

THE REACTIONS OF BENZENEDIAZONIUM IONS WITH SOME PURINES AND PYRIMIDINES¹

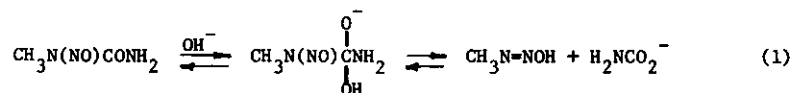
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Abstract — The reactions of certain pyrimidines and purines with benzenediazonium ions in basic solution have been investigated to determine whether the reactions provide triazenes, azo coupling, or phenylation products. Under these conditions, the anion of uracil forms the 5-azo coupling product. No reaction occurs with the anion of uridine or 5'-uridylic acid. A complex mixture of products is obtained with cytosine, but cytidine and 5'-cytidylic acid fail to yield either azo coupling or phenylation products. Xanthine readily undergoes the 8-arylazo coupling reaction. Xanthosine is unreactive. In contrast, inosine yields 8-phenylinosine. Purine itself is converted to 6-phenylpurine. The structure of the product was proved by deuterium labeling experiments. The factors governing the reactivity of these compounds are briefly noted.

INTRODUCTION

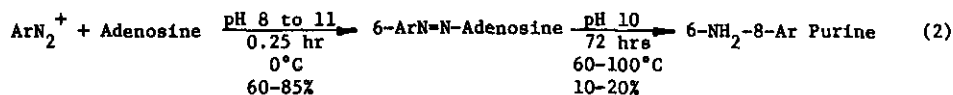
The N-alkyl-N-nitrosoureas decompose in neutral and basic solution to yield alkyldiazohydroxides.²⁻⁴ These compounds which can also be formed from other carcinogenic alkylating agents may



be important intermediates in the alkylation of the nucleic acids with the nitrosoureas and the nitrosoamines. The available evidence strongly suggests that these alkylation reactions occur without the intervention of free carbonium ions.⁵ These observations imply that the diazohydroxides or the diazonium ions which they form may be intercepted by the nucleic acid constituents. To examine this idea, we have studied the reactions of the purines and the pyrimidines with the relatively stable aryldiazohydroxides rather than the extremely reactive alkyldiazohydroxides⁶ to gain perspective on the course of the reaction and the extent to which triazenes are significant reaction intermediates.

Adenine, adenosine, and 5'-adenylic acid react with a variety of benzenediazonium ions in basic

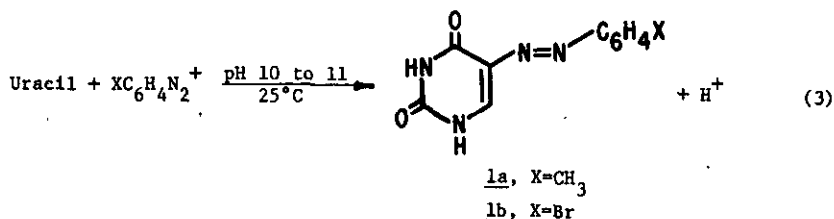
solution to give N-6 triazenes as the initial products.⁷ These compounds decompose thermally to



yield 8-aryladenines via intermolecular free radical substitution reactions. The ribose fragment is cleaved from the heterocycle during the substitution reaction. Guanine, guanosine, and 5'-guanylic acid react differently. Guanine yields the 8-azo coupling product at pH 8.5 and at pH 10.5 as expected.⁸⁻¹¹ Under the same conditions, guanosine gives the 8-arylguanosines through a free radical substitution reaction.⁸ In contrast to the results for adenosine and 5'-adenylic acid, the ribose moiety is not readily cleaved during this reaction. The benzenediazonium ions with electron withdrawing groups react with 5'-guanylic acid to yield N-2 triazenes.^{8,12} At higher temperature, these compounds decompose to give 8-aryl-5'-guanylic acids.⁸ The diverse character of these reactions prompted us to study the reactions of other selected purines and pyrimidines with the aryldiazohydroxides.

