldiazo hydroxide and a carbamic acid derivative (eq 1).[1-3] The alkyldiazo



(1)

hydroxides are in equilibrium with unstable diazonium ions which either react directly with nucleophilic reagents to produce alkylated products or decompose to carbonium ions which then react with nucleophilic reagents.[4] The carcinogenic activity of the alkylnitrosoureas depends at least in part on the fact that they are relatively stable in aqueous solution at pH 7. Consequently, these molecules can be transported into the cell and its nucleus prior to the formation of a highly reactive alkylating agent. Recent work suggests that the reactions proceed without rearrangement, *N*,*N*-di-*n*-propylnitrosoamine yields 7-*n*-propylguanine in rat liver DNA[5a] and *N*-*n*-butyl-*N*-nitrosourea yields only the *n*-butyl derivatives of calf thymus DNA.[5b] These results suggest that free carbonium ions are not involved in the alkylation reactions. Because the alkyldiazo hydroxides are very unstable, we have examined the reactions of several benzenediazonium hydroxides with the purines to identify the key intermediates in the reaction and to provide a broader basis for the characterization of the processes leading to alkylation and arylation. In the first phase of this study, we found that adenine, adenosine, and 5'-adenylic acid react with arenediazonium ions in neutral and basic solution to yield N-6 triazenes.[6] These triazenes decompose readily in basic solution to give the 8-aryladenines via intermolecular, free-radical substitution reactions in which the ribose moiety is removed from the purine (eq 2).[6] We have now extended the study to the reactions of the benzenediazonium ions with guanine and its derivatives. Certain of



(2)

these reactions have been studied previously. Kössel and his co-workers reported that aromatic diazonium salts reacted with several nucleotides and nucleic acids to form highly colored products.[7,8] More recently, a method for linking RNA and DNA covalently to finely divided cellulose through a diazotized arylamine has been described.[9] It was postulated that the diazotized arylamines react chiefly with guanine residues to form triazene derivatives.[7-9] However, the basis for this structural assignment does not seem secure. Kössel concluded that several benzenediazonium ions with electron-withdrawing substituents reacted with 3'- and 2'-guanylic acid and deoxy-5'-guanylic acid to yield triazenes largely on the basis of the electronic spectra of the products. In contrast, Moudrianakis and Beer reported that deoxy-5'-guanylic acid reacted with 2,5-disulfobenzenediazonium ion to produce the 8-azo coupling product.[10] Several other groups have reported that guanine, xanthine, and isoguanine react readily with arenediazonium ions.[11-13] The reaction products were reduced without isolation to provide the three 8-amino derivatives. In related work, Kössel assigned the structure of the yellow addition product obtained from the reaction of deoxyguanosine as the N-2 triazene.[7b] But Hoffmann and his co-workers argued that the products prepared from guanosine were 8-arylguanosines.[14] These differences prompted us to investi-

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Table I. Spectroscopic Properties for 8-(4-Substituted-phenylazo)guanines[d]

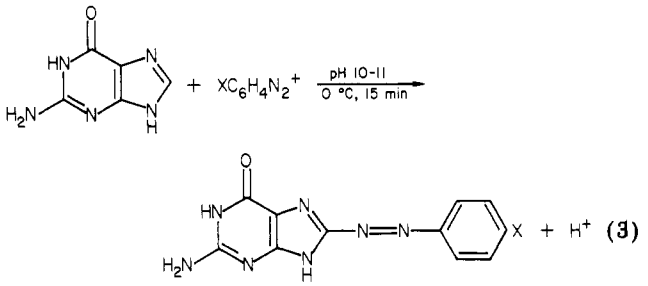
compd	NMR,[a] chemical shifts, ppm: NH_2	phenyl	other	UV-vis,[b] nm
1a, H	6.97 (s, br)	7.89 (d, 2 H), 7.60 (m, 3 H)		453
1b	6.76 (s, br)	7.39 (d), 7.77 (d, J = 8.3 Hz)	2.41 (s, CH_3)	456
1c, Br	6.81 (s, br)	7.76, 7.84 (br)[c]		455
1d, SO_3H	6.89 (s, br)	7.79 (d), 7.84 (d, J = 8.6 Hz)		455

[a] In dimethyl-d_6 sulfoxide. [b] In basic aqueous solution, pH 10.5. [c] Unresolved AB spectrum. [d] Satisfactory microanalyses were reported for compounds 1a-c.

gate the reactions of benzenediazonium ions with guanine, guanosine, and 5′-guanylic acid in basic solution to establish the structures of the products with more confidence and to gain perspective on the factors governing the course of the reactions.

