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Synthesis and Studies of a Carbon-13 Labelled Chemical Carcinogen

Robert E. Royer

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Dean, Graduate School

December 8, 1977
Synthesis and Studies of a Carbon-13 Labelled Chemical Carcinogen

BY

ROBERT E. ROYER

B.A., Gannon College, Erie, Pennsylvania, 1966

DISSERTATION

Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Chemistry in the Graduate School of The University of New Mexico

Albuquerque, New Mexico December, 1977
ACKNOWLEDGEMENT

I would like to thank Dr. Guido H. Daub who is responsible for my initial interest in this project. The synthetic work described here was his concept. I would also like to thank Dr. David L. Vander Jagt whose ideas and direction made the kinetic and structural studies possible. Both of these men provided me every possible assistance in the pursuit of this research project.

For providing the carbon-13 precursor used in this work, I am grateful to Dr. Donald Ott and H-division of Los Alamos Scientific Laboratory. Dr. Thomas W. Whaley's assistance in obtaining a number of CMR spectra is appreciated.

This research was supported in part by U.S. Public Health Service Grants CA 17298 and CA 16871.
Synthesis and Studies of a Carbon-13 Labelled Chemical Carcinogen

BY
ROBERT E. ROYER

ABSTRACT OF DISSERTATION

Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Chemistry in the Graduate School of The University of New Mexico Albuquerque, New Mexico December, 1977
ABSTRACT

Derivatives of 6-methylbenzo[a]pyrene enriched to 90% carbon-13 at the methyl carbon were synthesized. Reactions of the carcinogen, 6-chloromethylbenzo[a]pyrene, with nucleophiles under solvolytic conditions were studied kinetically and, using labelled 6-chloromethylbenzo[a]pyrene, the reaction products were studied by CMR. The purpose was to characterize the selectivity of the benzo[a]-pyrenyl-6-methyl carbonium ion in its reactions with some simple nucleophiles and with nucleosides and other nucleic acid components.

Although the solvolysis of 6-chloromethylbenzo[a]pyrene in aqueous/organic solution is first order, information about the course of the reaction can be obtained, under certain circumstances, from rate constants. Thus, if the rate of solvolysis is slowed down by the addition of chloride ion, it can be increased by the addition of nucleophiles which compete for the carbonium ion. By using this "inhibition of the common ion effect" method, it was determined that the selectivity of the benzo[a]pyrenyl-6-methyl carbonium ion for reaction with various nucleophiles is generally in line with, yet distinct from that of other relatively stable carbonium ions. The order of nucleophilic strength of the simple nucleophiles studied is: aniline > N₃⁻ > Cl⁻ > pyridine > n-propylamine > OH⁻ > diethylamine > H₂O. Nucleosides, nucleotides and their deoxy analogs containing adenine, guanine or cytosine were found to be nucleophilic toward the carbonium ion while those containing uracil and thymine were not.
Product distribution studies indicated that two conjugates are formed when 6-chloromethylbenzo[a]pyrene is solvolyzed in 50% aqueous acetone or dioxane in the presence of guanosine, adenosine or cytidine. No detectable conjugates were formed with uridine or thymidine. The chemical shifts of the labelled carbons in the conjugates indicate that in each case they are bonded to nitrogen. Consideration of the chemical shifts, basicities and chromatographic properties of the adenosine conjugates led to the assignment of their structures as 1-(benzo[a]pyrenyl-6-methyl)adenosine and N^6-(benzo[a]pyrenyl-6-methyl)adenosine. These assignments were confirmed by the conversion of the 1-substituted adenosine to the N^6-substituted adenosine by treatment with base. Chemical shifts and other properties of the conjugates with guanosine and cytidine, in analogy with those of the adenosine conjugates, led to the assignment of the structures 7-(benzo[a]pyrenyl-6-methyl)guanosine, N^2-(benzo[a]pyrenyl-6-methyl)guanosine, 3-(benzo[a]pyrenyl-6-methyl)cytidine and N^4-(benzo[a]pyrenyl-6-methyl)cytidine to those conjugates.

It was found that, due to the sensitivity of CMR in conjunction with carbon-13 enriched compounds, it was possible to obtain structural information about conjugates produced under solvolytic conditions on a reasonably small scale.
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INTRODUCTION

The problem of determining how chemicals cause cancer has proven to be a difficult one. It has been shown that many chemical carcinogens react covalently with biomolecules, and some of these chemical reactions are undoubtedly involved in the carcinogenic process. The extent of these reactions in living systems, or even in vitro, is often very small, however, and it is possible that even among these, some of the relatively minor reactions may be the critical ones. It is apparent, then, that the best methods available should be used to determine the nature of the reactions and the structures of the resulting conjugates.

This dissertation describes the synthesis of carcinogenic 6-methylbenzo[a]pyrene derivatives labelled with $^{13}$C at the methyl carbon and the application of $^{13}$C nuclear magnetic resonance spectroscopy (CMR) and other methods to the study of the reactions of the carcinogen, 6-chloromethylbenzo[a]pyrene, with some biomolecules. The purpose of the work was to find out as much about the reactions of this carcinogen as possible while evaluating the usefulness of CMR in this and similar work.

There have been numerous reviews of chemical carcinogenesis by leading workers in the field, reviewing and interpreting the volumes of experimental results in the literature. Some general principles about the way in which most chemical carcinogens act have been established, although the exact series of steps by which a carcinogen transforms a cell is not known for any chemical. Any
work in the field should be approached in the light of these ideas. The background information which follows discusses these principles primarily in relation to methylated polycyclic aromatic hydrocarbons. It will be shown that 6-chloromethylbenzo[a]pyrene, in addition to being a carcinogen itself, may be a model for the ultimate carcinogenic metabolites of some methylated polycyclic aromatic hydrocarbons or even, possibly, of benzo[a]pyrene (BAP) itself. The applications of CMR to this type of work are also discussed.
BACKGROUND

CHEMICAL CARCINOGENS

Most discussions in the field of chemical carcinogenesis include the name of Percivall Pott, a London surgeon who, in 1775, made observations on the connection between continual contact with soot and scrotal cancer in chimney sweeps. The number of known and suspected carcinogens has since become large and keeps on growing. Due to their number and diversity, they might only be a confusing list of chemical names. It is now generally believed, however, that most of these chemicals are powerful electrophiles as encountered in the environment or are metabolized to electrophilic intermediates in living organisms. This unifying concept is due largely to the work of Elizabeth and James Miller. Recent work has generally confirmed this idea and the related concept that chemical carcinogens act by forming covalently bonded conjugates with cellular substituents.

During the fifties, a great deal of interest developed concerning the possibility that some carcinogens act by intercalation between the bases in DNA or by other noncovalent interactions with biomolecules. Lerman stimulated interest with evidence that intercalation can cause mutations. In most cases, however, even those carcinogens which are capable of intercalation, such as the polycyclic aromatic hydrocarbons (PAHs), are also covalently bound to cellular
substituents, and their potency correlates poorly with their ability to intercalate. At least for the present, the thrust of the inquiry into chemical carcinogenesis is in the direction of chemically reactive species and covalent interactions.

Polycyclic Aromatic Hydrocarbons. Among the chemical carcinogens, PAHs have attracted special interest. This is partly because they are widely dispersed in the environment from a variety of sources. BAP (I), a potent carcinogen in laboratory animals, for example, was isolated from coal tar by Cook and co-workers in 1933.

\[
\text{I}
\]

This compound is now known to be produced in many combustion processes, including cigarette smoking, and has even been found in ancient ocean sediments, possibly as a result of forest fires.

The covalent binding of chemically inert compounds in biomolecules must be preceded by metabolic activation. Evidence regarding the nature of this activation has existed in the literature for some time. For example, in 1950, Boyland and Wolf found that phenanthrene (II) is metabolized in rats and rabbits to trans-dihydrodiols including \text{trans}-9,10-dihydroxy-9,10-dihydrophenanthrene (IV). This type of metabolite strongly suggests the existence of
an intermediate arene oxide (III). (Fig. 1) Arene oxides are epoxide derivatives of one of the "double bonds" of aromatic rings. They are reactive electrophiles and good candidates for the role of being ultimate carcinogenic metabolites.¹⁸

Figure 1. The isolation of trans-9,10-dihydroxy-9,10-dihydronaphthanthrene (IV) as a metabolite of phenanthrene suggests the existence of phenanthrene 9,10-oxide (III) as an intermediate.

In 1968, Jerina and co-workers demonstrated convincingly that PAHs are metabolized by way of arene oxides by isolating naphthalene 1,2-oxide from rat liver preparations treated with naphthalene.¹⁹ The isolation of this arene oxide explained the formation of further metabolites of naphthalene, not only dihydrodiols, but also phenols and glutathione conjugates.²⁰ They also found that the formation of phenols from arene oxides is consistent with the pattern of deuterium migration noted during the metabolic formation of phenols from PAHs, called the NIH shift. (Fig. 2)
Figure 2. This is an example of the NIH shift which accompanies the isomerization of 1-D-naphthalene-1,2-oxide (V) to 2-D-1-naphthol (VI). Similar deuterium migrations in the metabolic formation of phenols indicate the intermediacy of an arene oxide. (Ref. 22)

The formation of arene oxides is catalyzed by enzymes located on the endoplasmic reticulum of the cell called as a group, cytochrome P-450 or P-448, depending on the absorption maximum of their complex with carbon monoxide. The enzymes are inducible in a complex manner and the system shows differences from species to species and tissue to tissue. Cytochrome P-450 has been purified and the minimum requirements for the system to operate have been determined.
Since 1968, numerous epoxide derivatives of PAHs have been isolated from living systems or have been shown to exist as intermediates by examination of further metabolites. The use of high pressure liquid chromatography has been instrumental in the separation and identification of these compounds which are formed in small amounts and are not always distinguishable by thin layer chromatography.\textsuperscript{23}

Initially it was believed that epoxide derivatives at the position on a PAH which has the highest electron density, the so-called "K" region, were likely ultimate carcinogens. This idea came from the work of the Pullmans, who correlated regions of high electron density with carcinogenicity for various PAHs.\textsuperscript{24} In vivo conjugates of BAP with nucleic acids, however, do not appear to correspond to those from the reaction of benzo[a]pyrene-4,5-oxide (the "K region" epoxide) with DNA in the test tube.\textsuperscript{25,26,27} On the other hand, in vivo conjugates might correspond to those formed when 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]-pyrene (VII) is allowed to react with DNA.\textsuperscript{28} Recently, Jeffrey
and co-workers have isolated a conjugate from tissue cultures incubated with BAP which appears to be a conjugate of the diol epoxide with guanosine.\textsuperscript{29} The implication of the 1,2-epoxy-3,4-dihydroxy-tetrahydrophthalene moiety in chemical carcinogenesis has stimulated an interest in its chemistry\textsuperscript{30,31} similar to the earlier flurry of interest in "K region" oxides. Since \textit{in vivo} and even \textit{in vitro} conjugates are only formed in very small amounts, their identification is difficult and further evidence might shift the focus of attention to other PAH metabolites. It is possible that several other types of intermediates might be involved, including various types of free radicals\textsuperscript{32,33} and ketonic derivatives.\textsuperscript{34}

\textbf{Methylated Polycyclic Aromatic Hydrocarbons.} Of the methylated PAH carcinogens, 7,12-dimethylbenz[a]anthracene (DMBA) (VIII) has been most widely studied, along with the two corresponding monomethyl derivatives. DMBA is a potent carcinogen\textsuperscript{35,36} which also

![VIII](image_url)

causes adrenal necrosis in rats.\textsuperscript{37} Since it has been found that pretreatment of the rats with several PAHs and other compounds "protects" them from the effects of DMBA\textsuperscript{38,39} interest developed in its
metabolism. The protecting effect presumably results from the induction of enzymes affecting the rate or route of metabolism.

