Reactivity–Selectivity Properties of Reactions of Carcinogenic Electrophiles with Biomolecules. Kinetics and Products of the Reaction of Benzo[a]pyrenyl-6-methyl Cation with Nucleosides and Deoxynucleosides

Robert E. Royer,[1a] T. A. Lyle, G. G. Moy, Guido H. Daub,* and David L. Vander Jagt*[1b]

Departments of Biochemistry and Chemistry, University of New Mexico, Albuquerque, New Mexico 87131

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The nucleophilicities of the nucleosides and deoxynucleosides toward the carbonium ion generated by solvolysis of the chemical carcinogen 6-(chloromethyl)benzo[a]pyrene were measured by the method of inhibition of the common-ion effect. Nucleosides or deoxynucleosides containing adenine, guanine, or cytosine were able to trap the carbonium ion in competition with water while those containing uracil or thymine were not. Product distribution studies indicated that two products are formed when the carbonium ion is trapped by adenosine, guanosine, or cytidine (or their deoxy analogues). No detectable products were formed with uridine or thymidine. The chemical shifts of the arylmethyl carbon of labeled products prepared by trapping the carbonium ion from solvolysis of 6-(chloromethyl-^{13}C)benzo[a]pyrene indicated that in each case the labeled carbon is bonded to a nitrogen. Consideration of the carbon chemical shifts, pH effects on the UV–vis spectra, chromatographic properties, and chemical properties of the products led to the assignment of structures as follows: (1) from trapping with adenosine, 1-(benzo[a]pyrenyl-6-methyl)adenosine and N^6-(benzo[a]pyrenyl-6-methyl)adenosine; (2) from trapping with guanosine, 7-(benzo[a]pyrenyl-6-methyl)guanosine and N^2-(benzo[a]pyrenyl-6-methyl)guanosine; (3) from trapping with cytidine, 3-(benzo[a]pyrenyl-6-methyl)cytidine and N^4-(benzo[a]pyrenyl-6-methyl)cytidine. The order of nucleophilicity is guanosine > adenosine > cytidine.

Known chemical carcinogens represent a variety of chemical structures. Owing to the work of Miller and Miller,[3] it is now generally recognized that the unity behind this diversity of structures is the fact that all chemical carcinogens are electrophiles, either as encountered in the environment or after metabolic activation within the target tissue. Many chemical carcinogens react covalently with isolated biomolecules, especially DNA, and some of these reactions undoubtedly are related to those in vivo reactions which lead to chemically induced cell transformation. The extent of these covalent modifications of DNA in vivo is often very small, and, even among these, some relatively minor reactions, in terms of amount, may be the critical reactions in chemical carcinogenesis. The lack of correlation between extent of covalent modification and carcinogenicity suggests that the selectivity of attack of carcinogenic electrophiles on biomolecules is critical.

Among the methylated polycylic aromatic hydrocarbons, 7,12-dimethylbenz[a]anthracene and the two corresponding monomethyl compounds have been studied most widely. These carcinogens are metabolized both by way of arene oxide mediated ring hydroxylation and by way of methyl group hydroxylation. Dipple et al. have suggested that methylated polycyclic aromatic hydrocarbons might be converted into carcinogenic metabolites by formation of hydroxymethyl derivatives which can be converted into esters capable of generating arylmethyl cations.[4] The carcinogenicity of methylated polycyclics appears to correlate with the stability of the arylmethyl cation. 6-Methylbenzo[a]pyrene has been reported to be a more potent carcinogen than benzo[a]pyrene or than other methylbenzo[a]pyrenes.[5] 6-Methylbenzo[a]pyrene can be converted into 6-(hydroxymethyl)benzo[a]pyrene,[6] and both 6-(hydroxymethyl)benzo[a]pyrene and 6-(bromomethyl)benzo[a]pyrene are carcinogens.[7] In addition, benzo[a]pyrene can be hydroxymethylated in certain systems by a pathway which does not require cytochrome P-450.[8]

Chemical carcinogens comprise a range of electrophiles which may be expected to span the reactivity scale from limiting S_N1 to limiting S_N2. It is well recognized that the reactivity–selectivity principle predicts that the relative abilities of a group of nucleophiles to compete with water for an electrophile differ widely along the reactivity span from S_N1 to S_N2. In the previous paper,[9] we discussed the methodology used to study the reactivity–selectivity of the carbonium ion generated by solvolysis of 6-(chloromethyl)benzo[a]pyrene (6-CMBP).[2] We report here on the application of this methodology to studies of the selectivity of attack of this electrophile on nucleosides, deoxynucleosides, and nucleotides.