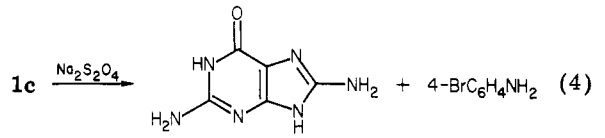
Results

Previous work established that the reaction between adenine and the benzenediazonium ions proceeded readily in basic aqueous solution to give N-6 triazenes.[6] Under the same conditions guanine reacts with the diazonium ions to yield the 8-azo coupling products, 1, in 60–85% yield (eq 3). The structural assignments of the azo compounds

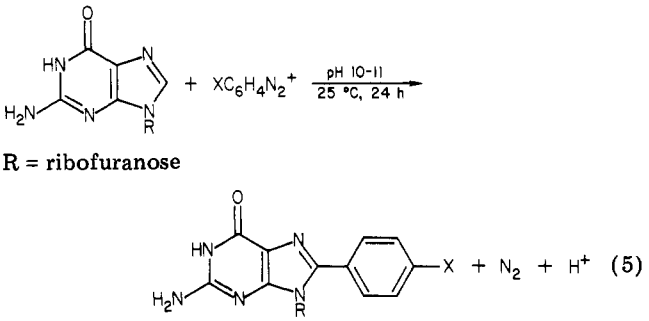


1a, X = H
b, X = CH_3
c, X = Br
d, X = SO_3H

are based upon their spectroscopic properties (Table I) and their reaction chemistry. For example, the product, 1c, obtained from 4-bromobenzenediazonium ion and guanine has a maximum in the electronic spectrum near 455 nm (ϵ 2220) in basic solution (pH 10.5). The NMR spectrum of this compound in dimethyl-d_6 sulfoxide is particularly informative because the resonances of all the protons can be observed. The characteristic frequency of the proton at the 8-position of guanine is absent from the spectrum, whereas the frequencies of all the NH protons are present. The protons of the benzene nucleus appear at δ 7.70–7.90. This chemical shift is characteristic of the shift observed for the protons of the (4-bromophenyl)azo fragment in other compounds. The azo compounds, 1, unlike the isomeric triazenes are stable in acidic and basic solution. To illustrate, the 4-bromophenyl derivative 1c was recovered unchanged after being heated in acid solution (pH 1) or basic solution (pH 10) for 24 h at 90–100 °C. The reduction of this compound with sodium dithionite[11-13] gave 8-aminoguanine (eq 4), which was characterized by its simple four-line NMR spectrum with relative itensity 1:1:2:2 and by its electronic spectrum.[13]



Under exactly the same conditions, guanosine reacts much more slowly than guanine with the benzenediazonium ions and it was necessary to allow 24 h for the completion of the reaction. Unfortunately, the benzenediazonium ions slowly decompose to form the familiar condensation products; however, we were able to isolate the 8-arylguanosines, 2, in 20–32% yield from the reaction mixtures (eq 5). These compounds were also obtained at



2a, X = H
b, X = CH_3
c, X = Br
d, X = SO_3H
e, X = NO_2

pH 8.5 in somewhat lower yield. Only compound 2d has been reported previously. The structures of the other products were established by their spectroscopic properties (Table II). Except for the nitro compound, the 8-arylguanosines, in contrast to the 8-(arylazo)guanines, do not exhibit absorption maxima above 300 nm. The absence of the 8-proton in their NMR spectra and the readily assigned resonances of the NH protons and the protons

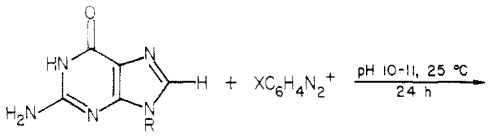
Table II. Spectroscopic Properties for 8-(4-Substituted-phenyl)guanosines and 8-(4-Substituted-phenyl)guanines[c]