Probably the first metabolite of DMBA to be identified was 4-hydroxy-7,12-dimethylbenz[a]anthracene from rat feces by Dickens in 1945. In 1965, Boyland and Sims reported that rat liver homogenates converted DMBA into 7-hydroxymethyl-12-methylbenz[a]anthracene (IX) and 7-methyl-12-hydroxymethylbenz[a]anthracene. The 7-hydroxymethyl derivative is more destructive to the adrenal gland than the parent hydrocarbon. Isolation of other derivatives, including dihydrodiols and glutathione conjugates ensued, and Keysell and co-workers isolated the "K-region" epoxide of DMBA. It is apparent, therefore, that DMBA is metabolized in rats by way of arene oxide mediated ring hydroxylations and, in addition, by way of methyl group hydroxylations. The hydroxymethyl derivatives can be further metabolized by ring hydroxylations and it appears that ring hydroxylation can be followed by methyl group hydroxylation.

Yang and Dower have recently applied high pressure liquid chromatography to the separation and quantification of DMBA metabolites from rat liver microsome preparations. They used microsomes
from control rats and from rats pretreated with methylcholanthrene. In both cases, they confirmed the fact that a whole spectrum of metabolites is produced, including both ring and methyl hydroxylated derivatives in comparable amounts. Other methylated PAH carcinogens are also metabolized by way of hydroxymethyl compounds.43

Dipple and co-workers have propounded the theory that methylated PAHs can be converted to carcinogenic metabolites either by direct formation of arylmethyl carbonium ions or by formation of esters of the hydroxymethyl metabolites which are capable of spontaneous dissociation to the carbonium ion.48 Broad correlations have tended to show that the carcinogenic potency of methylated PAHs increases as the stability of the corresponding arylmethyl carbonium ion increases.49,50 Similar correlations have been made between carcinogenic potency of other types of alkylating agents and their tendency to react by way of an $S_{N1}$ process.51

If hydroxymethyl PAHs are esterified to form incipient carbonium ions, it is possible that sulfate esters are involved. Sulfate ester formation is a method by which foreign compounds are made more polar and therefore more water soluble in order that they might be excreted. Although it has not been shown that arylmethanols are metabolized by sulfate ester formation, several aliphatic alcohols are metabolized this way.52,53 The sulfate esters of hydroxymethyl PAHs could represent activated compounds produced by a biological system which normally detoxifies.

If carcinogenic activity is mediated by arylmethyl carbonium ions,
then preformed alkylating agents of the type ArCH₂X, where X is a good leaving group, should be very potent carcinogens. Dipple and Slade found that under the conditions of their tests, 7-bromomethyl-12-methylbenz[a]anthracene is a potent carcinogen. Results of other groups have not always agreed entirely with those of Dipple's group. Administration of a possible ultimate carcinogen externally is not the same as having a similar species arise at specific locations inside the cell. The carcinogenic potency of an externally administered reactive chemical would depend on its ability to diffuse into the cell and react with a critical target before becoming deactivated. This in turn would depend not only on the chemical but on the conditions of the test. Despite this complication, Peck and co-workers have recently shown that a number of direct acting alkylating agents of the type in question are much more potent carcinogens than their parent hydrocarbons in mouse lung. Also, Rayman and Dipple found that 7-bromomethyl-12-methylbenz[a]anthracene is a carcinogen for mouse skin and that some conjugates with DNA formed in vivo appeared to be the same as those formed when this same alkylating agent was allowed to react with DNA in the test tube.

6-Methylbenzo[a]pyrene Derivatives. 6-Methylbenzo[a]pyrene (X) has been reported to be a more potent carcinogen than BAP and a more potent carcinogen than other methylated BAPs studied except for 2-methylbenzo[a]pyrene which has approximately the same potency. The arylmethyl carbonium ion at the 6-position should be the most stable one, and thus, carcinogenic potency correlates at least
roughly with carbonium ion stability. Flesher and Sydnor have reported that 6-methylbenzo[a]pyrene can be converted to 6-hydroxy-methylbenzo[a]pyrene (XI) by rat liver homogenates. Natarajan and Flesher have reported that 6-hydroxymethylbenzo[a]pyrene and 6-bromomethylbenzo[a]pyrene (XII, X = Br) are potent carcinogens. This data suggests that 6-hydroxymethylbenzo[a]pyrene might be a proximate carcinogen which is converted to an incipient carbonium ion for which 6-halomethyl derivatives are models, a situation analogous to that of 7-hydroxymethyl-12-methylbenz[a]anthracene.

![Chemical Structures]

Figure 3. 6-Methylbenzo[a]pyrene (X) and benzo[a]pyrene (I) might be metabolized by way of a compound resembling 6-chloromethyl-benzo[a]pyrene (XII, X = Cl).
There is an additional way in which 6-hydroxymethylbenzo[a]-pyrene might arise in the cell, namely, by hydroxymethylation of BAP itself. This idea has arisen chiefly from the work of Sloane and co-workers who have reported the hydroxymethylation of other aromatic compounds including benzene and aniline. The hydroxymethylation of BAP at the 6-position has been reported to occur in rat liver preparations by Flesher and Sydnor and in rat and guinea pig liver preparations by Sloane and Davis. Recently, Sloane has reported studies which indicate that whereas hydroxylation of alkyl side chains require the cytochrome P-450 system, hydroxymethylation does not. Interest in this route of metabolism, as judged by the literature, has not been comparable to the great interest in ring hydroxylations of PAHs.

The scheme in Figure 3 indicates the possible relevance of 6-chloromethylbenzo[a]pyrene (XII, X = Cl) as a model for possible carcinogenic metabolites of methylated PAHs and BAP as well as its being a direct acting carcinogen.

CELLULAR TARGETS

A critical difference between cancer cells and normal cells is the lack of cancer cell response to normal restraints to proliferation. This characteristic and others are usually passed down from mother to daughter cells as the tumor develops, suggesting that the genetic material is altered and that the critical event in carcinogenesis is a type of somatic mutation. In early work, many
carcinogens, such as the PAHs could not be shown to be mutagenic in bacteria. It was not known that bacteria do not have the enzymes to activate them. Also, it had not been shown during the early fifties that carcinogens bind to DNA in vivo, although the in vivo binding of carcinogenic aminoazo dyes to cellular protein was already reported by the Millers in 1947.

Studies of covalent binding concentrated on proteins; and protein deletion theories of carcinogenesis developed. Carcinogenic hydrocarbons appear to bind to one type of soluble protein, called the h protein, more than to others. Heidelberger has suggested that this protein might be a gene repressor.

In 1957, Wheeler and Skipper reported the covalent binding of carcinogens to DNA in vivo. Later, Brookes and Lawley found a correlation between carcinogenicity and degree of binding of PAHs to mouse skin DNA.

The link between carcinogenesis and mutagenesis has been further substantiated with the finding that metabolically activated forms of carcinogens which bind to DNA also cause mutations in bacteria. The correlation between mutation and oncogenesis is such that systems have been devised whereby compounds are tested for carcinogenic potency by observing bacterial mutations in the presence of the suspected carcinogen and a microsomal activating system.

Although carcinogenic and mutagenic potency are comparable in some systems, attempts to make exact correlations between the two have failed. In addition, some mutagens are not carcinogens. It appears
that the mutagenic and carcinogenic processes involve very specific interactions with biomolecules which are not identical, at least in some cases.

With respect to mutagenesis, this idea is supported by the work of Loveless\textsuperscript{71} who found that although methyl methanesulfonate and ethyl methanesulfonate react with the DNA of certain phage\textsuperscript{72} only ethyl methanesulfonate is mutagenic, but both N-methyl-N-nitrosourea and N-ethyl-N-nitrosourea are mutagenic in phage.\textsuperscript{73} Likewise, Swann and Magee have shown that a simple relationship between amount of methylation and carcinogenic potency of methylating agents in rat liver did not exist in their experiments.\textsuperscript{74} It appears that substitution at specific sites on specific nucleotide residues rather than just general covalent binding to DNA might be important. Since the major site of alkylation for some carcinogens and noncarcinogens is the N-7 position of guanine, it seems that a minor product or products might be the important ones.

This idea was put into concrete terms by Loveless\textsuperscript{75} who suggested that for N-methyl-N-nitrosourea and N-ethyl-N-nitrosourea, the important reaction might be alkylation of the O-6 of guanine which would reduce the hydrogen bonding sites from 3 to 2 as shown in Figure 4. This could lead to a base pair change during replication due to faulty base pairing.

It is well known that there are enzyme systems which can repair DNA. For example, Kirtikar and co-workers have studied the enzymatic excision of alkylated purine bases from DNA treated with 7-bromo-methyl-12-methylbenz[a]anthracene.\textsuperscript{76} It appears that some base
Figure 4. The three hydrogen bonding sites in guanosine residues are reduced to two by 0^-alkylation.

modifications are repaired much more efficiently than others. Frei has recently reviewed some of the diverse evidence which indicates that oncogenesis occurs when DNA replication occurs before DNA repair takes place. If this is true, then again, those modified residues which are more slowly repaired would be more critical.

**Selectivity in Nucleophilic Substitution Reactions.** Thus far, cellular nucleophiles have been considered targets for "attack" by potentially carcinogenic electrophiles. It is common, however, to consider $S_N^1$ reactions as processes in which a nucleophile attacks a substrate (electrophile) with a pair of electrons, replacing another group which leaves with a pair of electrons. The scheme in Figure 5 shows the range of possibilities which are believed to exist between limiting $S_N^2$ reactions with relatively unreactive substrates and limiting $S_N^1$ reactions with relatively stable carbonium ions. The distinction between $S_N^1$ and $S_N^2$ reactions was originally proposed by
Hughes, Ingold and Patel. The existence of intimate and solvent separated ion pairs in addition to free ions was postulated by Winstein. The scheme as pictured does not include the possible

<table>
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<tr>
<th>Actual Substrate</th>
<th>R-X</th>
<th>R⁺X⁻</th>
<th>R⁺⁺ X⁻</th>
<th>R⁺</th>
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<tbody>
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<td>Increasing Reactivity</td>
<td>Increasing Reactivity</td>
<td>Limiting S⁺₁</td>
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<tr>
<td>High Swain - Low Swain -</td>
<td>Low $k_N$</td>
<td>High $k_N/k_{H₂O}$</td>
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<td>Scott s value</td>
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Figure 5. Selectivity, as measured by the Swain-Scott equation for $S_n$ reactions, decreases with increasing reactivity of the substrate. For $S_N$ reactions, selectivity as measured by $k_N/k_{H₂O}$ values increases with increasing carbonium ion stability.

interactions of solvent molecules with nucleophile, substrate and leaving group. In total, the range of possibilities represents a complex picture. The theory and experimental evidence involved are reviewed in several textbooks on organic mechanisms (see for example Refs. 80 and 81). Fortunately, selectivity in $S_N$ reactions correlates fairly well with reactivity in general and can be approached without consideration of exact mechanistic details.

For substrates which undergo $S_n$ reactions, Swain and Scott used the following equation to describe selectivity:
\[ \log \frac{k}{k_0} = sn \]

k = rate of reaction

\( k_0 \) = rate of reaction of the same substrate with \( H_2O \)

s = a value assigned to each substrate, set at 1.0 for \( CH_3Br \)

n = a value assigned to each nucleophile indicating nucleophilicity

This equation correlates \( S_N^2 \) reactions in protic solvents quite well. For the most part, relatively unreactive substrates are highly selective because a good nucleophile is required to "push" the leaving group off. In reactive substrates, the leaving group needs little assistance, and weaker nucleophiles can attack.