Experimental Section

Materials. The syntheses of several 6-(substituted-methyl)benzo[a]pyrenes, labeled and unlabeled, were reported previously.[9,10] 6-(Bromomethyl-^{13}C)benzo[a]pyrene (6-BMBP-^{13}C) was prepared from 6-(hydroxymethyl-^{13}C)benzo[a]pyrene (90% enriched in the hydroxymethyl carbon) by heating the alcohol (0.3 g, 1.06 mmol), PBr_3 (0.5 g, 1.85 mmol), and anhydrous benzene (18 mL) at reflux temperature for 1 h. After concentration to 10 mL, the solution was cooled and the resulting precipitate was recrystallized from benzene giving 0.22 g (60%) of product; mp 225–226 °C (lit.[7] 225–226 °C). The acetone was reagent grade and was used without further purification. Dimethylacetamide was redistilled and was stored over molecular sieves. Dioxane was redistilled immediately before use. Aqueous acetone and

(1) (a) Taken in part from the Ph.D. thesis of R.E.R., submitted to the Graduate School of the University of New Mexico in partial fulfillment of the requirements for the Ph.D. degree, 1977. (b) Author to whom correspondence should be addressed at the Department of Biochemistry, University of New Mexico School of Medicine, Albuquerque, N.M. 87131. (c) This work was supported by NIH Grant CA 16871 and by a Research Career Development Award (CA 70939) to D.L.V.J., both grants from the National Cancer Institute.

(2) Abbreviations: 6-CMBP, 6-(chloromethyl)benzo[a]pyrene; BAP, benzo[a]pyrene; 6-BMBP, 6-(bromomethyl)benzo[a]pyrene.

(3) E. C. Miller and J. A. Miller, *Annu. Rev. Biochem.*, **28**, 291 (1959); J. A. Miller, *Cancer Res.*, **30**, 559 (1970).

(4) A. Dipple, P. D. Lawley, and P. Brookes, *Eur. J. Cancer*, **4**, 493 (1968).

(5) A. Dipple and T. A. Slade, *Eur. J. Cancer*, **7**, 473 (1971).

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(7) R. K. Natarajan and J. W. Flesher, *J. Med. Chem.*, **16**, 714 (1973).

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Table I. R_f Values for Methyl-Substituted 6-Methylbenzo[a]pyrenes

compd	R_f values			
	silica gel		microcrystalline cellulose	
	A[b]	B[b]	C[b]	D[b]
6-(chloromethyl)benzo[a]pyrene[a]	0.75			
6-(hydroxymethyl)benzo[a]pyrene[a]	0.70	0.79	0.96	0.56
6-[(n-propylamino)methyl]benzo[a]pyrene[a]	0.42			
(benzo[a]pyrenyl-6-methyl)triethylammonium chloride[a]	0		0.81	
S-(benzo[a]pyrenyl-6-methyl)-N-acetylcysteine[a]	0.60			
(benzo[a]pyrenyl-6-methyl)pyridinium chloride[a]	0	0.33		
6-(azidomethyl)benzo[a]pyrene[a]	0.81			
6-(anilinomethyl)benzo[a]pyrene[a]	0.82			
adenosine				
product A		0.18	0.47	0.19
product B		0.46	0.70	0.40
cytidine				
product A		0.51	0.49	0.30
product B		0.80	0.95	0.45
guanosine				
product A		0.10	0.39	0.24
product B		0.30	0.74	0.42

[a] See ref 9 for details of the syntheses of these compounds. [b] Solvent systems: A, acetone; B, 9:1 acetone-methanol; C, 86:14 1-butanol-H_2O; D, 7:2 methanol-concentrated HCl-H_2O.

aqueous dioxane solutions were prepared volume to volume. The nucleosides, deoxynucleosides, and nucleotides (Sigma) were used without further purification.

Kinetics. Reaction rates for solvolysis of 6-CMBP in aqueous organic solvents, with or without added nucleophiles, were determined from changes in optical density between reactant and products. Details were reported previously.[9]

Chromatography. Thin-layer chromatography was carried out on silica gel and microcrystalline cellulose plates, 5 × 20 cm, precoated on glass (Applied Science Laboratories, Inc.). Chromatograms were developed in the dark in covered jars in an atmosphere saturated with developing solvent. Solvents used are listed in Table I. All products containing the benzo[a]pyrenyl-6-methyl moiety gave fluorescent spots which were viewed under UV light. Unreacted nucleosides were visible as absorbing spots.

Column chromatography of the products produced by trapping of the carbonium ion from 6-CMBP was carried out on Sephadex LH-20 (Pharmacia), using methanol to elute the samples off the column. The column (75 × 1.5 cm) was run at 4 °C in the dark. Samples (5 mL) were collected and monitored at 392 nm for the benzo[a]pyrene moiety. The fractions containing a given product were pooled, and the methanol was removed on a rotary evaporator. Products were stored in Me_2SO-d_6 for those samples which were analyzed by ^{13}C NMR.

^{13}C NMR Samples of Products from Trapping Reactions. Reactions run under solvolytic conditions to prepare ^{13}C NMR samples of the products from trapping the carbonium ion of 6-CMBP with nucleosides were run as follows. To 10 mL of a solution containing 7.5 mL of water, 2.5 mL of dioxane, and 25 μmol of appropriate nucleoside was added dropwise and with stirring a solution of 25 μmol of 6-CMBP (^{13}C enriched) in 5 mL of dioxane. The addition was accomplished over a period of about 10 min. By this procedure, the composition of the solvent averaged 50% dioxane, comparable to the kinetic studies which were carried out in 50% aqueous organic solvents. After completion of the reaction and concentration of the reaction mixutre by rotary evaporation, column chromatography of the reaction mixture as described above gave sufficient material for ^{13}C NMR studies of the first products eluted off Sephadex LH-20 when adenosine or guanosine was used as the nucleoside. These procedures were repeated with acetone in place of dioxane except that in the case of guanosine, only 5 μmol could be used, owing to its limited solubility in 50% acetone. TLC and column chromatography indicated that the product composition did not vary if acetone replaced dioxane in the reaction mixture.