compd	NMR,[a] chemical shifts, ppm: NH_2	phenyl	other	UV-vis,[b] nm
2a, H	6.56 (s, br)	7.51 (d, 3 H), 7.67 (t, 2 H)		281
2b, CH_3	6.44 (s, br)	7.34 (d), 7.55 (d, J = 7.9 Hz)	2.39 (s, CH_3)	276
2c, Br	6.50 (s, br)	7.63 (d), 7.75 (d, J = 7.4 Hz)		295
2d, SO_3H	6.65 (s, br)	7.65 (d), 7.72 (d, J = 8.0 Hz)		298
2e, NO_2	6.62 (s, br)	7.99 (d), 8.38 (d, J = 8.6 Hz)		256, 363
3a, H	7.06 (s, br)	7.44 (m, 3 H), 8.08 (d, 2 H)		236, 312
3b, CH_3	7.57 (s, br)	7.38 (d), 8,03 (d, J = 7.8 Hz)	2.38 (s, CH_3)	241, 314
3c, Br	7.13 (s, br)	7.75 (d), 8.01 (d, J = 8.5 Hz)		242, 316
3d, SO_3H	7.40 (s, br)	7.80 (d), 8.08 (d, J = 8.2 Hz)		240, 328
3e, NO_2	6.81 (s, br)	8.26 (d), 8.34 (d, J = 8.8 Hz)		254, 395

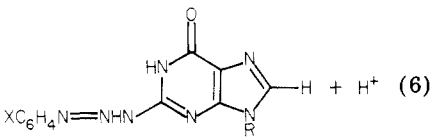
[a] In dimethyl-d_6 sulfoxide. [b] In basic aqueous solution, pH 10.5. [c] Satisfactory analyses were reported for compounds 2a-c, 2e, and 3c.

of ribose fragment support the structural assignment. The structural proof was completed by the acid hydrolysis of the 8-arylguanosines to 8-arylguanines (**3a–3e**). Three of these compounds, **3a**, **3b**, and **3e**, have been described previously.[15-16] The properties of the compounds obtained by hydrolysis were identical with the properties of the compounds reported in the literature.

A precipitate forms almost immediately upon the addition of the benzenediazonium ions to basic solutions of 5′-guanylic acid at ambient temperature. The solids exploded on the filter frit after they were collected and washed with chloroform. The resonance signals of the diazonium ions are evident in the NMR spectra of these compounds in solution. Presumably, these initial, very unstable solids are aryldiazonium phosphate derivatives. In subsequent experiments, heterogeneous reaction mixtures were stirred for 24 h until the unstable products had been consumed in other reactions. 5′-Guanylic acid reacts very slowly with the benzenediazonium ions in basic solution under these conditions. Consequently, the side reactions of the diazonium ions which yield highly colored condensation products consume a considerable quantity of this reagent, and the other products of the reaction of the benzenediazonium ions with 5′-guanylic acid are formed in quite low yield. Careful study of the NMR spectra of the crude reaction products isolated after 24 h at ambient temperature indicated that the 8-aryl-5′-guanylic acids were not formed. However, we were able to obtain evidence for the conversion of 5′-guanylic acid to triazenes **4d** and **4e**, with 4-sulfo- and 4-nitrobenzenediazonium ions in about 10% yield (eq 6). Several lines



R = ribose 5′-phosphate



(6)

4d, X = SO_3H
e, X = NO_2

of evidence support this assignment of structure. First, the reaction mixtures exhibit a broad absorption between 350 and 425 nm. This absorption is characteristic of triazenes and distinguishes these compounds from the 8-aryl and 8-arylazo derivatives. Second, the resonances of the triazene are discernible in the NMR spectra of the isolated solid products (Table III). Although these products are contaminated with 5′-guanylic acid, the resonance of the 8-proton of the triazene and the resonances of the aromatic protons of the 4-nitro- and 4-sulfophenyl fragments of the triazene are sufficiently separated from the other signals to enable identification. Third, these solid products are unstable in acid solution and decompose to 5′-guanylic acid and the benzenediazonium ion. Indeed, the azo coupling product of 2-naphthol is obtained from these triazenes in acid solution (pH 3).

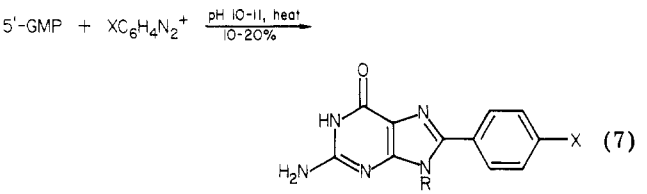
The benzenediazonium ions with less electron-withdrawing substituents were also reacted with 5′-guanylic acid. These reactions do not yield N-2 triazenes or 8-aryl-5′-guanylic acids under the same conditions.