For \( S_N^1 \) reactions, Sneen and co-workers have correlated carbonium ion stability with selectivity.\(^{83}\) Using a series of alkyl chlorides, they plotted the log of the solvolysis rate in 80% acetone, 20% water versus \( \log \frac{k_n}{k_w} \) where \( \frac{k_n}{k_w} \) is the ratio of the reaction rate with a good nucleophile, azide ion, to the reaction rate with a poorer nucleophile, water. Their correlations have been extended by Raber and co-workers.\(^{84}\) Sneen's plot results in a line which shows that more stable carbonium ions as indicated by higher solvolysis rates show greater selectivity for azide ion.

The following orders of nucleophilic strength were determined by Swain's group for a) \( S_N^2 \) reactivity\(^{82}\) and b) reaction with triphenylmethyl carbonium ion.\(^{85}\)
a) $\text{I}^{-} > \text{C}_6\text{H}_5\text{NH}_2^- > \text{HO}^- > \text{N}_3^- > \text{Cl}^- > \text{H}_2\text{O}$

b) $\text{S}_2\text{O}_3{=}^2 > \text{N}_3^- > \text{HO}^- > \text{C}_6\text{H}_5\text{NH}_2^- > \text{Cl}^- > \text{H}_2\text{O}$

Two facts should be noted. First, the order of nucleophilic strength is different for the two types of substrate, and second, weak bases such as azide and iodide are often kinetically better nucleophiles than strong bases such as hydroxide. The second fact introduces hard and soft acid and base theory into consideration.\textsuperscript{86} Polarizability or softness is an important factor in nucleophilic power toward the soft acid, carbon, whereas nonpolarizable or hard bases are more nucleophilic toward the hard acid, the proton. The exact mechanistic significance of polarizability has been interpreted differently by different workers.

In considering biological targets, it is important to note that electrophiles are expected to show a range of specificity for different biological nucleophiles, and that the principles involved are at least partly understood but cannot be expected to be entirely predictive for complex biomolecules.

Selective Attack on Biomolecules: Examples. Much of the early work on alkylation of biomolecules concerned sulfur and nitrogen mustards and similar compounds and their reactions in high concentrations with amino acids and proteins.

At or near physiological pH, the carboxyl groups of amino acids and proteins are in the anionic form and react extensively with various alkylating agents.\textsuperscript{87,88} Imidazole groups,\textsuperscript{89} sulfhydryl groups\textsuperscript{90}
and amino groups are also involved even though most of the amino groups are in the RNH₃⁺ form.

Levene and Tipson had already studied the methylation of adenosine with dimethyl sulfate in 1932. In this and other early studies with nucleic acid substituents, again, the alkylating agent was used in excess, and multialkylated products were obtained. This did not shed much light on which positions are most subject to alkylation. In addition, the products were usually inadequately characterized. In 1963, Jones and Robins reported studies of the mono- and di-methyl derivatives of guanosine, deoxyguanosine, adenosine and deoxyadenosine formed by reaction with dimethyl sulfate and methyl iodide in dimethylsulfoxide and dimethylformamide. They established that alkylating agents of this type attack guanine at the 7-position and adenine at the 1-position (see Fig. 6 for base numbering). The ribose and deoxyribose moieties were unaffected. Young and Campbell had already noted that guanine competes for alkylating agents better than other bases. Brooks and Lawley found that cytidine is alkylated at the 3-position.

Recently, considerable information has been forthcoming on the alkylation of nucleic acids and their components, with very different product distributions of variously alkylated products. It is useful to consider these products as deviations from 7-alkylguanine, 1-alkyl-adenine and 3-alkylcytosine as the standard products and attempt to relate the differences to special alkylating agent or reaction conditions.
Singer and Fraenkel-Conrat have found that ethyl methanesulfonate and diethyl sulfate alkylate primarily the ring nitrogens of the base residues of RNA, with 7-ethylguanine, 1-ethyladenine and 3-ethylcytidine being the primary products. In this respect they confirmed some of the earlier work. By using $^{14}$C labelled alkylating agents, however, they were able to show that minor products were also
formed, including some $\mathrm{O}^6$-ethylguanine. The older studies usually concentrated on one or two major products, and in cases where nucleic acids were hydrolyzed, some conjugates including O-alkylated bases and phosphate esters were also probably hydrolyzed.

In contrast to those two alkylating agents, these same workers found that ethylnitrosourea has a marked tendency toward O-alkylation, with $\mathrm{O}^6$-ethylguanine being the major alkylated base and a significant amount of phosphate ethylation occurring. Methylnitrosourea was found by Lawley to methylate the phosphate groups of nucleic acids in vitro and in vivo.

The N-alkyl-N-nitrosoureas are a special class of alkylating agents since they spontaneously decompose to form alkyl diazonium compounds as shown in Figure 7. The leaving group in this case, the $\mathrm{N}_2$ molecule, is exceptional, giving the nitrosamides their special alkylating properties. The N,N-dialkylnitrosamines act similarly to the nitrosamides after being metabolically hydroxylated (Fig. 7). Thus it appears that a large class of carcinogenic compounds may preferentially alkylate nucleic acids at the oxygens.

The experiments of Pound and co-workers indicate that ethyl carbamate, a carcinogen in mice, ethylates mainly the phosphate groups of DNA in vivo. They did not detect any ethylation of the bases. Elmore and co-workers had already suggested in 1948 that phosphotriesters might be formed during alkylation of DNA. Phosphotriester formation might lead to single strand breaks in the DNA. Only recently has it been confirmed that phosphotriesters can be formed in appreciable amounts in vivo.
Figure 7. N-alkyl-N-nitrosoureas decompose to form alkyl diazonium compounds as do N,N-dialkyl nitrosamines after enzymatic hydroxylation.

Another case which demonstrates an exceptional specificity is that of N-acetoxy-2-acetylamino fluorene (XIII). The carcinogen 2-acetylamino fluorene is N-hydroxylated in the cell and then esterified, sometimes with sulfate. Thus N-acetoxy-2-acetylamino fluorene is a model for some of the activated metabolites of acetylamino fluorene and some other aromatic amide and amine carcinogens. It is not an alkylating agent, but rather reacts by way of the
electrophilic nitrogen and shows a preference for attack at the 
C^8-position of guanine (Fig. 8).^{103}

\[ \text{Figure 8. N-acetoxy-2-acetylaminofluorene (XIII) preferentially}
\text{attacks the 8-position of guanine residues in nucleic acids.} \]

Recent work has indicated that the highly mutagenic epoxide 
derivatives of polycyclic aromatic hydrocarbons react to a signifi-
cant extent with the amino groups of nucleic acid bases in vivo.\textsuperscript{29,104}
The specificity of these reactions may depend on intrinsic properties 
of the electrophile and also on reaction conditions. Benzyl bromide 
was shown by Brooks and co-workers to form 7-benzylguanosine, 
1-benzyladenosine, and 3-benzylcytidine when it reacts with the 
nucleosides in dimethylacetamide (DMA).\textsuperscript{105} Dipple and co-workers 
found that the carcinogen 7-bromomethylbenz[a]anthracene also alkyl-
ates the ring nitrogens of the nucleosides in DNA. In aqueous solu-
tion, however, the amino groups of guanine, adenine and possibly of
cytosine were attacked. Structural determination of conjugates between nucleosides and PAH derivatives is complicated by the fact that absorption by the base in the ultraviolet region is swamped by the PAH moiety. Simple alkylated derivatives of the bases can be identified by comparison of their ultraviolet absorption spectra with those of compounds substituted at the same positions whose structures have been worked out, sometimes with difficulty, over the years. The identity of the conjugate resulting from attack of 7-bromomethylbenz[a]anthracene at the N6 position of adenosine was confirmed by Dipple's group by an alternate synthesis. The other derivatives were identified by less direct methods.

In another recent study, Shapiro and Shiuey have compared the reactions of 7-bromomethylbenz[a]anthracene, p-methoxybenzyl bromide, and benzyl bromide with cytidine in DMA and in aqueous solution. They concluded that conditions which favor the development of a positive charge on the alkylating agent favor substitution on the amino group rather than the ring nitrogen of cytidine. Thus, in aqueous solution, p-methoxybenzyl bromide gave more amino group substitution than benzyl bromide, and neither compound produced any detectable amount of amino substituted product in DMA. In all cases, only a small amount of conjugate was produced in aqueous solution.

Close correlations between relative carcinogenicity and specificity of attack on certain positions of nucleic acids have not yet been found. Research in this area has increased, however, with the expectation that some relationships will become apparent.
THE USE OF CARBON-13 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY IN STRUCTURE DETERMINATION

The easiest way to establish a perspective on the usefulness of carbon-13 nuclear magnetic resonance spectroscopy (CMR) in determining the structures of organic molecules is to compare it with proton magnetic resonance spectroscopy (PMR) which has become a very familiar tool to the chemist. The $^{13}$C nucleus, like the proton has a spin of 1/2. Nuclei with nonzero spins have magnetic moments which may have various orientations with respect to an applied magnetic field. Nuclei with spin 1/2 may have two orientations with respect to the direction of an applied field, generally aligned with or against the field. If a sample is irradiated perpendicular to the field at a frequency which corresponds to the difference in energy between the two possible orientations of the nuclei, called the resonance frequency, the sample will absorb energy due to the transition of some nuclei from the lower to the higher energy state.

**Chemical Shifts.** The resonance energy of a nucleus depends on the strength of the magnetic field according to the following formula:

$$\Delta E = g\beta_N H_0$$

$\beta_N$ (the nuclear magneton) = $5.050 \times 10^{-27}$ Joules/Tesla

$H_0$ = the applied field

$g$ = an empirically determined value for each nucleus
This formula is explained in a particularly lucid manner by Banwell.\textsuperscript{108} The resonance frequency is given by:

\[ \nu_o = \frac{g \beta _N H_o}{h} \]

The g value for $^{13}$C is 1.404; at a field strength of 2.35 Tesla, which is a practical value, the resonance frequency is 25.2 MHz.

The field which is actually felt by nuclei is usually less than the applied field because the electron cloud around the nucleus shields it to some extent. The shielding is proportional to the applied field and the phenomenon is often generalized by the expression:

\[ H_i = H_o (1 - \sigma) \]

- $H_i$ = the field strength actually felt by the nucleus
- $H_o$ = the applied field
- $\sigma$ = a shielding constant

The difference between the resonance frequency of a carbon nucleus in a particular chemical environment and a reference carbon nucleus is called the chemical shift. If measured in Hertz, the chemical shift will depend on the field strength at which the instrument operates. The chemical shift in Hertz is usually divided by the resonance frequency in megaHertz, therefore, to give the familiar parts per million (ppm) which is not dependent
on field strength. The reference most commonly used in CMR, as in PMR, is now TMS, and downfield shifts are given positive values. This was not always the case.

A great advantage of CMR is that $^{13}$C chemical shifts occur over a range of over 200 ppm in common organic compounds whereas proton chemical shifts usually occur over a range of about twelve ppm in the same compounds.

**Coupling Information.** Of the three types of information most commonly obtained from PMR studies, chemical shifts, coupling information, and peak area, the first has been the most important in CMR. Coupling constants between $^{13}$C and protons are large, resulting in overlapping multiplets in any but the simplest compounds. Decoupling protons in CMR spectra increases the sensitivity by consolidating the multiplets into single peaks and the process of decoupling itself further enhances the signal by a phenomenon called the nuclear Overhauser effect. Protons are decoupled by irradiating them at their resonance frequency while taking the CMR spectrum. Multiplet overlap can be avoided while peak multiplicity information is still retained by the process of off resonance decoupling. Peak area is very often not an indication of the relative number of equivalent nuclei in CMR as it is in PMR. In addition to these three kinds of information commonly used in PMR, the determination of spin-lattice relaxation times for $^{13}$C nuclei is attracting increasing interest as a means of obtaining information about molecular mobility. 109

**Sensitivity.** The sensitivity of natural abundance CMR is much
lower than PMR because $^{13}\text{C}$ represents only about 1.1% of the carbon nuclei. In addition, the $^{13}\text{C}$ nucleus gives weaker NMR signals than the proton due to its lower magnetic moment. Overall the sensitivities differ by a factor of about 5,700.\textsuperscript{110} This difference is largely made up for by the application of Fourier transform technique to CMR. Using this technique, the sample can be pulsed briefly over a broad frequency range and the signals from many pulses can be accumulated. The resulting complex signal contains the NMR spectral information which is derived from it mathematically.\textsuperscript{111} The sample may be enriched in $^{13}\text{C}$ to further increase the sensitivity.