In order to prepare sufficient quantities for ^{13}C NMR analysis of the late-eluting product off the LH-20 column when cytidine was the trapping agent, we used the same procedure except that 6-CMBP and cytidine were increased fourfold and the solvent volume was doubled. A larger LH-20 column (3-cm diameter) was required for the chromatography. The method used to prepare ^{13}C NMR samples of the late-eluting peak when adenosine was the trapping agent is discussed in the Results.

Reaction of 6-BMBP (^{13}C Enriched) with Nucleosides, in Dimethylacetamide. In order to prepare sufficient quantities for ^{13}C NMR analysis of the late-eluting product off the LH-20 column when guanosine was the trapping agent, it was necessary to carry out a reaction under nonsolvolytic conditions. ^{13}C-enriched 6-BMBP (25 μmol) was dissolved in 5 mL of dimethylacetamide containing 17 mg of guanosine. After 16 h, the mixture was chromatographed on LH-20 and also tested by TLC. The same two trapped products were formed as are formed under solvolytic conditions. Since very little alcohol formed, this procedure in dimethylacetamide provided larger amounts of products with respect to the amount of starting material than was obtained by solvolysis. Somewhat surprisingly, the relative amounts of the two products were similar to the amounts trapped under solvolytic conditions. This same procedure was repeated with cytidine. Only the early-eluting product was produced in significant amounts.

Spectra. Emission and excitation spectra of TLC samples and fractions from the Sephadex LH-20 columns were obtained with a Perkin-Elmer MPF-44a fluorescence spectrophotometer with corrected spectra accessory and a Houston Instruments Omnigraphic 2000 X-Y recorder. UV-vis spectra were obtained with a Cary 219 recording spectrophotometer. ^{13}C NMR spectra were obtained either with a Varian XL-100 CW/FT (Nicolet TT 100 FT) spectrometer or with a Varian CFT-20 spectrometer, on FT mode with proton decoupling.

Samples purified by column chromatography showed single spots on thin-layer chromatography and, for ^{13}C-enriched samples, single peaks by ^{13}C NMR analysis. The following UV-vis parameters were recorded for the various purified products obtained by trapping with adenosine, guanosine, or cytidine. **(1) From Trapping with Adenosine:** 1-(benzo[a]pyrenyl-6-methyl)-adenosine (peak A), λ_{max} (log ε) (in 95% ethanol) 408 (4.31), 400 (4.41), 380 (4.42), 361 (4.16), 303 (4.73), 291 (4.68), 278 (4.58), 267 (4.79), 254 (4.74) nm; N^6-benzo[a]pyrenyl-6-methyl)adenosine (peak B), 406 (4.93), 393 (4.46), 373 (4.42), 354 (4.11), 300 (4.70), 288 (4.67), 276 (4.58), 266 (4.73) nm.

(2) From Trapping with Guanosine: 7-(benzo[a]pyrenyl-6-methyl)guanosine (peak A), λ_{max} (log ε) (in 95% ethanol) 406 (4.18), 397 (4.42), 384 (4.30), 376 (4.42), 358 (4.15), 301 (4.75), 289 (4.72), 277 (4.59), 266 (4.80), 257 (4.75) nm; N^2-(benzo[a]pyrenyl-6-methyl)guanosine (peak B), 406 (3.89), 392 (4.47), 371 (4.42), 353 (4.13), 299 (4.70), 287 (4.68), 275 (4.59), 266 (4.76), 255 (4.74) nm.

(3) From Trapping with Cytidine: 3-(benzo[a]pyrenyl-6-methyl)cytidine (peak A), λ_{max} (log ε) (in 95% ethanol) 407 (4.20),

Table II. Solvolytic Reactivity of 6-CMBP in 50% Acetone, 500 mM LiCl, in the Presence of Added Nucleosides, Nucleotides, and Deoxynucleosides

nucleophile[a,f]	concn, mM	k,[b] s^{-1}	k_{Nu}/k_{H_2O}[e]
...	...	0.97×10^{-2}	
UMP	10	1.0×10^{-2}	
U	10	0.99×10^{-2}	
U	50	0.99×10^{-2}	
TMP	10	0.98×10^{-2}	
T	10	0.99×10^{-2}	
T	50	1.0×10^{-2}	
CMP	10	1.1×10^{-2}	
dCMP	10	1.0×10^{-2}	
C	10	1.0×10^{-2}	
dC	10	1.1×10^{-2}	
C	50	1.4×10^{-2}	8
AMP	10	1.1×10^{-2}	
dAMP	10	1.5×10^{-2}	
A	10	1.2×10^{-2}	
dA	10	1.2×10^{-2}	
A	50	2.4×10^{-2}	30
dGMP	10	1.5×10^{-2}	
G	10	1.3×10^{-2}	
dG	10	1.4×10^{-2}	
G	50	2.6×10^{-2} [c]	35
GMP	10	1.4×10^{-2} [d]	
G	10	1.6×10^{-2} [d]	