Table III. Spectroscopic Properties of 2-[3-(4-Substituted-phenyl)-2-triazen-1-yl]-6-hydroxypurine Ribonucleoside 5′-Phosphates

compd	chemical shifts, ppm [a,b] aromatic protons	C_8-H	UV-vis, [c] λ_{max}
4d, SO_3H	7.80, 7.87 [d]	8.29 (s)	368
4e, NO_2	7.55 (d), 8.20 (d, J = 9.0 Hz)	8.27 (s)	404

[a] Sodium 3-(trimethylsilyl)propionate was used as the internal standard. [b] In aqueous solution, pH 13.5. [c] In aqueous solution, pH 10.5. [d] An unresolved AB spectrum was observed.

Basic solutions containing 4-bromo- or 4-sulfobenzenediazonium ion and 5′-guanylic acid were heated to accelerate the decomposition of the diazonium ion. Under these more vigorous conditions, phenylation occurred. The structures of the reaction products were proven by acid-catalyzed hydrolysis of the 8-arylguanylic acids to yield 8-arylguanines (eq 7).



(7)

5c, X = Br
d, X = SO_3H

R = ribose 5′-phosphate

The difference in reactivity of 5′-guanylic acid and 5′-adenylic acid was assessed by comparison of the rates of formation of the N-2 and N-6 triazenes in dilute basic aqueous solution at pH 8.0 and 10.5. Only the absorption of 6-[3-(4-sulfophenyl)-2-triazen-1-yl]purine ribonucleoside 5′-phosphate at 390 nm at pH 8 and 398 nm at pH 10.5 could be detected in these solutions.

Discussion

Benzenediazonium ion and its derivatives react rapidly with adenine, adenosine, and 5′-adenylic acid in dilute basic solution to give N-6 traizenes.[6] The pattern of reactivity of guanine, guanosine, and 5′-guanylic acid is distinctly different. These differences may be conveniently discussed on the basis of the reactions outlined in Scheme I.

Scheme I

$$PhN_2^+ \rightleftarrows PhN{=}NOH \rightleftarrows PhN{=}NO^- \quad (8)$$

$$PhN_2ON_2Ph \rightarrow Ph\cdot + N_2 + PhN{=}NO\cdot \quad (9)$$

$$\text{purine} + PhN_2^+ \rightarrow \text{8-azo compound} \quad (10)$$

$$\text{purine} + PhN_2^+ \rightleftarrows \text{N-2 triazene} \quad (11)$$

$$\text{purine} + Ph\cdot \rightarrow \text{8-phenyl compound} \quad (12)$$

The equilibria shown in eq 8 are pH dependent with the diazotate present in strongly basic solution and the diazonium ion present in neutral and weakly basic solution. The available evidence favors the view that the phenyl radical is formed via eq 9.[17] This irreversible reaction proceeds rather slowly at ambient temperature. Conse-

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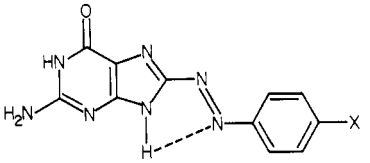
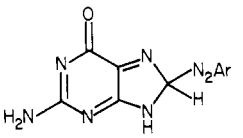
quently, the reactive nucleophilic sites in the purines compete for the electrophilic diazonium ion. The differences in the reaction patterns observed for adenine and guanine and their derivatives arise because there are major differences in the nucleophilicities of the N-2 and N-6 amino groups and because there are also major differences in the rates of formation of the σ complexes obtained via the electrophilic reactions of the benzenediazonium ions at the 8-positions. The fact that triazene formation is a reversible process whereas the coupling and phenylation reactions are essentially irreversible also influences the ultimate course of the reaction.