CMR With $^{13}\text{C}$ Labelled Compounds. The labelling of a specific position in a molecule with $^{13}\text{C}$ makes it possible to follow the fate of a particular carbon unequivocally as the molecule undergoes chemical changes. This is particularly useful when the labelled carbon is the reactive center of the molecule. It is only necessary to determine which of the possible structures corresponds to the resulting chemical shift. The other advantage of using $^{13}\text{C}$ labelled compounds is that smaller samples can be used concomitant with the amount of enrichment.

The Chemical Shift in Structure Determination. Chemical shift information in CMR is often unambiguous. For example, saturated carbons are well separated from singly unsaturated carbons. Saturated carbons have chemical shifts of less than 100 ppm while singly unsaturated carbons, including aromatics lie between about 100 and 230 ppm downfield from TMS. In order to pin down the chemical environment of some carbon atoms, however, careful correlations must
be made. Thus, for example, if the substituted carbon atom of a substituted alkane has a chemical shift \( x \) ppm downfield from the same carbon in the parent alkane, the substituent may be expected to have a similar effect on other alkane carbons and \( x \) is the substituent parameter. The substituent might be an alkyl group, a heteroatom or an organic functional group. Substitution for hydrogen often has a measurable effect at the substituted carbon and also at the next two carbons in the chain. The chemical shifts of all carbons in unsubstituted alkanes can be predicted within about one ppm by considering each carbon as a substituted methane and adding the appropriate parameter for each carbon up to three bonds away.\(^{112}\)

Parameters describing the effect of various substituents on the chemical shifts of the three nearest carbons in alkanes are listed by Levy and Nelson.\(^{113}\) These parameters which were devised for compounds of the type \( R-X \) where \( R \) is an alkyl group do not work well for compounds of the type \( \text{Ar-CH}_2-X \) which are of interest here. Recently, however, Shapiro has shown that there is a linear relationship between the chemical shifts of substituted alkanes and arylmethyl carbons in substituted toluenes.\(^{114}\) Thus, if the chemical shifts of variously substituted alkanes are plotted versus the chemical shifts of correspondingly substituted toluenes, an excellent straight line results. From such a plot, the chemical shift of a substituted arylmethyl carbon can be predicted by extrapolation if the chemical shift of an alkane carbon with the same or a similar substitutent is known. As a more general approach, alkane substituent parameters may be plotted against the known arylmethyl substituent
parameters. This relationship should prove valuable for predicting the chemical shifts of substituted arylmethyl carbons. This is especially true because different arylmethyl carbons may have chemical shift values which are quite dissimilar, so that one is not necessarily a good reference for the other.
SYNTHESIS

SYNTHETIC SCHEME

The labelled and unlabelled BAP derivatives used in this research were synthesized by formylation of BAP and subsequent chemical conversions of the formyl group. The BAP used was synthesized in this laboratory.

Benzo[a]pyrene. This polycyclic aromatic hydrocarbon was synthesized according to literature methods for the most part, as indicated in Figure 9. The synthesis is basically that of Cook and Hewett,\textsuperscript{115} with the first step, succinoylation of pyrene (XIV), being run according to their procedure. The ketone function was removed from the resulting β-(1-pyrenoyl)propionic acid (XV) by the Huang Minlon modification of the Wolf-Kishner reduction as described by Norman and Waters.\textsuperscript{116} This step, using hydrazine and potassium hydroxide in high boiling solvent is an improvement over the Clemmensen type reduction described by Cook and Hewett. Cyclization of the γ-(1-pyrenyl)butyric acid (XVI) was improved by Fieser and Novello\textsuperscript{117} who used phosphorus pentachloride to form the acid chloride and then cyclized with stannic chloride. However, in this work, better yields were obtained by simply adding cold hydrogen fluoride to XVI and stirring than by the acid chloride route. The 7-oxo-7,8,9,10-tetrahydrobenzo[a]pyrene (XVII) was reduced to 7-hydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene (XVIII) as described by Bachmann and co-workers\textsuperscript{118} who used aluminum isopropoxide in isopropyl alcohol
Figure 9. Synthetic scheme for benzo[a]pyrene.
(the Meerwein-Pondorf-Verley reduction). Bachmann and co-workers also introduced the method used in the final step for dehydration and dehydrogenation of XVIII which involves heating with palladium on charcoal.

\[ ^{13}C \text{ Labelled N-Methylformanilide.} \] Carbon-13 was obtained from Los Alamos Scientific Laboratory as isopropyl formate enriched in \(^{13}C\) to approximately 90% at the formate carbon. Since aromatic amines will not generally react with esters to form amides directly,\(^{119}\) the isopropyl formate-\(^{13}C\) was saponified with sodium hydroxide and the resulting sodium formate-\(^{13}C\) was allowed to react with N-methylaniline hydrochloride to form the labelled N-methylformanilide. (Fig. 10)

\[
\begin{align*}
\text{H-C-OCCH(CH}_3\text{)_2} & \quad \rightarrow \quad \text{H-C-ONa}^+ \\
\text{H-C-ONa}^+ + \text{CH}_3\text{NH}_2\text{Cl}^- & \quad \rightarrow \quad \text{CH}_3\text{NH-N-CO-H}
\end{align*}
\]

Figure 10. Synthesis of N-methylformanilide-1-\(^{13}C\).
\[ ^{13}C \text{ Labelled 6-Substituted Benzo[a]pyrenes.} \]

Fieser and Hershberg\textsuperscript{120} used the Vilsmeier-Haack reaction to prepare 6-benzo[a]pyrene carboxaldehyde using N-methylformanilide as formylating agent and o-dichlorobenzene as solvent. Dewhurst and Kitchen\textsuperscript{121} obtained a purer product by using dimethylformamide as solvent. Since dimethylformamide is also a formylating agent, it was not used in this work in order to avoid dilution of the \( ^{13}C \) label. Good results were obtained by warming BAP, \( ^{13}C \) labelled N-methylformanilide, and phosphorus oxychloride on a steam bath without additional solvent.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{synthesis_13c_labelled_6-substituted_benzo[a]pyrenes}
\caption{Synthesis of \( ^{13}C \) labelled 6-substituted benzo[a]pyrenes.}
\end{figure}
The conversion of 6-benzo[a]pyrenecarboxaldehyde-formyl-\textsuperscript{13}C (XIX) to 6-hydroxymethyl-\textsuperscript{13}C-benzo[a]pyrene (XXI) by the Meerwein-Pondorf-Verley reduction followed the procedures of Dewhurst and Kitchen\textsuperscript{121} for the unlabelled compounds.

The conversion of XXI to 6-chloromethyl-\textsuperscript{13}C-benzo[a]pyrene (XXII) with thionyl chloride and to 6-bromomethyl-\textsuperscript{13}C-benzo[a]pyrene (XXIII) with phosphorus tribromide followed the procedures of Natarajan and Flesher,\textsuperscript{59} described for the unlabelled compounds, with minor modifications. Each compound was also synthesized without the \textsuperscript{13}C label for studies not involving CMR.

9-Substituted Anthracenes. Unlabelled 9-chloromethylantracene (XXIV) was synthesized as a reference for comparison of the reactivity of 6-chloromethylbenzo[a]pyrene. 9-Anthraaldehyde was reduced to

![Anthracene](image)

XXIV

9-hydroxymethylantracene, which was converted to the chloride by methods similar to those used in the conversion of XIX to XXII. This sequence has been described by Hunter and co-workers.\textsuperscript{122}

**EXPERIMENTAL**

\(\beta-(1\text{-pyrenoyl})\text{propionic Acid (XV).}\) Succininc anhydride (125 g, 1.24 mol) was suspended in nitrobenzene (1,250 ml) and cooled in
an ice bath. Aluminum chloride (330 g, 2.48 mol) was added slowly with continued cooling and stirring. Pyrene (250 g, 1.25 mol) was added slowly while the reaction mixture was maintained at 0-10° and then stirred for 3 h in an ice bath. The reaction mixture was poured slowly, with stirring, into aqueous hydrochloric acid and ice was added periodically to keep the mixture at 0°. The mixture was then steam distilled to remove the nitrobenzene. The solid product was washed with water and recrystallized from water once as the sodium salt and once as the ammonium salt. After each recrystallization, the acid was reprecipitated from aqueous solution by addition of HCl. The dry product weighed 299.5 g (80%) mp 182-183° (lit. 183°). 115

\[ \text{γ-(1-pyrenyl)butyric Acid (XVI)} \]

β-(1-pyrenoyl)propionic acid (XV) (25 g, 82.8 mmol), mp 182-183°, potassium hydroxide (15.9 g, 0.283 mol), hydrazine hydrate (10.4 ml, 0.228 mol) and 114 ml of diethylene glycol were heated together for 1.5 h at 100° and then heated at 150° for 4.5 h, distilling off the water which was produced. The product was precipitated from aqueous solution with hydrochloric acid and collected by filtration. It was recrystallized from ethyl acetate (Norit) to give 15 g (63%) of XVI, mp 184.5-186° (lit. 185-186°). 116

7-Oxo-7,8,9,10-tetrahydrobenzo[a]pyrene (XVII). γ-(1-pyrenyl)-butyric acid (XVI) (70.8 g, 0.261 mol), mp 184.5-186°, was placed in a polyethylene beaker and cold hydrogen fluoride (710 ml) was added and the mixture was stirred periodically while the hydrogen fluoride evaporated. The product was washed thoroughly with 5% sodium bicarbonate solution and dried. Recrystallization from benzene (Norit)
afforded 40.0 g of XVII, mp 169-170° (lit. 168-171°). A second crop (5.7 g), mp 166-167°, was obtained from the mother liquor for a total yield of 82.5%.

7-Hydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene (XVIII). A solution of 7-oxo-7,8,9,10-tetrahydrobenzo[a]pyrene (XVII) (30 g, 0.11 mol), mp 169-170°, and aluminum isopropoxide (122 g, 0.60 mol) in 600 ml of dry isopropyl alcohol was refluxed for 30 min, then distilled slowly for 2.5 h, using a Hahn condenser. The volume was maintained by periodic addition of isopropyl alcohol. The reaction mixture was hydrolyzed with 5% HCl and the solid was filtered, washed with dilute ammonium hydroxide and dried. The product, 22.5 g (75%), mp 135-137° (lit. 141.5-142°), was suitable for aromatization.

Benzo[a]pyrene (I). 7-Hydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene (XVIII) (5 g, 18.4 mmol), mp 135-137°, was placed in a test tube equipped with an inlet, outlet and cold finger. Palladium (5%) on charcoal (0.5 g) was added and the tube was held at 300° for 1.5 h in a Wood's metal bath with a slow stream of nitrogen passing through the tube. The tube was then cooled and the inside washed down with benzene; the benzene was evaporated and heating at 300° was continued for another 2 h. The product was crystallized from benzene to give 4.30 g of I. This was further purified by way of the picrate to give 2.70 g (58%) of I, mp 176.5-177.5 (lit. 178.6-179.8 corr.).