RESULTS AND DISCUSSION

Weng previously reported that uracil reacts with 4-methyl- and 4-bromobenzenediazonium ions to give the corresponding 5-arylazouracil derivatives, 1.¹³ His results were confirmed in the course of this work by a study of the nmr spectra of the reaction products. To illustrate the characteristic absorption of the proton at the 5 position of uracil is absent from the nmr spectrum of the



compound formed in the reaction of 4-bromobenzenediazonium ion and uracil and the signal for the proton at the 6 position is a sharp singlet. The protons of the benzene nucleus yield an AB pattern at 270 MHz. The chemical shifts observed for these protons in 1b are characteristic of the shifts observed for the protons of the 4-bromophenylazo fragment in other azo derivatives.^{7,8} The electronic absorptions and nmr data observed for the reaction products are summarized in Table I.

Uridine was reacted with 4-bromobenzenediazonium ion under the same conditions for as long as 48 hours. Only the familiar deeply colored condensation products which are formed during the decomposition of the benzenediazonium ion were obtained. No coupling or substitution products of uridine could be detected by thin layer chromatography and between 85 and 90% of this starting

TABLE I
Spectroscopic Properties for 5-(4-Substituted phenylazo)uracils

Compound	NMR Spectra ^a			UV-VIS spectra, ^b nm
	C ₆ -H	Chemical Shifts, ppm Phenyl	Other	
<u>1a</u> , CH ₃	8.24(s)	7.56(d), 7.27(d) [J=8.0 Hz]	2.35(s,CH ₃)	363, 286(sh)
<u>1b</u> , Br	8.44(s)	7.60(d), 7.53(d) [J=8.6 Hz]	-	369,288(sh), 236(sh)

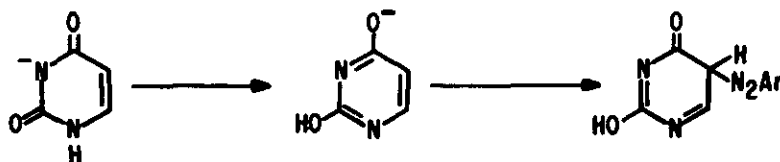
^aIn dimethyl-d₆ sulfoxide. ^bIn basic aqueous solution at pH 10.5.

material was recovered from the reaction mixture in replicate experiments. Kössel previously reported that a mixture of 3'- and 2'-uridylic acid and deoxy-5'-uridylic acid did not react with several different benzenediazonium ions.^{12a-c}

In contrast to uracil, cytosine reacted with the aryldiazonium ions in basic solution to form a highly colored product mixture. Some of these products are quite unstable and darken rapidly in air and we have not been able to isolate pure cytosine derivatives from the mixture. However, it should be noted that the electronic spectra of one product obtained in low yield with an absorption maximum at 370 nm was compatible with that expected for 5-(4-bromophenylazo)cytosine.

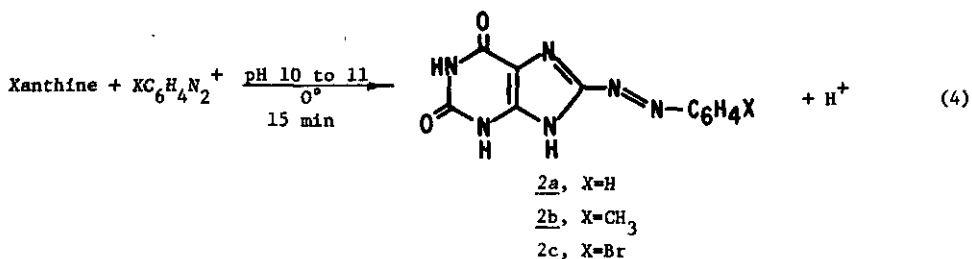
Cytidine is much less reactive and like uridine does not yield a detectable quantity of coupling or phenylation products after 48 hours. About 85 to 90% of the cytidine could be recovered from the reaction mixture. Kössel found that the cytidine residues in RNA and DNA did not react with benzenediazonium ions.^{12d} He also noted that a mixture of 2'- and 3'-cytidylic acid and deoxy-5'-cytidylic acid reacted with benzenediazonium ions to yield a light yellow product but the compounds were not identified.^{12a-c}