[a] All solutions of the nucleophiles were adjusted to pH 7 before addition of acetone and 6-CMBP. [b] All rate data at 25 °C; rate constants reproducible to ±5%. [c] Extrapolated rate constant for G from rate date at 1–10 mM. [d] Measured in 50% dioxane containing 500 mM liCl. [e] k_{Nu}/k_{H_2O}, defined in eq 4, is the second-order rate constant for reaction of R^+ with Nu divided by the first-order constant for reaction of R^+ with H_2O. [f] Abbreviations: U, T, C, A, and G for uridine, thymidine, cytidine, adenosine, and guanosine; dC, dA, and dG for the deoxyribose analogues; UMP, TMP, CMP, dCMP, AMP, dAMP, and dGMP for the nucleotide 5′-phosphates.

398 (4.44), 378 (4.42), 359 (4.14), 343 (3.78), 301 (4.84), 277 (5.10), 268 (5.12) nm; N^4-(benzo[a]pyrenyl-6-methyl)cytidine (peak B), 406 (3.87), 391 (4.48), 371 (4.42), 353 (4.11), 299 (4.74), 287 (4.67), 266 (4.73), 256 (4.70) nm.

Results and Discussion

Kinetic Analysis of the Nucleophilicities of Nucleosides, Deoxynucleosides, and Nucleotides by Inhibition of the Common-Ion Effect. If a solvolytic reaction proceeding by way of a limiting S_N1 process leads to the formation of a stabilized carbonium ion and shows a strong common-ion effect, as is observed with 6-CMBP, then the nucleophilicity of an added nucleophile can be evaluated kinetically by measuring the ability of this nucleophile to inhibit the common-ion effect (eq 1). It

$$RX \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} R^+ + X^- \xrightarrow[k_2]{H_2O} ROH \qquad R^+ \xrightarrow[k_{Nu}]{Nu} RNu \tag{1}$$

was shown previously[9] that the nucleophilicity of an added nucleophile can be evaluated (eq 2) relative to the nu-

$$\frac{k_{obsd}'}{k_{obsd}} = \frac{k_1k_{H_2O} + k_1k_{Nu}[Nu]}{\dfrac{k_{-1}[X^-]}{k_1k_{H_2O}}} = 1 + \frac{k_{Nu}[Nu]}{k_{H_2O}} \tag{2}$$

cleophilicity of water by measuring the effect of the nucleophile on the solvolysis rate k_{obsd}' compared to the rate in the absence of the nucleophile (k_{obsd}).

Table III. Comparison of the Selectivity of Trapping of the Carbonium Ion from 6-(Chloromethyl)benzo[a]pyrene by Nucleosides and Other Nucleophiles[b]

nucleophile	k_{Nu}/k_{H_2O}	nucleophile	k_{Nu}/k_{H_2O}
aniline	1.7×10^3 [a]	adenosine	30
azide	1.4×10^3 [a]	n-propylamine	20[a]
chloride	4.8×10^2 [a]	hydroxide	16[a]
N-acetylcysteine	40[a]	cytidine	8
pyridine	40[a]	diethylamine	3[a]
guanosine	35		

[a] From ref 9. [b] All data at 25 °C in 50% acetone containing 500 mM LiCl.

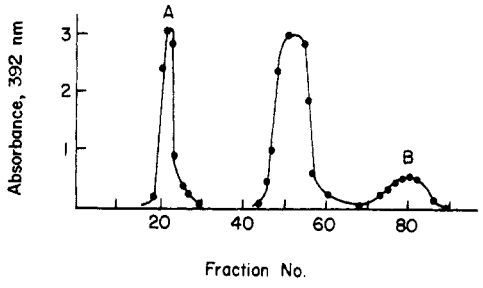


Figure 1. Elution profile on Sephadex LH-20 of the reaction mixture from the solvolysis of 6-CMBP in the presence of adenosine. Products A and B (1 and 2) correspond to the first and third products eluted from the column. The second product corresponds to the alcohol 6-(hydroxymethyl)benzo[a]pyrene.

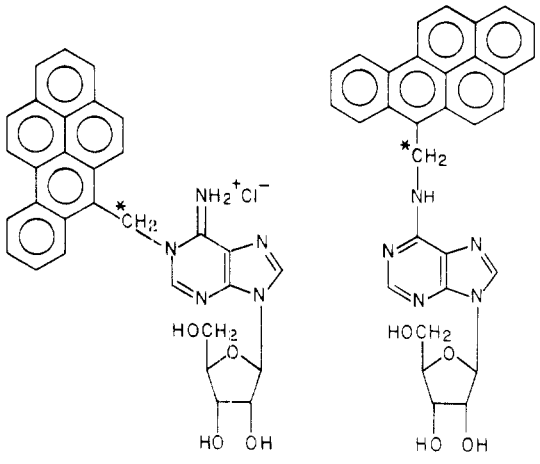
Table II lists the effects of nucleosides, deoxynucleosides, and nucleotides on the rate of solvolysis of 6-CMBP in 50% aqueous acetone containing 500 mM LiCl. Within experimental error (rates reproducible to ±5%), uridine, thymidine, UMP, and TMP show no effect on the rate of solvolysis of 6-CMBP even at concentrations of uridine or thymidine up to 50 mM. CMP, dCMP, cytosine, and deoxycytosine show borderline effects at 10 mM concentrations. At 50 mM concentration, cytosine has a definite effect on the solvolysis rate leading to a calculated selectivity value $k_{Nu}/k_{H_2O} = 8$. AMP, dAMP, adenosine, deoxyadenosine, dGMP, guanosine, and deoxyguanosine all affect the solvolysis rate. For adenosine, this leads to a calculated selectivity $k_{Nu}/k_{H_2O} = 30$; for guanosine, $k_{Nu}/k_{H_2O} = 35$. GMP was not sufficiently soluble in 50% acetone to evaluate by this procedure. However, GMP is sufficiently soluble in 50% dioxane. Solvolysis of 6-CMBP in 50% dioxane containing 500 mM lithium chloride is almost identical with solvolysis in the acetone system. Table II shows that both GMP and deoxyguanosine inhibit the common-ion effect in 50% dioxane.