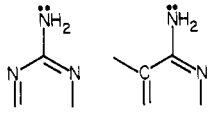
Guanine reacts rapidly with the diazonium ions as expected[11-13] to provide the stable 8-arylazoguanines, **1**, in good yield. Presumably the *E* isomers are obtained, and it is likely that the preferred conformer is stabilized by hydrogen bonding as shown.[18] At pH 10.5 guanine is



almost completely converted to the reactive anion. The competition experiments reveal that this anion is about 50-fold more reactive for the formation of the azo coupling product with 4-bromobenzenediazonium ion than the adenine anion is for the formation of the N-6 triazene. The

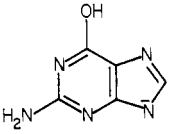


difference in reactivity stems in part from the formation of a highly stabilized σ complex in the reaction of the guanine anion and in part from the modest nucleophilicity of the N-2 amino group in this molecule. In contrast, adenine does not yield as stable a σ complex as guanine, but the nucleophilicity of the N-6 amino group of adenine is distinctly greater than that in guanine or its anion. This point is well illustrated by the very slow rate of reaction between 2-aminopyrimidine and 4-bromobenzenediazonium ion to form the N-2 triazene. This reaction is only about 10% complete after 24 h under the experimental conditions used in this work. Clearly, the nucleophilicity of the 2-aminopyrimidine fragment of the guanines is significantly less than that of the 2-aminopyridine fragment of the adenines. The difference in reactivity at the



8-position of guanine and adenine observed in this study parallels the difference in reactivity observed in the electrophilic iodination of these compounds and their derivatives.[19] It is also relevant that the product distribution ultimately realized may depend upon the fact that the triazenes are formed in reversible reactions, whereas the azo compounds are the products of irreversible reactions.

Guanosine which is also present in basic solution (pH 10.5) as the anion reacts very slowly with the benzenediazonium ions. Neither the N-2 triazene nor the 8-azo coupling product are formed. It is evident that the 9-ribose fragment considerably slows the electrophilic substitution reaction. Purines with two activating groups such as guanine and xanthine react rather readily with benzenediazonium ions to yield 8-azo coupling products, whereas the less reactive purines, for example, hypoxanthine and adenine, do not form coupling products.[20] However, none of the purines with substituents in the 9-position yield azo coupling products. This fact strongly suggests that tautomers without protons in the 9-position are key intermediates in the coupling reactions.[20] This structural re-



quirement is augmented by the deactivating polar effect[21] and steric requirements[22] of the ribose fragment. The rate of formation of the azo compound is slowed to such a degree that the decomposition reactions of the benzenediazonium ions to yield condensation products and phenyl radicals become the dominant reactions. Thus, guanosine is converted under these conditions to the 8-arylguanosines, **2**, in 20–30% yield in 24 h. The reaction proceeds in essentially the same way at pH 8.5 except that about twice as much 8-(4-bromophenyl)guanosine is formed at pH 10.5 than at pH 8.5. This modest difference in yield suggests that the phylation reaction proceeds only moderately more rapidly with the anion of guanosine than with guanosine. The phenylation of guanosine like the phenylation of adenosine[6] and other free-radical substitution reactions of the purines occurs selectively at the 8-position.[23]

The ribose moiety is not cleaved from guanosine during the phenylation reaction at ambient temperature. However, when the reaction of 4-bromobenzenediazonium ion and guanosine is carried out at 95 °C, 8-(4-bromophenyl)guanosine and 8-(4-bromophenyl)guanine are formed in a 2:1 ratio. Guanosine and the product, 8-(4-bromophenylguanosine, do not decompose under these experimental conditions. Consequently, we infer that the loss of the ribose fragment occurs at an intermediate stage of the reaction presumably via a base-catalyzed β-scission reaction similar to that proposed to account for the conversion of adenosine to 8-aryladenine.[6]

5′-Guanylic acid is apparently even less reactive than guanosine under these experimental conditions and phenylation does not occur detectably at 25 °C. With the more stable 4-substituted benzenediazonium ions, 5′-guanylic acid reacts to give the N-2 triazenes slowly and in low yield. These compounds which are formed in reversible reactions are presumably the strongly hydrogen bonded *E* isomers.

The modes reactivity of 5′-guanylic acid is well illustrated by the fact that the N-6 triazene of 5′-adenylic acid

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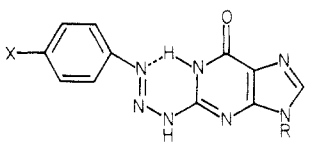

is formed in great preference to the N-2 triazene of 5′-guanylic acid at pH 8 and 10.5. As already noted, the difference in reactivity stems from the major difference in nucleophilicity of the N-2 and N-6 amino groups in these molecules. However, at elevated temperatures, the 8-phenylation products of 5′-guanylic acid are formed from 4-sulfobenzenediazonium ions and from 4-bromobenzenediazonium ion even though the latter compounds does not form a triazene in detectable amounts. As discussed previously[6] and as outlined in Scheme I, the phenylation products are formed in intermolecular free-radical reactions.