Under the conditions of these experiments the reaction occurs between the anion of uracil and the benzenediazonium ion. The decreased reactivity of the anion of uridine toward this reagent is striking. It is unlikely that the ribose moiety deactivates the anion through its polar effect rather it seems that the ribose substituent in the 1 position prevents the transformation of the anion to a reactive tautomer which can form a stable σ -complex with the diazonium ion.¹⁴ The difference in reactivity of uracil and uridine parallels the difference in reactivity of guanine and



and guanosine except that the steric factor important in the reaction of the adjacent position in guanosine has no impact on the reaction of uridine. Indeed, the reaction of the anion of uracil is somewhat slower than the reaction of the anion of guanine with the benzenediazonium ion. This difference in reactivity is reflected in the lower yield of the 5-arylazouracils (40-60%) compared to the 8-arylazoguanines (60-85%). It presumably stems from the fundamental difference in carbon-nucleophilicity of the purines and the pyrimidines.

Cytosine is not converted to an anion under the experimental conditions used in this study and, as expected, it is much less reactive than the anions of uracil and guanine. As a consequence, the condensation products of the aryldiazonium ions are formed in preference to the azo coupling product of cytosine. Cytidine and the cytidylic acids are also unreactive and none of these compounds yield either the azo coupling product or the phenylation product in detectable quantity. We also studied certain other purines to assess the relative importance of the azo coupling and phenylation reactions. Xanthine couples readily with benzenediazonium ion and its 4-methyl and 4-



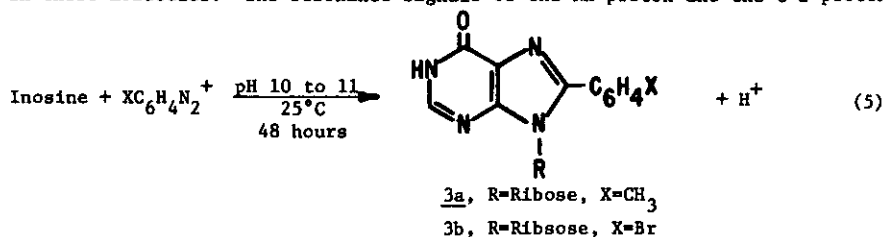
bromo derivatives to form azo compounds as expected.¹⁵ The yields of these azo compounds ranged from 24 to 60%. The absence of the resonance signal of the 8 proton in the NMR spectra confirmed the site of the substitution reaction. The spectroscopic properties of these compounds are presented in Table II. The related nucleoside, xanthosine, does not react with benzenediazonium ion to give an azo compound or the 8 phenylation product.

TABLE II
Spectroscopic Properties for 8-(4-Substituted phenylazo)xanthines

Compound	NMR Spectra Chemical Shifts, ppm		UV-VIS Spectra nm
	Phenyl	Other	
$\underline{2a}$, H	7.71(br,2H), 7.46(br,3H) ^b		410,273(sh) ^c
$\underline{2b}$, CH ₃	7.82(d,2H), 7.44(d,2H) ^a [J=8.0 Hz]	2.43(s,CH ₃) ^a	410,276(sh) ^c 234(sh)
$\underline{2c}$, Br	7.84(br) ^a		

^aIn dimethyl-d₆ sulfoxide. ^bIn basic solution, pH 13.5. ^cIn basic solution, pH 10.5.

Hypoxanthine unlike xanthine is not active enough to couple with benzenediazonium ion to give the azo product. However, the corresponding nucleoside, inosine, reacts with 4-methyl and 4-bromobenzenediazonium ion in basic solution at room temperature to give the 8-arylation product, 3, in about 10% yield in 48 hours. The electronic spectra of 3 indicate that the azo linkage is not present in these molecules. The resonance signals of the NH proton and the C-2 proton are readily



identified in the NMR spectra of these compounds, whereas the resonance signal of the 8 proton is clearly absent. Thus, all the spectroscopic properties summarized in Table III support the structural assignment.