N-methylformanilide-1-13C. Isopropyl formate enriched at the formate carbon to 90.93% 13C was obtained from the Los Alamos Scientific Laboratory as an 82% w/w solution with isopropyl alcohol.
The solution, (39.3 g, 0.35 mol isopropyl formate) was refluxed with 100 ml of a solution containing 40% water, 60% ethanol v/v and 0.35 mol of sodium hydroxide for 2 h. The solvents were removed on a rotary evaporator and the sodium formate was used without further purification in the same flask. N-methylaniline hydrochloride (65 g, 0.45 mol) was added to the flask and the mixture was heated at reflux for 1 h using an oil bath at 150°. After this, the water formed was removed by distillation. After cooling, 50 ml of 5% HCl was added to the flask along with 50 ml of ether. The organic layer was separated and the aqueous layer extracted three more times with 50-ml portions of ether. The combined ether extracts were dried over magnesium sulfate and the ether was removed over a steam bath. The $^{13}$C labelled N-methylformanilide was distilled at 8 torr. The fraction boiling between 114-121° (36.4 g, 76%) was collected. (lit. bp 114-121/8 torr)$^{123}$

6-Benz[a]pyrene-carboxaldehyde-formyl-$^{13}$C (XIX). Benzo[a]pyrene (4 g, 15.9 mmol), N-methylformanilide-1-$^{13}$C (4.5 g, 33 mmol) and phosphorus oxychloride (4.5 g, 29 mmol) were warmed on a steam bath for 2.5 h in a flask fitted with a reflux condenser. The system was flushed with dry nitrogen and swirled periodically. The apparatus was also protected from light as a precaution. After cooling, the reaction mixture was poured into 200 ml of 10% aqueous sodium acetate and stirred until the red oil became a yellow solid. This crude product was filtered, dried and recrystallized twice from chloroform giving 2.35 g of product mp 202-203°. The residue from the mother liquors was chromatographed on alumina with benzene, giving an
additional 0.7 g mp 202.5-203.5 (lit. 202.5-203.5). The total yield was 88%.

6-Methyl-13C-benzo[a]pyrene (XX). 6-Benzo[a]pyrene carboxyaldehyde-formyl-13C (0.7 g, 2.5 mmol), hydrazine hydrate (0.66 ml, 13 mmol), diethylene glycol (40 ml) and KOH (0.44 g, 7.9 mmol) were heated with stirring at 100° for 30 min and then at 140-150° for 2 hr, collecting water by distillation. Water was added to the cooled reaction mixture to precipitate the product which was filtered, dried and chromatographed on alumina with benzene. The product was crystallized by concentrating the benzene solution and adding ethanol to give 0.42 g (60%) of product mp 214-215°. Following sublimation and a second recrystallization from benzene/ethanol, the product melted at 215-216° (lit. 216.2-216.7°).

6-Hydroxymethyl-13C-benzo[a]pyrene (XXI). 6-Benzo[a]pyrene carboxyaldehyde-formyl-13C (3.25 g, 11.6 mmol), aluminum isopropoxide (16.25 g, 79 mmol) and isopropyl alcohol (325 ml) were boiled for 0.5 h and then distilled slowly, using a Hahn condenser until no more acetone came over as determined by testing with 2,4-dinitrophenylhydrazine test solution. The remaining solution (150 ml) was poured into cold 5% HCl (600 ml) and stirred for 0.5 h. The solid was filtered and recrystallized three times from benzene, giving 3.1 g (95%) of product which melted at 228-229° (d). In a sealed evacuated tube, it melted at 230-231° without decomposing and upon resolidifying, it melted again at 290° with decomposition (lit. 232-233°).

6-Chloromethyl-13C-benzo[a]pyrene (XXII). A mixture of 6-hydroxymethyl-13C-benzo[a]pyrene (XXI) (0.2 g, 0.71 mmol), thionyl chloride
(0.2 g, 1.7 mmol) and anhydrous benzene (7 ml) were refluxed for 30 min with stirring. The solvent was removed on a rotary evaporator and the crude product was recrystallized twice from benzene, giving 0.17 g (80%) of product mp 218-219° (lit. 215-218° (d)).

6-Bromomethyl-13C-benzo[a]pyrene (XXIII). A mixture of 6-hydroxymethyl-13C-benzo[a]pyrene (XXI) (0.3 g, 1.06 mmol), phosphorus tribromide (0.5 g, 1.85 mmol) and anhydrous benzene (18 ml) was refluxed with stirring for 1 h. The reaction mixture was concentrated to 10 ml and the product which crystallized on cooling was recrystallized from benzene to give 0.22 g (60%) of XXXIII, mp 225-226° (lit. 225-226°).

9-Hydroxymethylanthracene. 9-Anthraldehyde (2.5 g, 12 mmol) was heated with 25 ml of isopropyl alcohol until solution was obtained. Aluminum isopropoxide (7 g, 34 mmol) in 25 ml of isopropyl alcohol was added and the solution was distilled using a Hahn condenser until no more acetone distilled. The volume was maintained by periodic addition of isopropyl alcohol. The reaction mixture was stirred with 1 liter of 5% HCl for 1 h. The solid was filtered, dried and recrystallized from ethanol to give 1.8 g (72%) of 9-hydroxymethylanthracene, mp 154-156° (lit. 162-164° from benzene).

9-Chloromethylanthracene (XXIV). 9-Hydroxymethylanthracene (1.7 g, 6 mmol), thionyl chloride (0.98 g, 8.2 mmol) and benzene (12 ml) were refluxed with stirring for three hours. The solvent was then removed on a rotary evaporator and the residue recrystallized twice from benzene-cyclohexane to give 1.21 g (70%) of XXIV melting sharply at 139° (lit. 141-142.3°).
KINETIC STUDIES

SPECTROPHOTOMETRIC MEASUREMENT OF REACTION RATE

The absorption spectra of 6-chloromethylbenzo[α]pyrene and 6-hydroxymethylbenzo[α]pyrene in acetone over the wavelength region 350-430 nanometers are shown in Figure 12. This region of the spectrum

Figure 12. Electronic absorption spectra of 6-chloromethylbenzo[α]-pyrene (a) and 6-hydroxymethylbenzo[α]pyrene (b) over the wavelength region 350-430 nm.
Table 1

Wavelengths and extinction coefficients of the absorption maxima for 6-chloromethylbenzo[a]pyrene and 6-hydroxymethylbenzo[a]pyrene in acetone in the wavelength region 350 to 430 nm.

<table>
<thead>
<tr>
<th></th>
<th>6-Chloromethylbenzo[a]pyrene</th>
<th>6-Hydroxymethylbenzo[a]pyrene</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>$\varepsilon$ (M$^{-1}$ cm$^{-1}$)</td>
<td>$\lambda_{\text{max}}$ (nm)</td>
</tr>
<tr>
<td>365</td>
<td>$1.33 \times 10^4$</td>
<td>360</td>
</tr>
<tr>
<td>378</td>
<td>$2.58 \times 10^4$</td>
<td>376</td>
</tr>
<tr>
<td>388</td>
<td>$2.14 \times 10^4$</td>
<td>392</td>
</tr>
<tr>
<td>397</td>
<td>$2.72 \times 10^4$</td>
<td>407</td>
</tr>
<tr>
<td>409</td>
<td>$1.69 \times 10^4$</td>
<td></td>
</tr>
</tbody>
</table>

was found to be somewhat sensitive to changes in the methyl group substituent and free of interference from the solvents which were used. The difference in absorbance at 409 nanometers provides a "window" in which the solvolysis of 6-chloromethylbenzo[a]pyrene in aqueous solution can be conveniently followed. The BAP ring system is a good chromophore, as indicated by the extinction coefficients given in Table 1, allowing reactions to be followed in very dilute solution. A plot of absorbance versus time for the solvolysis reaction in 80% acetone 20% water (V/V) at 25°C is shown in Figure 13. The starting concentration of 6-chloromethylbenzo[a]pyrene was $3.33 \times 10^{-5}$ M.
Figure 13. A plot of absorbance at 409 nm versus time for the solvolysis of 6-chloromethylbenzo[a]pyrene in 80% acetone, 20% water (V/V) at 25°C.

This reaction would be expected to proceed by way of a carbonium ion (an $S_{N}1$ process). This type of reaction can be described by the following sequence:

$$RX \xrightleftharpoons[k_{-1}]{k_{1}} R^{+} + X^{-} \quad \text{Step 1}$$

$$R^{+} + H_{2}O \xrightarrow{k_{2}} ROH + H^{+} \quad \text{Step 2}$$
Step 1 is the slow or rate determining step. As the reaction proceeds, the concentration of $X^-$ builds up in solution, and the reverse of the first step, which is

$$R^+ + X^- \xrightarrow{k_{-1}} RX$$

often becomes important, especially for relatively stable carbonium ions for which $k_{-1}/k_2$ can be large. The rate equation must take this into account, and takes the form:

$$-\frac{d[RX]}{dt} = \frac{k_1[RX]}{k_{-1}[X^-] + \frac{k_2[H_2O]}{1}} \quad (eq. \ 1)$$

Some authors include ($H_2O$) in $k_2$ (see reference 124). Very early in the reaction, when $X^-$ is still negligible, the following simple first order rate law is followed:

$$-\frac{d[RX]}{dt} = k_1[RX] \quad (eq. \ 2)$$

Since the starting concentrations of substrate in the solvolyses discussed here were very low, the chloride ion concentration remained essentially unchanged and the reactions followed simple first order kinetics throughout. The integrated form of equation 2 is:

$$\log[RX] = \log[RX_0] - \frac{k_1}{2.303} t$$
Thus, the first order rate constant $k_1$ can be calculated from the slope of the line obtained by plotting the log of substrate concentration versus time. In this work, the line was fitted to the data by the least squares method with a computer program. This program also produced the coefficient of correlation and $k_1$. Absorbance was used as a measure of substrate concentration. The data from Figure 13 yield a first order rate constant of $9.4 \times 10^{-3} \text{ s}^{-1}$. The coefficient of correlation as well as those for all the other first order rate determinations presented here was greater than 0.99.

The $k_1$ value for the solvolysis of 9-chloromethylandthracene under the same conditions and other parameters for the solvolysis of 9-chloromethylandthracene in aqueous acetone solutions were determined in order to provide a comparison for 6-chloromethylbenzo[a]pyrene in addition to those in the literature. Absorption spectra for 9-chloromethylandthracene and 9-hydroxymethylandthracene in the same region used for 6-chloromethylbenzo[a]pyrene are shown in Figure 14. In this case, the absorbance change was followed at 391 nanometers. The molar extinction coefficients for the anthracene derivatives (Table 2) are smaller than those for the BAP derivatives and starting concentrations of $8.33 \times 10^{-5}$ were used.
Figure 14. The electronic absorption spectra of 9-chloromethylanthracene (a) and 9-hydroxymethylandanthracene (b) over the wavelength region 350-410 nm.

Table 2

Wavelengths and extinction coefficients of the absorption maxima for 9-chloromethylandanthracene and 9-hydroxymethylandanthracene in acetone in the wavelength region 340-420 nm.

<table>
<thead>
<tr>
<th>9-Chloromethylandanthracene</th>
<th>9-Hydroxymethylandanthracene</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max}} ) (nm)</td>
<td>( \varepsilon ) (M(^{-1}) cm(^{-1}))</td>
</tr>
<tr>
<td>353</td>
<td>5.34 x 10(^3)</td>
</tr>
<tr>
<td>371</td>
<td>7.81 x 10(^3)</td>
</tr>
<tr>
<td>392</td>
<td>6.97 x 10(^3)</td>
</tr>
</tbody>
</table>
Table 3 compares the first order rate constants for these two compounds with others obtained from the literature. The reactivity of 6-chloromethylbenzo[a]pyrene falls among various systems which have been used as models for reactive $S_N1$ substrates.