Experimental Section

The chemicals and solvents used in this work were obtained from commercial sources and were purified as necessary prior to use. The electronic and NMR spectra were recorded on Cary 219, Bruker 270-MHz, and Chicago 500-MHz spectrometers.

8-(Arylazo)guanines (1). The 8-azo coupling products were prepared by the same general method. For example, a solution of 4-bromobenzenediazonium chloride (10 mmol) was prepared as described previously[6] and was added dropwise to a solution of guanine (0.75 g, 5 mmol) in 0.62 N sodium hydroxide (40 mL) at 0 °C. The pH was maintained between 10 and 11 by the dropwise addition of aqueous sodium hydroxide. After the addition of the diazonium ion was complete, the solution was stirred for 15 min. The solution was then neutralized to pH 7 with 1.0 N hydrochloric acid, and the precipitate which formed was filtered, washed thoroughly with chloroform and water, and air-dried to yield the crude red product. This product was dissolved in 1.0 N potassium hydroxide and reprecipitated with 1.0 N hydrochloric acid. After filtration, the desired product was washed with chloroform and water repeatedly and air-dried to yield 8-[(4-bromophenyl)azo]guanine (1.25 g, 75%) as an orange solid. This compound had an absorption maximum at 455 nm (ϵ 2220) in pH 10.5 aqueous solution.

Compounds **1a**, **1b**, and **1d** were obtained in 64, 61, and 84% yield, respectively, after 15 min. Some unidentified byproducts were formed during the preparation of **1a** and **1b**; these byproducts were formed in much smaller quantity when purified samples of benzenediazonium tetrafluoroborate and the related 4-methyl compound were used as the starting material. The principal features of the electronic and NMR spectra of the 8-(arylazo)-guanines are presented in Table I.

Reduction of 8-[(4-Bromophenyl)azo]guanine. 8-[(4-Bromophenyl)azo]guanine (0.668 g, 2 mmol) was dissolved in 0.38 N sodium hydroxide (50 mL). Sodium dithionite (8.70 g, 50 mmol) was added in small portions. The solution was stirred and heated at 90 °C for 48 h. The solution which eventually became pale yellow was maintained at about pH 9 during the reaction. The cooled mixture was neutralized and the precipitate which formed was collected, washed with water and chloroform, and dried to yield 8-aminoguanine (0.2 g, 60%) as a white solid. The NMR spectrum was recorded in dimethyl-d_6 sulfoxide: δ 10.93 (s, 1 H), 10.26 (s, 1 H), 6.12 (s, 2 H), 6.00 (s, 2 H). The electronic spectrum was recorded in basic aqueous solution (pH 9): λ_{max} 249, 291 nm.

Competitive Reaction of Adenine and Guanine with 4-Bromobenzenediazonium Ion. This benzenediazonium ion was prepared as already described and added to a solution of guanine and adenine in basic solution (pH 10.5). About 15 min were allotted for the reaction. Hydrochloric acid (0.5 N) was added to precipitate the reaction products. Samples of the products were dissolved in methanol containing a small quantity of sodium methoxide and analyzed by thin-layer chromatography for the presence of 6-[3-(4-bromophenyl)-2-triazen-1-yl]purine, using 20% methanol–80% chloroform. This compound was not detected when the ratio of adenine to guanine was 20:1. However, a small quantity of this product was obtained when adenine (25 mmol) and guanine (0.5 mmol) were reacted with 4-bromobenzenediazonium ion (0.5 mmol) in basic solution (80 mL). Thus, guanine is about 50-fold more reactive than adenine under these experimental conditions.

8-Arylguanosines (2). The reaction mixtures of guanosine and the benzenediazonium ions were prepared in the same way as the guanines. However, the reaction was much slower, and the reaction mixtures were allowed to stir for 24 h at room temperature prior to neutralization with 1.0 N hydrochloric acid. The precipitate which formed was collected and washed with chloroform and water to yield the crude product. This material was washed with hot methanol, water, and chloroform and air-dried to yield the light yellow 8-arylguanosines. Compounds **2a–d** and **2e** were obtained in 21, 27, 32, 22, and 30%, respectively. Unreacted guanosine was recovered from these reactions in 50, 48, 55, 52, and 50%. The spectroscopic properties of the reaction products are presented in Table II.