TABLE III

Spectroscopic Properties for 8-(4-Substituted phenyl)inosines

Compound	NMR Spectra Chemical Shifts, ^a ppm			UV-VIS Spectra, ^b nm
	C ₂ -H	Phenyl	Other	
<u>3a</u> , CH ₃	8.13(s)	7.62(d,2H), 7.40(d,2H) [J=7.8 Hz]	2.41(s,CH ₃)	272
<u>3b</u> , Br	8.16(s)	7.82(d,2H), 7.68(d,2H) [J=8.6 Hz]	-	278

^aIn dimethyl-d₆ sulfoxide. ^bIn methanol.

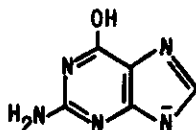
Purine also reacts with the 4-bromobenzenediazonium ion at room temperature in basic solution to give the somewhat unexpected 6-(4-bromophenyl)purine, 4. The electronic spectrum ($\lambda_{\text{max}}^{\text{CH}_3\text{OH}} = 278 \text{ nm}$) is typical of the purine skeleton.¹⁶ The nmr spectrum of compound 4 revealed that the characteristic frequency of the C₆-H proton of purine at $\delta 9.21$ was not present in the product. Although the identification of this resonance seems secure,¹⁷ we investigated the reaction of purine-8-d. This compound yields 6-(4-bromophenyl)purine-8-d. The resonances of both the C₆-H and C₈-H protons are absent from the nmr spectrum of this product securing the structural assignment as the 6 phenylation product.

Modern localization energy data apparently are not available for the free radical substitution reactions of purine, however, the electron density distribution calculated for purine implies that substitution by an electrophilic radical should occur at the 8 position.^{18,19} In any event, the phenylation reaction of the unsubstituted compound proceeds rather selectively at the 6 position

under the conditions of our experiments. Additional work will be necessary to establish whether or not other radical reagents also substitute preferentially at the 6 position.

It has been known for sometime that the purines with two activating groups such as guanine and xanthine react rather readily with benzenediazonium ions in basic solution to yield 8-azo coupling products whereas the less reactive purines such as hypoxanthine and adenine react differently.¹⁴

On the other hand, none of the purines with 9 substituents yield azo coupling products. This ob-



servation suggests that tautomers without protons in the 9 position are key intermediates in the coupling reactions.¹⁴

One objective of this research was the determination of the course of the reactions of the diazohydroxides and diazonium ions with the nucleic acid constituents. The reaction pathway for the aryl derivatives can now be reasonably formulated. Among the compounds investigated in this study, adenosine is the most reactive nucleoside. It rapidly forms the N-6 triazene with a variety of aryl diazonium compounds.⁷ Only 5'-guanylic acid also forms an isolable N-2 triazene.^{8,11} The contrasting reactivity is well illustrated by the observation that guanosine forms the 8-phenylation product rather than the N-2 triazene. Inosine without a basic amino group also slowly forms the phenylation product. When the N-6 triazene of adenosine is heated, it also decomposes to yield the 8-phenyl derivative. Prior work suggests that the N-2 and N-6 triazenes are formed in reversible reactions^{7,8} and the diazonium ions with which they are in equilibrium decompose in dilute basic solution via reaction with the diazohydroxides to yield phenyl radicals.²⁰ The radicals react selectively at the 8 position of the purine nucleosides to yield substitution products. The ribose moiety is cleaved from adenosine during the free radical substitution reaction, but this cleavage reaction does not occur during the phenylation of inosine or, more importantly, guanosine.

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 and Bruker 270 MHz spectrometers.