Several conclusions can be drawn from these kinetic data: (1) the presence or absence of the 5′-phosphate or 2′-hydroxyl on the ribose does not appear to affect the nucleophilicity; (2) nucleophilicity is dependent upon the presence of guanine, adenine, or cytosine in the order G > A > C. No kinetic evidence was obtained to indicate any appreciable nucleophilicity for U or T.

Table III compares the nucleophilicities of G, A, and C with other nucleophiles measured by inhibition of the common-ion effect.[9] Guanosine and adenosine are comparable to pyridine in this test system.

Product Identification from Trapping with Adenosine. Solvolysis of 6-CMBP-^{13}C in 50% dioxane containing 25 mM adenosine was carried out as described in the Experimental Section. The reaction mixture was chromatographed on Sephadex LH-20 with methanol (Figure 1). The fractions constituting peak A in Figure 1 were pooled, the solvent was removed by rotary evap-

oration, and the residue was dissolved in 5 mL of Me_2SO-d_6 for ^{13}C NMR analysis. A single band with chemical shift 47.5 ppm downfield from Me_4Si was observed, under proton-decoupling conditions, consistent with trapping by nitrogen.[9] The literature[11-13] concerning the alkylation of adenosine suggests that relatively stable carbonium ions might alkylate adenosine at either N-1 or N-6. Thus the expected products have structures 1 and 2.



1 2

When 1-alkyladenosines are heated with base, they often rearrange to form N^6-alkyladenosines via the Dimroth rearrangement.[14,15] Product A in Figure 1 was treated with base under two sets of conditions. In one procedure, product A was placed in a sealed tube with concentrated ammonium hydroxide and heated in a boiling water bath for 1 h. In the other procedure, product A was maintained at 50 °C for 24 h in 50% methanol containing 50 mM sodium hydroxide. Either treatment converted product A into a compound which appears identical with the product in peak B of Figure 1. It had the same elution volume on Sephadex LH-20 and the same UV spectrum as product B and was inseparable from product B by TLC. Consequently, product A appears to be the product of trapping by N-1 (structure 1).

Product B of Figure 1 was not affected by treatment with ammonium hydroxide or sodium hydroxide. The chemical shift of the labeled carbon in product B was determined on a sample containing a mixture of product B isolated by chromatography of the solvolytic reaction mixture (Figure 1) and product B formed by the base-catalyzed rearrangement of product A. A single resonance was observed at a chemical shift 46.0 ppm downfield from Me_4Si. Structure 2, corresponding to trapping by N-6 of adenosine, was thus assigned to product B.

Additional support for the assignment of 1 and 2 to products A and B was obtained from the pH effects on the absorption spectra. Product A appears to lose a proton at roughly neutral pH, an event which is reversible and can be followed by changes in the absorption spectrum (Figure 2). Within experimental error, the change in pH does not alter the chemical shift of the labeled carbon, suggesting that the structure of the protonated form is best

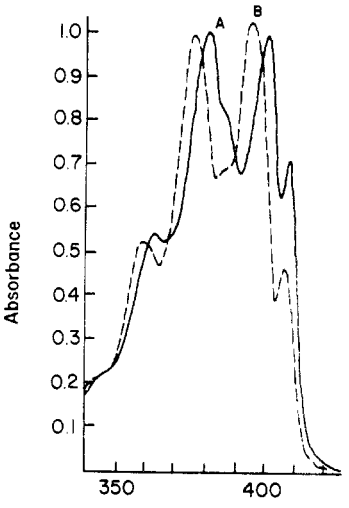
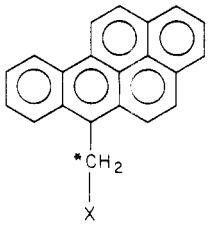


Figure 2. Absorption spectra of product A (Figure 1) from trapping the carbonium ion from 6-CMBP with adenosine: spectrum A, low pH; spectrum B, high pH. The concentration was ca. 4×10^{-5} M.