The reaction between guanosine and 4-bromobenzenediazonium chloride was also carried out at 95–100 °C for 24 h. Under these much more vigorous conditions, 8-(4-bromophenyl)guanosine (**2c**) and 8-(4-bromophenyl)guanine (**3c**) were formed in a 2:1 ratio as determined by analysis of the NMR spectrum of the initial reaction products.

The reaction with 3.3×10^{-3} M guanosine and 6.6×10^{-3} M 4-bromobenzenediazonium chloride was also carried out in aqueous solution at pH 8.5. The pH was maintained by the dropwise addition of dilute sodium hydroxide. 8-(4-Bromophenyl)guanosine (**2c**) was obtained in 14% yield after 24 h.

Hydrolysis of the 8-Arylguanosines. Each of the 8-arylguanosines obtained in the reactions of the benzenediazonium ions with guanosine were treated with 0.1 N hydrochloric acid for 1 h at 100 °C. The reaction mixtures were worked up in the usual way to give a virtually quantitative yield of the corresponding 8-arylguanines. The properties of the compounds are presented in Table II.

Reaction of 2-Aminopyrimidine with 4-Bromobenzenediazonium Ion. 2-Aminopyrimidine was reacted with this diazonium ion in aqueous solution at pH 10.5 in the same manner as described for guanine. Aliquots of the reaction mixture were periodically removed and analyzed for the triazene by thin-layer chromatography on silica gel, using 80% chloroform–methanol. The product was first detected 1 h after the reaction was initiated. The reaction continued slowly for 24 h. The entire reaction was then neutralized with 1.0 N hydrochloric acid.

The precipitate which formed was collected, washed thoroughly with chloroform and water, and air-dried to give pale-yellow 2-[3-(4-bromophenyl)-2-triazen-1-yl]pyrimidine hemihydrate in about 10% yield. A satisfactory microanalysis was obtained. The NMR spectrum was recorded in dimethyl-d_6 sulfoxide: δ 8.67 (d, 2 H, C_4-H, C_6-H), 7.67 (d, 2 H), 7.52 (d, 2 H, J = 8.5 Hz), 7.18 (t, 1 H, C_5-H). The compound absorbed at λ 348 and 389 nm (sh) in basic solution (pH 10.5).

2-[3-(4-Substituted-phenyl)-2-triazen-1-yl]-6-hydroxypurine Ribonucleoside 5′-Phosphates (4). In preliminary experiments, the benzenediazonium ions were prepared and added to 5′-guanylic acid as described for 1. The solids which precipitated during the addition were collected and washed with chloroform. The products exploded on the filter frit when dried. In subsequent experiments, the heterogeneous reaction mixtures were stirred for 24 h at room temperature prior to the examination of the reaction products. The electronic spectra of the final reaction mixtures were recorded following dilution with pH 10.5 aqueous buffer. The absorption maximum for 4-sulfo and 4-nitro derivatives appeared at 368 and 404 nm, respectively. In separate experiments, water was removed from the reaction products in vacuum. The remaining solid was washed thoroughly with chloroform and air-dried. The NMR spectra of the N-2 triazenes obtained in this manner are presented in Table III.

The benzenediazonium ions with less electron-withdrawing substituents react with 5′-guanylic acid to form a different product in quite low yield. It has not been possible to separate these products from 5′-guanylic acid. However, the spectroscopic properties and chemical properties of the materials exclude several possible compounds. First, the NMR spectra and the lack of an absorption maxima above 300 nm in the ultraviolet spectra negate the N-2 triazene. This possibility is also excluded by the fact that

acid hydrolysis of the product in the presence of 2-naphthol fails to yield the coupling product. Second, the compounds do not yield 8-arylguanines after acid hydrolysis; hence they are not 8-aryl-5′-guanylic acids. Third, the compounds are not hydrolyzed in acidic or basic solution to yield phenols. One product that has not been rigorously excluded is the 2′- or 3′-phenyl ether.

About 90% of the 5′-guanylic acid was accounted for in these experiments.

Comparison of the Reactivity of 5′-Adenylic Acid and 5′-Guanylic Acid. Solutions of 5′-adenylic acid (2.85×10^{-3} M), 5′-guanylic acid (2.85×10^{-3} M), and 4-sulfobenzenediazonium ion (5.71×10^{-3} M) was prepared at pH 8.0 and 10.5.