The Pyrimidines.--The azo coupling products were prepared by the same general method. For example, a solution of 4-bromobenzenediazonium chloride (10 mmole) was prepared as described previously⁷ and was added dropwise to a solution of uracil (0.56g, 5 mmole) in 0.62 N sodium hydroxide (40 ml)

at 0°C. The pH was maintained between 10 to 11 by the dropwise addition of aqueous sodium hydroxide. After the addition of the diazonium ion was complete, the solution was stirred for 24 hours. The solution was then neutralized to pH 7 with 1.0 N hydrochloric acid, the precipitate which formed was collected on a filter, washed thoroughly with ether, ethyl acetate, chloroform and water, and air-dried to give 5-(4-bromophenylazo)uracil, 1b, in 40% yield. Compound 1a was obtained in 57% yield. The principal features of the spectra of the 5-(arylo)uracils are presented in Table I.

When cytosine was used as a substrate, the mixture of products obtained darkened rapidly in air during washing process and we were unable to isolate pure 5-(arylo)cytosine, although the spectroscopic properties of the crude product suggest that this material was produced in low yield. Uridine and cytidine were also used as substrates. In these cases, the reaction mixtures were neutralized with 1.0 N hydrochloric acid first, then chloroform was added to the solution. The layers were separated and the solvents were removed under vacuum, the residues were carefully examined for new products by nmr spectroscopy and thin layer chromatography. Between 85 and 90% of the cytidine was subsequently recovered from aqueous portion. Only the familiar condensation products resulting from diazonium ion decomposition were identified in the chloroform extract.

The Purines.--The benzenediazonium ion solution was prepared as already described and added to a solution of xanthine in basic solution (pH 10.5). The azo coupling products formed within 15 minutes. The solution was then neutralized with 1.0 N hydrochloric acid to pH 7.0 and the precipitate which formed was collected, washed thoroughly with water and chloroform. This product was purified by solution in 1.0 N potassium hydroxide and reprecipitation with 1.0 N hydrochloric acid. The desired product was collected by filtration and washed with chloroform and water repeatedly, and air-dried to give the 8-(4-substituted phenylazo)xanthines. Compounds 2a, 2b, and 2c were obtained in 51, 24 and 61% yield, respectively. The principal features of the spectra of 3 are presented in Table II.

The reaction mixtures of inosine and the benzenediazonium ions were prepared in the same way as the xanthines. However, the reaction was much slower and reaction mixtures were stirred for 48 hours at room temperature prior to neutralization with 1.0 N hydrochloric acid. The precipitate which formed was collected, washed with chloroform, water and cold methanol to give the light yellow 8-arylinosines. Compounds 3a and 3b were obtained in 10% yield. Their spectra are presented in Table III.

A solution of 4-bromobenzenediazonium chloride (20 μ mole) was prepared as described previously and was added to a solution of purine (1.2 g, 10 μ mole) in 0.62 N sodium hydroxide (80 ml). The reaction mixture was stirred for 48 hours at room temperature. The pH was maintained between 10 to 11. The product was isolated and purified as described to yield 6-(4-bromophenyl)purine (0.4 g, 15%).

The nmr spectrum of this compound was recorded in dimethyl-d₆ sulfoxide: 8.97(s, C₂-H), 8.67(s, C₈-H), 8.83(d,2H), 7.82(d,2H), J=8.3 Hz. Purine-8-d was prepared as described by Schweizer and his coworkers¹⁷ in quantitative yield. When purine-8-d was used as the substrate, the nmr spectrum of 6-(4-bromophenyl)-8-d-purine in dimethyl-d₆ sulfoxide (8.95(s,C₂-H), 8.83(d,2H), 7.83(d,2H), J=8.2 Hz] contained one less resonance.

Acknowledgement.--This research was supported by Grant No. CA-20049, awarded by the National Cancer Institute, DHEW. The NMR work was assisted by Grant NO. CA-14599, awarded by the National Cancer Institute of the Public Health Service.

1. This article is contributed in the honor of Professor Herbert C. Brown on the occasion of his 70th birthday.
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Received, 12th September, 1981