**THERMODYNAMIC ACTIVATION PARAMETERS**

The activation energy ($E^*$) of the Arrhenius equation

$$k = Ae^{-E^*/RT}$$

can be estimated for substitution reactions, if rate constants at more than one temperature are known, by equation 3.

$$E^* = \frac{2.303 R}{(T_2 - T_1)/T_1 T_2} \log \frac{k_{T_2}}{k_{T_1}}$$  \hspace{1cm} (eq. 3)

The enthalpy and entropy of activation, $\Delta H^*$ and $\Delta S^*$, can be estimated by equations 4 and 5.

$$\Delta H^* = E^* - RT$$  \hspace{1cm} (eq. 4)

$$\Delta S^* = (\log A - 13.23) \times 2.303 R$$  \hspace{1cm} (eq. 5)

where $\log A = \log k + \frac{E^*}{2.303 RT}$

The rate constants for the solvolyses of 6-chloromethylbenzo[a]pyrene in 80% acetone at 25°C and 43°C are $9.40 \times 10^{-3}$ s$^{-1}$ and $5.96 \times 10^{-2}$ s$^{-1}$ respectively. From equation 3,
Table 3

Comparison of the first order rate constants ($k_1$) for the solvolysis of some reactive chlorides in 80° acetone at 25°. In cases where $k_1$ values were extrapolated from solvolyses in different solvents or at different temperatures, the original conditions and $k_1$ values are also noted.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$k_1$ (s$^{-1}$)</th>
<th>Conditions</th>
<th>$k_1$ (s$^{-1}$) in 80% acetone at 25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triphenylmethyl chloride</td>
<td>1.7 x 10$^{-2}$</td>
<td>85% acetone</td>
<td>8.75 x 10$^0$ est</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-33°C</td>
<td></td>
</tr>
<tr>
<td>$p,p'$-Dimethylbenzydryl chloride</td>
<td>1.35 x 10$^{-3}$</td>
<td>0°C</td>
<td>2.70 x 10$^{-2}$ est</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\alpha$-(9-Anthryl)ethyl chloride</td>
<td>8.95 x 10$^{-5}$</td>
<td>90% acetone</td>
<td>1.6 x 10$^{-2}$ est</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>6-Chloromethylbenzo[a]pyrene</td>
<td></td>
<td></td>
<td>9.40 x 10$^{-3}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9-Chloromethylanthracene</td>
<td></td>
<td></td>
<td>2.96 x 10$^{-4}$</td>
</tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Benzhydryl chloride</td>
<td></td>
<td></td>
<td>7.00 x 10$^{-5}$</td>
</tr>
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<td></td>
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</tr>
<tr>
<td>$\alpha$-(2-Naphthyl)ethyl chloride</td>
<td></td>
<td></td>
<td>4.76 x 10$^{-6}$</td>
</tr>
<tr>
<td></td>
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<tr>
<td>Tert-butyl chloride</td>
<td></td>
<td></td>
<td>1.97 x 10$^{-6}$</td>
</tr>
</tbody>
</table>

$^a$Ref. 85  $^b$Ref. 84  $^c$Ref. 125  $^d$Ref. 126  $^e$Ref. 127  $^f$Ref. 128
E* = \frac{2.30 \times 1.99 \text{ cal mol}^{-1} \text{deg}^{-1}}{(316^\circ - 298^\circ)/316^\circ \times 298^\circ} \times \log \frac{5.96 \times 10^{-2} \text{s}^{-1}}{9.40 \times 10^{-3} \text{s}^{-1}}

= 19.2 \text{ kcal mol}^{-1}

From equations 4 and 5,

\Delta H^* = 19.2 \times 10^3 \text{ cal mol}^{-1} - (1.99 \text{ cal deg}^{-1} \text{mol}^{-1} \times 298^\circ

= 18.6 \text{ kcal mol}^{-1}

\log A = \log 9.40 \times 10^{-3} + \frac{1.93 \times 10^4 \text{ cal mol}^{-1}}{2.3 \times 1.99 \text{ cal mol}^{-1} \text{dec}^{-1} \times 298^\circ

= 12.2

\Delta S^* = (12.2-13.2) 1.99 \text{ cal mol}^{-1} \text{deg}^{-1} \times 2.3

= -4.58 \text{ cal mol}^{-1} \text{deg}^{-1}

Similar calculations for 9-chloromethylanthracene using rate constants at 25\(^\circ\)C and 44\(^\circ\)C of 2.96 \times 10^{-4} \text{s}^{-1} and 2.63 \times 10^{-3} \text{s}^{-1}

yield the following parameters:

E* = 21.6 \text{ kcal mol}^{-1}

\Delta H^* = 21.0 \text{ kcal mol}^{-1}

\Delta S^* = -3.6 \text{ cal mol}^{-1} \text{deg}^{-1}

These values for E* lie near the mid range of E*s for a great many nucleophilic substitution reactions, both S_N1 and S_N2. The \Delta S^* values for S_N1 solvolyses are generally less negative or more positive than those for comparable S_N2 solvolyses in the same solvents. The \Delta S^* values calculated above are somewhat less negative than for
many $S_N I$ processes in similar solvents. For example, Thornton\textsuperscript{129} has compiled a list of $\Delta S^*$ values for the solvolysis of substituted cumyl chlorides in 90% acetone 10% water. These values range from -21.1 to -9.3 cal mol\textsuperscript{-1}deg\textsuperscript{-1}. The work of Berliner and Shieh\textsuperscript{126} provides a closely analogous series of solvolyses for comparison. Their $\Delta S^*$ values for the solvolysis of $\alpha$-arylethyl chlorides in 80% acetone 20% water range from -14.2 cal mol\textsuperscript{-1}deg\textsuperscript{-1} for $\alpha$-phenethyl chloride to -5.04 cal mol\textsuperscript{-1}deg\textsuperscript{-1} for $\alpha$-(2-chrysene)ethyl chloride. The somewhat less negative values obtained for the two arylmethyl chlorides in this work might indicate that there is less ordering of solvating molecules in the transition state than in some other systems, because a major portion of the $\Delta S^*$ in $S_N I$ reactions is believed to arise from ordering of solvent molecules.\textsuperscript{130} The $\Delta S^*$ values suggest that the two arylmethyl systems in this work approach limiting $S_N I$ reactions involving "free" carbonium ions.

EFFECT OF ADDED NUCLEOPHILE

In aqueous solutions, a good nucleophile can compete with water molecules for carbonium ions and thus affect product distribution according to the following scheme:

\[
\text{RX} \underset{\text{H}_2\text{O}}{\xrightarrow{\text{ROH}}} \text{R}^+ + \text{X}^- \quad \text{Nuc:} \quad \text{R:Nuc}
\]

Since the nucleophile is not involved in the rate determining step, it can change the product distribution radically without affecting
the reaction rate. Azide ion is known to be a good probe in studies involving this principle.

Azide ion does compete very effectively with water for the benzo-\([a]\)pyrenyl-6-methyl carbonium ion. When 6-chloromethylbenzo[a]pyrene is solvolyzed in 80% acetone, 20% water in the presence of sodium azide concentrations of approximately 50 mM or greater, little alcohol is formed, as indicated by thin layer chromatography, and a new product appears. The absorption spectrum of the product is different from that of the alcohol and is not affected by further increases in azide ion concentration during solvolysis, indicating that the product is almost entirely 6-azidomethylbenzo[a]pyrene. The absorbance of the product mixture at the absorption maximum near 409 nanometers was used to estimate the concentrations of the products when low concentrations of sodium azide were used and thus to approximate \(k_{N_3^-}/k_2\). The molar extinction coefficient, \(\varepsilon\), for the alcohol and the azido product are \(7.2 \times 10^3\) and \(1.1 \times 10^4\) M\(^{-1}\) cm\(^{-1}\) respectively. When the initial substrate concentration is \(3.3 \times 10^{-5}\) M, and the sodium azide concentration is \(2 \times 10^{-4}\) M, the absorbance of the product mixture is 0.290, yielding the following calculation for product concentration.

\[
A = \varepsilon_{\text{alcohol}} [\text{alcohol}] + \varepsilon_{\text{azido product}} [\text{azido product}]/\text{product}
\]

\[
0.290 = 7.2 \times 10^3 [\text{alcohol}] + 1.1 \times 10^4 (3.3 \times 10^{-5} - [\text{alcohol}])
\]

\[
[\text{alcohol}] = 1.9 \times 10^{-5}\text{ M}
\]

\[
[\text{azido product}] = 1.4 \times 10^{-5}\text{ M}
\]
The concentration of water is approximately 11 M. The following is a calculation of \( \frac{k_{N_3}}{k_2} \):

\[
\frac{k_{N_3}}{k_2} \times \frac{2.0 \times 10^{-4} \text{ M}}{1 \times 11 \text{ M}} = \frac{1.4 \times 10^{-5} \text{ M}}{1.9 \times 10^{-5} \text{ M}}
\]

\[
\frac{k_{N_3}}{k_2} = 4.1 \times 10^4
\]

The \( k_{N_3}/k_2 \) value is recalculated by a different method under "inhibition of the common ion effect".

In contrast to the large effect of azide ion on product distribution, the solvolysis rate of 6-chloromethylbenzo[a]pyrene in 80% acetone is little affected by added sodium azide.

Figure 15 is a graph of the rate enhancement caused by added sodium azide versus the azide concentration. The rate enhancement is indicated by the value of \( k_{obs} \), the observed first order rate constant, divided by \( k_1 \), the first order rate constant without added azide. Data for the solvolysis of 9-chloromethylnanthracene under the same conditions are included for comparison. Figure 16 is similar to Figure 15, but the added nucleophile in this case is N-acetyl cysteine. The very slight rate enhancement with sodium azide can be attributed to an increase in the ionic strength of the solution. Note that 9-chloromethylnanthracene is somewhat more sensitive to ionic strength effects than is 6-chloromethylbenzo[a]pyrene.
Figure 15. Graph of relative rate enhancement versus concentration of sodium azide in the solvolysis of 6-chloromethylbenzo[a]pyrene (●) and 9-chloromethylantracene (▼) in 80% acetone at 25°C.

Figure 16. Graph of relative rate enhancement versus concentration of N-acetylcysteine in the solvolysis of 6-chloromethylbenzo[a]pyrene (●) and 9-chloromethylantracene (▼) in 80% acetone at 25°C.
THE COMMON ION EFFECT

If the leaving group or ion in an $S_N1$ reaction is present in solution, and $k_1[X^-]$ is significant compared to $k_2[H_2O]$, then according to equation 1, the rate of reaction as measured by the disappearance of starting material will be slowed. Some of the carbonium ion being formed is diverted back to starting material instead of proceeding on to product. If the concentration of $X^-$ does not change significantly during the course of the reaction, the term

$$\frac{k_{-1}[X^-]}{k_2[H_2O]} + 1$$

is essentially constant, and the first order rate law is followed with a new, "observed" rate constant according to the equation

$$\frac{-d[RX]}{dt} = k_{obs}[RX] = \frac{k_1[RX]}{k_{-1}[X^-]} \frac{k_2[H_2O]}{k_{-1}[H_2O]} + 1$$

$$k_{obs} = \frac{k_1}{k_{-1}[X^-]} \frac{k_2[H_2O]}{k_{-1}[H_2O]} + 1$$

(eq. 6)

This slowing of $S_N1$ reaction rates is called the common ion effect or mass law effect. If the common ion effect is small, the competing ionic strength effect can make it difficult to detect. In the case of 6-chloromethylbenzo[a]pyrene, however, the ionic strength effect is minute compared to the common ion effect,
indicating a high degree of selectivity for chloride ion over water as a nucleophile. The extent of this effect is indicated in Table 4 which lists some observed first order rate constants for the solvolysis of 6-chloromethylbenzo[a]pyrene in 80% acetone with various concentrations of lithium chloride added to the solution.