The solutions were stirred for 15 min at room temperature. The electronic spectra of aliquots of the reaction mixture which had been diluted with the buffer were recorded. Only the absorption at 390 nm could be detected. 6-[3-(4-Sulfophenyl)-2-triazen-1-yl]purine ribonucleoside 5′-phosphate and 2-[3-(4-sulfophenyl)-2-triazen-1-yl]-6-hydroxypurine ribonucleoside 5′-phosphate exhibit absorption maxima at 390 and 360 nm, respectively, at pH 8.0.

The same experiment was carried out at pH 10.5, only the absorption at 398 nm, which is characteristic of 6-[3-(4-sulfophenyl)-2-triazen-1-yl]purine ribonucleoside 5′-phosphate,[6] was observed.

Phenylation of 5′-Guanylic Acid. The reaction mixtures prepared from 4-bromo- and 4-sulfobenzenediazonium ion and 5′-guanylic acid were also heated for 95 °C for 8 h. The solution was neutralized with 1.0 N hydrochloric acid to precipitate the products and residual 5′-guanylic acid. The solid was suspended in 0.1 N hydrochloric acid and heated at 100 °C for about 1 h. The mixture was cooled rapidly, and the solids were collected, washed with cold chloroform and ice water, and dried to yield 8-(4-bromophenyl)- and 8-(4-sulfophenyl)guanine in 18 and 10% yields, respectively. These products were identical in all respects with the materials obtained in the hydrolysis of the 8-arylguanosine.

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Registry No. 1a, 79953-00-7; **1b**, 79953-01-8; **1c**, 79953-02-9; **1d**, 75056-37-0; **2a**, 79953-03-0; **2b**, 79953-04-1; **2c**, 79953-05-2; **2d**, 79953-06-3; **2e**, 79953-07-4; **3a**, 7780-10-1; **3b**, 79953-08-5; **3c**, 79953-09-6; **3d**, 14677-69-1; **3e**, 14173-37-6; **4d**, 79953-10-9; **4e**, 79953-11-0; 4-bromobenzenediazonium chloride, 2028-85-5; guanine, 73-40-5; benzenediazonium tetrafluoroborate, 369-57-3; 4-methylbenzene diazonium tetrafluoroborate, 459-44-9; 8-aminoguanine, 28128-41-8; adenine, 73-24-5; guanosine, 118-00-3; 2-aminopyrimidine, 109-12-6; 2-[3-(4-bromophenyl)-2-triazen-1-yl]pyrimidine, 79953-12-1; 5′-guanylic acid, 85-32-5; 4-sulfodiazonium chloride, 6118-33-8; 4-nitrodiazonium chloride, 100-05-0; 5′-adenylic acid, 61-19-8; 6-[3-(4-sulfophenyl)-2-triazen-1-yl]purine ribonucleoside 5′-phosphate, 79982-46-0.

Evaluation of Superacid Strength from the Protonation of Benzene. Comparison of $HF-SbF_5$, $HF-TaF_5$, and $HBr-AlBr_3$ Systems[1]

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The degree of protonation of benzene in $HF-SbF_5$, $HF-TaF_5$, and $HBr-AlBr_3$ solutions has been investigated by carbon-13 NMR spectroscopy. Both 30:1 $HF-SbF_5$ and $HBr-AlBr_3$ are much stronger acids than 30:1 $HF-TaF_5$ (55% protonation at a TaF_5/benzene ratio of 3) or 4:1 $HF-TaF_5$ (68% protonation at a TaF_5/benzene ratio of 2.5). $HBr-AlBr_3$ protonates benzene completely down to a $AlBr_3$/benzene ratio of 2. Under HBr pressure, 3.5–4 mol of HBr for each mole of $AlBr_3$ are taken into the benzenium bromoaluminate solution. The "sludge" catalysts commonly encountered in hydrocarbon conversions are actually solutions of carbocations in the $HBr-AlBr_3$ superacid. The high acidity revealed by the benzene protonation is representative for such sludges. The acidity measurement by benzene protonation can in principle be extended to other superacid systems.

Protonation of mono- and polyalkylbenzenes and the NMR spectra of the corresponding cations in various acidic media have been extensively investigated.[3–7] Although all these acidic systems are classified as superacids,[8] their acid strengths vary widely. Thus, a small amount of mesitylene was indicated in addition to its protonated form in $HF-BF_3$,[9] while $HF-SbF_5$ was found to convert benzene

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