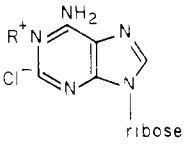
Table IV. Chemical Shifts of the Labeled Arylmethyl Carbon in Some Benzo[a]pyrenyl-6-methyl-^{13}C Derivatives



X	δ^a	X	δ^a
H	14.3[b]	$(CH_3CH_2)_3N^+$	56.8[b]
Cl	39.5[b]	adenosine-N-1 (1)	47.5
$CH_3CH_2CH_2NH$	45.6[b]	adenosine-N-6 (2)	46.0
HO	55.8[b]	cytidine-N-3 (4)	44.1
$AcNHCH(CO_2^-)CH_2S$	29.6[b]	cytidine-N-4 (5)	40.8
PhNH	40.2[b]	guanosine-N-7 (6)	45.7
N_3	46.5[b]	guanosine-N-7 (10)	44.6
pyr^+	55.2[b]	guanosine-N-2 (7)	45.6

[a] Chemical shifts are in parts per million downfield from Me_4Si. [b] Reference 9.

represented by the protonated imine (1) rather than as a quaternary ammonium salt (3). The chemical shifts of



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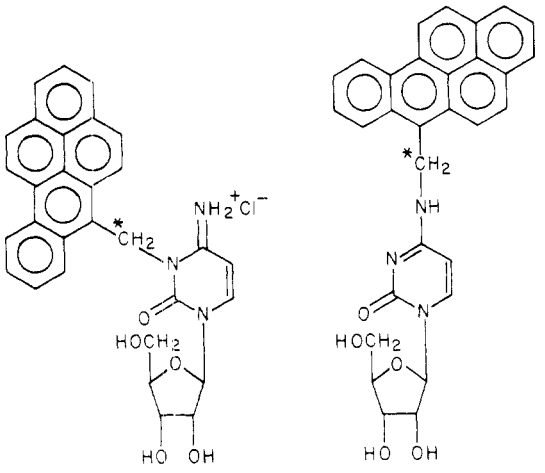
the arylmethyl carbon in a simple quaternary ammonium salt is well downfield of the chemical shift of product A (Table IV). Product B did not show a pH-dependent change in its absorption spectrum.

Product Identification from Trapping with Cytidine. Solvolysis of 6-CMBP-^{13}C in 50% dioxane containing cytidine was carried out as described in the Experimental Section. Chromatography on Sephadex LH-20 gave a profile similar to that observed from trapping the carbonium ion with adenosine (Figure 1) except that products A and B were obtained in smaller amounts relative to the amount of alcohol. A sample of product A for ^{13}C NMR analysis was obtained by allowing 6-BMBP-^{13}C to react with cytidine in dimethylacetamide (see Experimental Section). Its identity with product A

(11) A. M. Jeffrey, S. H. Blobstein, I. B. Weinstein, F. A. Beland, R. G. Harvey, H. Kasai, and K. Nakanishi, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 2311 (1976).
(12) P. Brookes, A. Dipple, and P. D. Lawley, *J. Chem. Soc.*, 2026 (1968).
(13) A. Dipple, P. Brookes, D. S. MacKintosh, and M. P. Rayman, *Biochemistry*, **10**, 4323 (1971).
(14) M. H. Fleysher, A. Bloch, M. T. Hakala, and C. A. Nichol, *J. Med. Chem.*, **12**, 1056 (1969).
(15) O. Dimroth, *Justus Liebigs Ann. Chem.*, **364**, 183 (1909).

was confirmed by TLC. The chemical shift of product A is 44.1 ppm downfield from Me_4Si, consistent with trapping by nitrogen[9] (Table IV). A sample of product B for ^{13}C NMR analysis was obtained from chromatography of material obtained from a large-scale solvolysis experiment, using 6-CMBP-^{13}C. The chemical shift of product B is 40.8 ppm downfield from Me_4Si. The literature[12,16] for trapping by cytidine suggests that attack by N-3 or N-4 will predominate. Thus the expected products have structures **4** and **5**.



4 **5**

By analogy with the products from trapping with adenosine, structures **4** and **5** are assigned to products A and B from trapping with cytidine. The nucleosides substituted on the primary amine (**2** and **5**) are eluted later from Sephadex LH-20 and have high R_f values by TLC on silica gel and microcrystalline cellulose (Table I). In addition, the chemical shifts of the labeled carbons in **2** and **5** are upfield of the ring-substituted products, **1** and **4**. In addition, product A from trapping with cytidine (**4**) shows a pH-dependent, reversible change in absorption spectrum similar to that of **1** (Figure 2).

An attempt was made to convert product A into product B by way of the Dimroth rearrangement. Treatment of product A with sodium hydroxide resulted in the formation of an unknown product with a chemical shift 37.3 ppm downfield from Me_4Si. This product shows low R_f values in the TLC systems used, unlike product B. No Dimroth rearrangements have been reported in the literature for 3-substituted cytidines.