Table 4

The observed first order rate constants ($k_{\text{obs}}$) for the solvolysis of 6-chloromethylbenzo[a]pyrene in 80% acetone, 20% water (V:V) at 25°C with various concentrations of lithium chloride present in solution.

<table>
<thead>
<tr>
<th>Concentration of LiCl (mM)</th>
<th>$k_{\text{obs}}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$9.4 \times 10^{-3}$</td>
</tr>
<tr>
<td>1</td>
<td>$4.3 \times 10^{-3}$</td>
</tr>
<tr>
<td>10</td>
<td>$1.1 \times 10^{-3}$</td>
</tr>
<tr>
<td>50</td>
<td>$4.3 \times 10^{-4}$</td>
</tr>
<tr>
<td>100</td>
<td>$3.3 \times 10^{-4}$</td>
</tr>
<tr>
<td>250</td>
<td>$1.4 \times 10^{-4}$</td>
</tr>
<tr>
<td>500</td>
<td>$9.2 \times 10^{-5}$</td>
</tr>
</tbody>
</table>

The value of $k_{-1}/k_2$ can be calculated from equation 6 and the data in Table 4. The use of a low concentration of chloride ion in the calculation insures that the effect of increased ionic strength will be small and the activity of chloride ion will not differ significantly from its concentration. At a chloride ion concentration of 1 mM, the observed first order rate constant is $4.27 \times 10^{-3}$ s$^{-1}$, giving a $k_{-1}/k_2$ value of $1.32 \times 10^4$. 

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Calculations analogous to those above were made for the solvolysis of 9-chloromethylantracene in 50% aqueous acetone. Table 5 lists some observed first order rate constants for the solvolysis of 9-chloromethylantracene in 50% aqueous acetone with various concentrations of lithium chloride present in solution.

Table 5
The observed first order rate constants ($k_{obs}$) for the solvolysis of 9-chloromethylantracene in 50% aqueous acetone (V:V) at 25°C with various concentrations of lithium chloride present in solution.

<table>
<thead>
<tr>
<th>Concentration of LiCl (mM)</th>
<th>$k_{obs}$ ($s^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$5.0 \times 10^{-2}$</td>
</tr>
<tr>
<td>5</td>
<td>$3.7 \times 10^{-2}$</td>
</tr>
<tr>
<td>10</td>
<td>$3.0 \times 10^{-2}$</td>
</tr>
<tr>
<td>50</td>
<td>$1.3 \times 10^{-2}$</td>
</tr>
<tr>
<td>100</td>
<td>$8.1 \times 10^{-3}$</td>
</tr>
<tr>
<td>300</td>
<td>$4.9 \times 10^{-3}$</td>
</tr>
<tr>
<td>500</td>
<td>$3.4 \times 10^{-3}$</td>
</tr>
</tbody>
</table>

At a chloride ion concentration of 5 mM, the observed first order rate constant is $3.70 \times 10^{-2}$, giving a $k_{-1}/k_2$ value of $2.00 \times 10^3$. Swain et al. have shown that $k_{-1}/k_2$ values are not greatly affected by the relative amounts of water and acetone in this solvent system. They have determined the $k_{-1}/k_2$ value for triphenylmethyl chloride in 85% acetone as $3.1 \times 10^3$ and estimated it for other $S_N^1$ substrates from data in the literature. Comparison of these
values with the ones obtained here indicate that the benzo[a]-pyrenyl-6-methyl carbonium ion and the anthracenyl-9-methyl carbonium ion are somewhat more selective for chloride ion than would be expected, although generally in line with the literature comparisons.

THE SPECIAL SALT EFFECT

It has been shown by Winstein and co-workers\(^9\) that lithium perchlorate, at low concentrations, can cause a large, nonlinear increase in solvolysis rates for some \(S^1\) reactions. At higher concentrations, lithium perchlorate causes a linear rate increase attributable to an increase in ionic strength. Winstein attributed the nonlinear increase to perchlorate ion interference with the rejoining of solvent separated ion pairs according to the following sequence:

\[
\begin{align*}
RS & \xrightarrow{\text{RS}} R^+X^- & \xrightarrow{\text{RS}} R^+X^- & \xrightarrow{\text{RS}} \text{product} \\
& \downarrow & \downarrow & \downarrow \\
& \downarrow & \downarrow & \downarrow \\
& R^+ & ClO_4^- & \\
\end{align*}
\]

Figure 17 is a graph of \(k_{\text{obs}}\) for the solvolysis of 6-chloro-methylbenzo[a]pyrene in 80% acetone versus the concentration of sodium perchlorate added. The value of \(k_{SS}\) obtained by extrapolating the linear portion of the graph to zero is an indication of the magnitude of the special salt effect, and the ratio \(k_{SS}/k_1\) can be used for purposes of comparison. Although there seems to be a
definite special salt effect on this solvolysis, the \( \frac{k_{ss}}{k_1} \) value of 1.20 is small compared to values of 3.1 and 3.3 for the acetolysis of 3-\( \mu \)-anisyl-2-butyl brosylate and 2-\( \mu \)-anisylethyl tosylate respectively which were determined by Hinterstein and Robinson.\textsuperscript{131} This might indicate that in this system, ion return from solvent separated ion pairs is relatively unimportant.

Figure 17. The special salt effect on the rate of solvolysis of 6-chloromethylbenzo[a]pyrene in 80\% acetone of added sodium perchlorate.
INHIBITION OF THE COMMON ION EFFECT

It is not generally possible to obtain data about the course of an $S_N1$ reaction from rate determinations. If a compound which forms a relatively stable carbonium ion is solvolyzed with some nucleophile present, the product composition could depend very strongly on the nucleophilicity and concentration of the nucleophile, but the reaction rate would depend on the slow step, formation of carbonium ion, which is independent of the nucleophile except for small effects on solvent polarity.

If the apparent rate of a solvolysis reaction is slowed down considerably by the presence of the common ion, the rate can be increased again by addition of a nucleophile which competes with the common ion for the carbonium ion.

For experiments described here in which 6-chloromethylbenzo[a]-pyrene is solvolyzed in 50% acetone, in the presence of 0.5 M lithium chloride, the quantity

$$\frac{k_1[X^-]}{k_2[H_2O]}$$

is large ($\sim 237$). Under these circumstances, equation 6:

$$k_{obs} = \frac{k_1}{k_1[X^-] + 1} \frac{k_2[H_2O]}{k_2[H_2O]}$$

becomes

$$k_{obs} \approx \frac{k_1}{k_1[X^-] + 1} \frac{k_2[H_2O]}{k_2[H_2O]}$$

60
and

\[ k_{\text{obs}} = \frac{k_1 k_2 [\text{H}_2\text{O}]}{k_{-1} [\text{X}^-]} \]

In the presence of added nucleophile,

\[ k_{\text{obs}} = \frac{k_1 k_2 [\text{H}_2\text{O}] + k_1 k_{\text{nuc}} [\text{nuc}]}{k_{-1} [\text{X}^-]} \]

This approximation can be arrived at in the following manner. Since all of the carbonium ion formed either goes on to form product or to reform starting material, the following equation applies:

\[ k_1 [\text{RX}] = k_2 [\text{R}^+][\text{H}_2\text{O}] + k_{\text{nuc}} [\text{R}^+][\text{nuc}] + k_{-1} [\text{X}^-][\text{R}^+] \]

This equation yields the equation for carbonium ion concentration:

\[ [\text{R}^+] = \frac{k_1 [\text{RX}]}{k_2 [\text{H}_2\text{O}] + k_{\text{nuc}} [\text{nuc}] + k_{-1} [\text{X}^-]} \]

The observed rate of conversion of starting material to products is given by:

\[ k_{\text{obs}}[\text{RX}] = k_2 [\text{H}_2\text{O}][\text{R}^+] + k_{\text{nuc}} [\text{nuc}][\text{R}^+] \]

Substitution of the carbonium ion concentration equation for the value of \([\text{R}^+]\) gives
\[ k_{\text{obs}}[\text{RX}] = \frac{k_2[H_2O]k_1[\text{RX}]}{k_2[H_2O] + k_{\text{nuc}}[\text{nuc}] + k_{-1}[X^-]} + \frac{k_{\text{nuc}}[\text{nuc}]k_1[\text{RX}]}{k_2[H_2O] + k_{\text{nuc}}[\text{nuc}] + k_{-1}[X^-]} \]

Under conditions where the reaction is slowed down greatly by the common ion effect, \( k_2[H_2O] + k_{\text{nuc}}[\text{nuc}] \) is much smaller than \( k_{-1}[X^-] \) and the following approximation can be made:

\[ k_{\text{obs}}[\text{RX}] \approx \frac{k_2[H_2O]k_1[\text{RX}]}{k_{-1}[X^-]} + \frac{k_{\text{nuc}}[\text{nuc}]k_1[\text{RX}]}{k_{-1}[X^-]} \]

Cancelling out \([\text{RX}]\) gives the approximation required.

Since the concentrations of chloride ion and water are essentially constant, a graph of \([\text{nuc}]\) versus \( k_{\text{obs}} \) will be a straight line with slope \( k_1k_{\text{nuc}}/k_{-1}[X^-] \) and intercept \( k_1k_2[H_2O]/k_{-1}[X^-] \). The fit of the data to a straight line should indicate the soundness of the method for the system in question. Also note,

\[
\frac{k_{\text{obs}} \text{ with nucleophile}}{k_{\text{obs}} \text{ without nucleophile}} = \frac{k_1k_2[H_2O] + k_{\text{nuc}}[\text{nuc}]}{k_1k_2[H_2O] / k_{-1}[X^-]} = 1 + \frac{k_{\text{nuc}}[\text{nuc}]}{k_2[H_2O]}
\]

From this relationship, \( k_{\text{nuc}}/k_2 \), a measure of the selectivity of the carbonium ion and nucleophilicity of the nucleophile is easily calculated. Ionic strength effects and activity coefficients of \( X^- \) are cancelled out.

Figure 18 is a graph of \( k/k_0 \) for the solvolysis of 6-chloromethylbenzo[a]pyrene and also for the solvolysis of 9-chloromethyl-
anthracene in 50% (V:V) aqueous acetone with 0.5 M lithium chloride versus the concentration of added sodium azide. It should be noted that within the limits of rate enhancements shown, the data is linear and very low concentrations of azide produce significant enhancements. Figure 19 is similar to Figure 18, but in this case the added nucleophile is N-acetylcysteine. Azide ion produces greater rate enhancements in the solvolysis of 9-chloromethylanthracene than in the solvolysis of 6-chloromethylbenzo[a]pyrene, but the opposite is true for N-acetylcysteine.

![Graph](image)

Figure 18. Rate enhancement by sodium azide on the solvolysis of 6-chloromethylbenzo[a]pyrene (●) and 9-chloromethylanthracene (▼) in 50% aqueous acetone at 25°C in the presence of 0.5 M lithium chloride. \( k/k_0 \) is the ratio of the observed first order rate constant with added azide to the observed first order rate constant with no azide present.
Figure 19. Rate enhancement by N-acetylcysteine on the solvolysis of 6-chloromethylbenzo[a]pyrene (o) and 9-chloromethylanthracene (▼) in 50% aqueous acetone at 25°C in the presence of 0.5 M lithium chloride. \( \frac{k}{k_0} \) is the ratio of the observed first order rate constant with added N-acetylcysteine to the observed first order rate constant with no N-acetylcysteine present.

For 6-chloromethylbenzo[a]pyrene at an azide concentration of 1 mM, the observed rate constant is \( 2.21 \times 10^{-2} \), giving a \( k_{N_3}^-/k_2 \) value of \( 3.6 \times 10^4 \). This agrees reasonably well with the value of \( 4.1 \times 10^4 \) calculated on p. 50 when it is considered that the solvent in that case was 80% acetone.