Product Identification from Trapping with Guanosine. Solvolysis of 6-CMBP-^{13}C in 50% dioxane containing guanosine was carried out as described for trapping with adenosine. Chromatography on Sephadex LH-20 again gave a profile similar to that for adenosine (Figure 1), except that larger amounts of product A were formed relative to alcohol or product B. The material eluted in the first peak (product A) was sufficient for ^{13}C NMR analysis. A single resonance with a chemical shift 45.7 ppm downfield from Me_4Si was observed, consistent with trapping by nitrogen (Table IV). Sufficient product B for ^{13}C NMR analysis was obtained by running the reaction with 6-BMBP-^{13}C in dimethylacetamide (see Experimental Section). The identity of this material with product B produced under solvolysis conditions was confirmed by chromatography. Product B showed a chemical shift for the labeled carbon which was 45.6 ppm downfield from Me_4Si. Literature data[12,13] for attack of electrophiles on guanosine suggest that carbonium ion type electrophiles

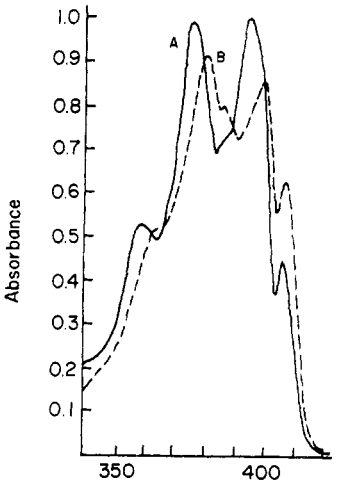
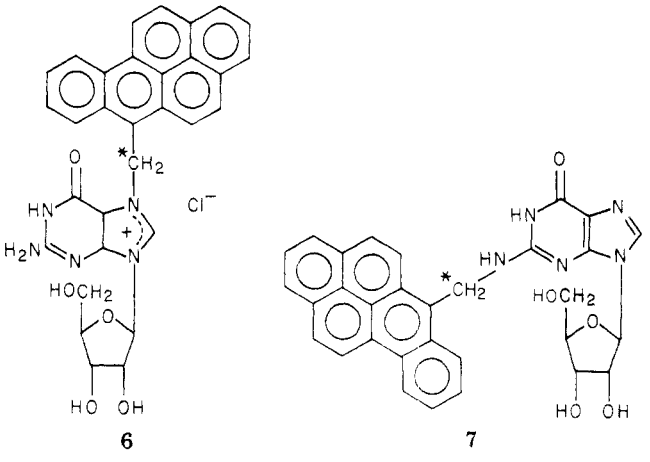


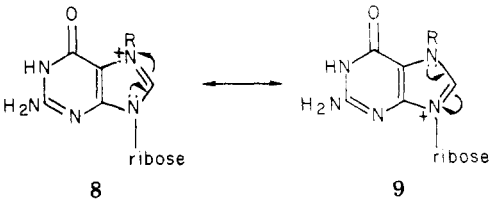
Figure 3. Absorption spectra of product A from trapping the carbonium ion from 6-CMBP with guanosine: spectrum B, low pH; spectrum A, spectrum of product A after addition of sodium hydroxide followed by solubilization of the precipitate in methanol. Addition of HCl to this sample results in a solution with an absorption spectrum identical with B. The concentration was ca. 4×10^{-5} M.

alkylate primarily at N-7 and N-2. The expected products have structures **6** and **7**. Products from attack at N-7 (**6**)



6 **7**

can also be represented by structures **8** and **9**.



8 **9**

Structure **6** resulting from attack at N-7 is assigned to product A, the early-eluting material. The absorption spectrum of product A in dilute acid (pH 5.5) is shown in Figure 3. When the pH was raised to 8.5, a yellow solid precipitated. Figure 3 shows the absorption spectrum of this precipitated material redissolved in methanol. Addition of acid converts the spectrum back to the low-pH form (Figure 3B). The pH-dependent changes in the spectra are quite different from those for **1** or **4** (Figure 2). It has been suggested[17] that guanosine substituted at N-7 can lose a proton from N-1 and form the betaine (**10**). Labeled product A, after precipitation with sodium hydroxide, has a chemical shift of the labeled carbon 44.6 ppm downfield from Me_4Si. This represents a 1.1-ppm

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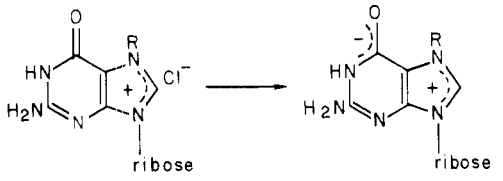

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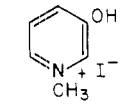
upfield shift on deprotonation. Takeuchi and Dennis[18] recently reported the chemical shifts of some 1-methylpyridinium 3-oxides and their betaines (11–14). De-



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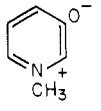

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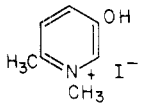

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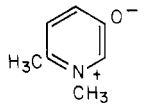

45.3

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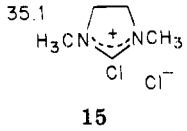
protonation of the 1-methylpyridinium 3-oxides moves the methyl carbon resonance upfield by approximately 2 ppm. These examples are significant because the effects of deprotonation on the chemical shifts are opposite to the downfield carbon shifts observed when amine hydrochlorides are converted into free amines.[19]

Representation of the electronic structure of product A as in structure **6** raises the question of whether the partial positive charge at N-7 would be expected to give a downfield shift, similar to the resonances of carbons in quaternary ammonium salts (Table IV). The reported chemical shift[20] of the methyl carbons in **15** is similar to



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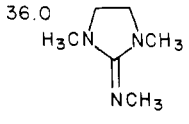

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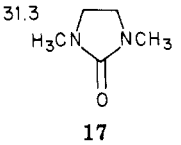

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the chemical shifts of the methyl carbons in the neutral compounds **16** and **17**. Consequently, the assignment of structure **6** to product A seems reasonable.

A number of 7-alkylpurines have been shown to lose the ribose readily upon heating in neutral solution.[17] When product A was heated in a boiling water bath, only the alcohol 6-(hydroxymethyl)benzo[*a*]pyrene was observed, suggesting that **6** loses the 7-alkyl group more readily than it loses the ribose.

Product B from trapping with guanosine shows chromatographic properties similar to those of **2** and **5** from trapping with adenosine and cytidine. Product B does not show the pH-dependent spectral changes of **1**, **4**, and **6**. Assignment of structure **7** to product B seems reasonable.

Solvent Effects on the Selectivity of Trapping. In the experiments described above, the amounts of product A and product B (**1** and **2**) from trapping with adenosine under solvolytic conditions were approximately the same, assuming that the extinction coefficients at 392 nm are similar for **1** and **2**. When the reaction of 6-BMBP with adenosine was carried out in dimethylacetamide, the same two products form also in approximately equal amounts. This is in contrast to the results of Dipple et al.,[13] who observed that 7-(bromomethyl)benzanthracene alkylated adenosine mainly on N-1 in aprotic solvents. The two products from trapping 6-CMBP with cytidine under solvolytic conditions also form in approximately equal amounts. However, in dimethylacetamide, reaction of cytidine with 6-BMBP produced mainly product A (**4**) resulting from attack on N-3, similar to the result of Dipple et al.[13] Solvolytic trapping by guanosine of the carbonium ion from 6-CMBP gave products A and B (**6** and **7**) in a ratio of about 2.5:1, again assuming similar extinction coefficients at 392 nm. However, in dimethylacetamide these two products were obtained in approximately equal amounts. Dipple et al. observed that the reaction of 7-(bromomethyl)benzanthracene with guanosine in aprotic solvents gave mainly 7-substituted guanosine. Consequently, the results here where less N-7 trapping was observed to trapping on N-2 when the reaction was carried out in dimethylacetamide are surprising. Dipple et al. observed that in aqueous solution the carbonium ion from 7-(bromomethyl)benzanthracene reacted mainly at the primary amino groups of adenosine, cytidine, and guanosine. Consequently, it appears that 6-CMBP in aqueous solution is less selective in its positions of attack. This result is not as expected. Since both 6-CMBP and 7-(bromomethyl)benzanthracene would be expected to proceed through limiting S_N1 reactions in aqueous solutions, the more stable carbonium ion from 6-CMBP would be expected to be more selective in its reactions with nucleophiles than the carbonium ion from 7-(bromomethyl)benzanthracene, based upon the reactivity–selectivity principle.[21]

In summary, this study has used the kinetic method of inhibition of the common-ion effect to evaluate the nucleophilicities of nucleosides and deoxynucleosides toward the carbonium ion generated by solvolysis of 6-CMBP as a model for the biological reactions of those carcinogenic electrophiles which proceed through limiting S_N1 reactions. The most nucleophilic site is N-7 of guanine, although N-2 of guanine, N-1 and N-6 of adenine, and N-3 and N-4 of cytosine also exhibit significant nucleophilicity toward the carbonium ion from 6-CMBP. Structures of the products formed in the trapping reactions were assigned from the carbon chemical shifts of the arylmethyl carbon, from the effects of pH on these chemical shifts and on the absorption spectra, from properties of the products on Sephadex LH-20 chromatography, and by reference to pertinent data in the literature.

Registry No. **1**, 70682-24-5; **2**, 70682-25-6; **4**, 70682-26-7; **5**, 70682-27-8; **6**, 70714-14-6; **7**, 70682-28-9; **10**, 70682-29-0; 6-(chloromethyl)benzo[*a*]pyrene, 49852-84-8; 6-(hydroxymethyl)benzo[*a*]pyrene, 21247-98-3; 6-[(*n*-propylamino)methyl]benzo[*a*]pyrene, 70682-30-3; (benzo[*a*]pyren-6-ylmethyl)triethylammonium chloride, 70682-31-4; *S*-(benzo[*a*]pyren-6-ylmethyl)-*N*-acetylcysteine, 70703-17-2; (benzo[*a*]pyren-6-ylmethyl)pyridinium chloride, 70682-32-5; 6-(azidomethyl)benzo[*a*]pyrene, 70682-33-6; 6-(anilinomethyl)benzo[*a*]pyrene, 70682-34-7; 6-(bromomethyl-^{13}C)benzo[*a*]pyrene, 70682-35-8; adenosine, 58-61-7; guanosine, 118-00-3; cytidine, 65-46-3; CMP, 63-37-6; dCMP, 1032-65-1; dC, 951-77-9; AMP, 61-19-8; dAMP, 653-63-4; dA, 958-09-8; dGMP, 902-04-5; dG, 961-07-9; GMP, 85-32-5; 6-(hydroxymethyl-^{13}C)benzo[*a*]pyrene, 62084-24-6.

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(21) For a review see A. Pross, *Adv. Phys. Org. Chem.*, **14**, 69 (1977).