In order to further characterize the selectivity of the benzo[a]-pyrenyl-6-methyl carbonium ion, several simple nucleophiles were examined by the "inhibition of the common ion effect" method. Table 6 lists the results for these along with results for the four nucleo-
Table 6

Data indicating the relative nucleophilic power of different types of nucleophiles compared to water ($k_{nuc}/k_2$) toward the benzo[a]pyrenyl-6-methyl carbonium ion.

<table>
<thead>
<tr>
<th>Nucleophile</th>
<th>Concentration (mM)</th>
<th>$k_{obs} \times 10^2$ s$^{-1}$</th>
<th>Half life (s)</th>
<th>$k_{obs}$ w/nucleophile $k_{obs}$ w/o nucleophile</th>
<th>$k_{nuc}/k_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>--</td>
<td>0.97</td>
<td>72</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Aniline</td>
<td>1</td>
<td>2.6</td>
<td>27</td>
<td>2.7</td>
<td>$4.7 \times 10^{-4}$</td>
</tr>
<tr>
<td>Sodium Azide</td>
<td>1</td>
<td>2.2</td>
<td>31</td>
<td>2.3</td>
<td>$3.6 \times 10^4$</td>
</tr>
<tr>
<td>Guanosine</td>
<td>10</td>
<td>1.5</td>
<td>46</td>
<td>1.5</td>
<td>$1.4 \times 10^3$</td>
</tr>
<tr>
<td>N-Acetylcysteine</td>
<td>10</td>
<td>1.4</td>
<td>51</td>
<td>1.4</td>
<td>$1.1 \times 10^3$</td>
</tr>
<tr>
<td>Pyridine</td>
<td>10</td>
<td>1.4</td>
<td>51</td>
<td>1.4</td>
<td>$1.1 \times 10^3$</td>
</tr>
<tr>
<td>Adenosine</td>
<td>10</td>
<td>1.2</td>
<td>58</td>
<td>1.2</td>
<td>$5.6 \times 10^2$</td>
</tr>
<tr>
<td>Cytidine</td>
<td>10</td>
<td>1.0</td>
<td>69</td>
<td>1.0</td>
<td>--</td>
</tr>
<tr>
<td>Sodium Hydroxide</td>
<td>10</td>
<td>1.0</td>
<td>69</td>
<td>1.0</td>
<td>--</td>
</tr>
<tr>
<td>Uridine</td>
<td>10</td>
<td>.99</td>
<td>70</td>
<td>1.0</td>
<td>--</td>
</tr>
<tr>
<td>Thymidine</td>
<td>10</td>
<td>.99</td>
<td>70</td>
<td>1.0</td>
<td>--</td>
</tr>
<tr>
<td>n-propylamine</td>
<td>100</td>
<td>2.9</td>
<td>24</td>
<td>3.0</td>
<td>$5.6 \times 10^2$</td>
</tr>
<tr>
<td>Sodium Hydroxide</td>
<td>100</td>
<td>2.5</td>
<td>28</td>
<td>2.6</td>
<td>$4.4 \times 10^2$</td>
</tr>
<tr>
<td>Diethylamine</td>
<td>100</td>
<td>1.3</td>
<td>52</td>
<td>1.3</td>
<td>$8.3 \times 10^1$</td>
</tr>
</tbody>
</table>
sides as "possible" nucleophiles. The best nucleophile tested was aniline, with sodium azide next. The "hard" nucleophiles, n-propylamine, diethylamine and sodium hydroxide were tested at 100 times the concentration used for aniline in order to get meaningful results since they were much weaker nucleophiles.

Solvolyases were run in 50% aqueous dioxane with 0.5 M lithium chloride in the presence of the "hard" bases, hydroxide ion and N-propylamine in order to be sure that their low nucleophilicity was not somehow due to the acetone solvent. In addition, guanosine monophosphate was tested in this solvent system because of its low solubility in aqueous acetone. The results (Table 7) are in line with those of solvolyases in aqueous acetone although n-propylamine appears to be a slightly better nucleophile in dioxane solution.

Table 7

Enhancement of the rate of solvolysis of 6-chloromethylbenzo[a]-pyrene in 50% aqueous dioxane with 0.5 M LiCl at 25°C by sodium hydroxide, n-propylamine and GMP.

<table>
<thead>
<tr>
<th>Nucleophile</th>
<th>Concentration (mM)</th>
<th>$k_{obs}$ ($s^{-1}$) $\times 10^2$</th>
<th>$\frac{k_{obs}}{k_{obs} \text{ w/o nucleophile}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>--</td>
<td>1.0</td>
<td>--</td>
</tr>
<tr>
<td>NaOH</td>
<td>100</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>n-Propylamine</td>
<td>100</td>
<td>3.9</td>
<td>3.9</td>
</tr>
<tr>
<td>GMP</td>
<td>10</td>
<td>1.4</td>
<td>1.4</td>
</tr>
</tbody>
</table>

66
Some of the nucleophiles tested for rate enhancements of the solvolysis of 6-chloromethylbenzo[a]pyrene in 50% aqueous acetone in the presence of 0.5 M lithium chloride were tested with 9-chloromethylanthracene under the same conditions. The results, which were quite similar, are listed in Table 8.

The order of relative nucleophilicity indicated by the data in Table 6 is quite different from that determined by Swain et al. for trityl and benzhydryl carbonium ions which were in turn different from each other. This data, then, along with that in Figures 18 and 19, support the conclusion of Swain and Scott that carbonium ions are not as systematic as $S_N^2$ substrates in their selectivity for various nucleophiles.

**Nucleic Acid Components as Nucleophiles.** The enhancement in the rate of solvolysis of 6-chloromethylbenzo[a]pyrene due to the presence of nucleosides, nucleotides, deoxynucleosides and deoxynucleotides was studied as an approach for predicting which of these residues might be preferentially attacked in nucleic acids. Table 9 lists the results obtained when each of these compounds was present in the reaction mixture at a concentration of 10 mM. This low concentration was used because of the low solubility of some of these prospective nucleophiles in aqueous acetone solutions. Each one was run at two different pH values because it was discovered during the course of these experiments that guanosine monophosphate is a much better nucleophile at pH 9.5 than at neutral pH. The pH study was done in order to shed some light on this phenomenon and to find out if it
Table 8

Data indicating the relative nucleophilic power of different types of nucleophiles compared to water ($k_{\text{nuc}} / k_2$) toward the anthryl-9-methyl carbonium ion.

<table>
<thead>
<tr>
<th>Nucleophile</th>
<th>Concentration (mM)</th>
<th>$k_{\text{obs}}$ (s$^{-1}$) x 10$^3$</th>
<th>Half life (s)</th>
<th>$k_{\text{obs}}$ w/nucleophile $k_{\text{obs}}$ w/o nucleophile</th>
<th>$k_{\text{nuc}} / k_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>--</td>
<td>3.4</td>
<td>204</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Sodium azide</td>
<td>1</td>
<td>8.7</td>
<td>80</td>
<td>2.6</td>
<td>4.4 x 10$^4$</td>
</tr>
<tr>
<td>Guanosine</td>
<td>10</td>
<td>4.8</td>
<td>144</td>
<td>1.4</td>
<td>1.1 x 10$^3$</td>
</tr>
<tr>
<td>N-acetylcysteine</td>
<td>10</td>
<td>4.3</td>
<td>161</td>
<td>1.3</td>
<td>8.3 x 10$^2$</td>
</tr>
<tr>
<td>n-Propylamine</td>
<td>100</td>
<td>4.9</td>
<td>141</td>
<td>1.4</td>
<td>1.1 x 10$^2$</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>100</td>
<td>5.5</td>
<td>127</td>
<td>1.6</td>
<td>1.7 x 10$^2$</td>
</tr>
<tr>
<td>Diethylamine</td>
<td>100</td>
<td>4.8</td>
<td>145</td>
<td>1.4</td>
<td>1.1 x 10$^2$</td>
</tr>
<tr>
<td>Triethylamine</td>
<td>100</td>
<td>3.6</td>
<td>191</td>
<td>1.1</td>
<td>2.8 x 10$^1$</td>
</tr>
</tbody>
</table>
Table 9

The nucleophilic power of nucleosides, deoxynucleosides, nucleotides and deoxynucleotides as indicated by their enhancement of the rate of solvolysis of 6-chloromethylbenzo[a]pyrene by inhibition of the common ion effect. Reactions were run in 50% aqueous acetone with 0.5 M LiCl at 25°C with potential nucleophiles present in 10 mM concentrations; pH was adjusted with dilute NaOH in 50% acetone, 0.5 M LiCl.

<table>
<thead>
<tr>
<th>Nucleophile</th>
<th>pH</th>
<th>$k_{\text{obs}}$ (s$^{-1}$) x 10$^2$</th>
<th>$k_{\text{obs}}$ w/nucleophile $/k_{\text{obs}}$ w/o nucleophile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uridine</td>
<td>7.0</td>
<td>1.0</td>
<td>1.03</td>
</tr>
<tr>
<td>monophosphate</td>
<td>9.5</td>
<td>1.2</td>
<td>1.24</td>
</tr>
<tr>
<td>Uridine</td>
<td>7.0</td>
<td>1.99</td>
<td>1.02</td>
</tr>
<tr>
<td>monophosphate</td>
<td>9.5</td>
<td>1.3</td>
<td>1.34</td>
</tr>
<tr>
<td>Thymidine</td>
<td>7.0</td>
<td>1.98</td>
<td>1.01</td>
</tr>
<tr>
<td>monophosphate</td>
<td>9.5</td>
<td>1.2</td>
<td>1.24</td>
</tr>
<tr>
<td>Thymidine</td>
<td>7.0</td>
<td>1.99</td>
<td>1.02</td>
</tr>
<tr>
<td>monophosphate</td>
<td>9.5</td>
<td>1.1</td>
<td>1.13</td>
</tr>
<tr>
<td>Cytidine</td>
<td>7.0</td>
<td>1.1</td>
<td>1.13</td>
</tr>
<tr>
<td>monophosphate</td>
<td>9.5</td>
<td>1.0</td>
<td>1.03</td>
</tr>
<tr>
<td>Deoxycytidine</td>
<td>7.0</td>
<td>1.0</td>
<td>1.03</td>
</tr>
<tr>
<td>monophosphate</td>
<td>9.5</td>
<td>1.0</td>
<td>1.03</td>
</tr>
<tr>
<td>Cytidine</td>
<td>7.0</td>
<td>1.0</td>
<td>1.03</td>
</tr>
<tr>
<td>monophosphate</td>
<td>9.5</td>
<td>1.0</td>
<td>1.03</td>
</tr>
<tr>
<td>Deoxycytidine</td>
<td>7.0</td>
<td>1.1</td>
<td>1.13</td>
</tr>
<tr>
<td>monophosphate</td>
<td>9.5</td>
<td>1.0</td>
<td>1.03</td>
</tr>
<tr>
<td>Adenosine</td>
<td>7.0</td>
<td>1.1</td>
<td>1.13</td>
</tr>
<tr>
<td>monophosphate</td>
<td>9.5</td>
<td>1.8</td>
<td>1.86</td>
</tr>
<tr>
<td>Deoxyadenosine</td>
<td>7.0</td>
<td>1.5</td>
<td>1.55</td>
</tr>
<tr>
<td>monophosphate</td>
<td>9.5</td>
<td>1.4</td>
<td>1.44</td>
</tr>
<tr>
<td>Adenosine</td>
<td>7.0</td>
<td>1.2</td>
<td>1.23</td>
</tr>
<tr>
<td>monophosphate</td>
<td>9.5</td>
<td>1.1</td>
<td>1.13</td>
</tr>
<tr>
<td>Deoxyadenosine</td>
<td>7.0</td>
<td>1.2</td>
<td>1.24</td>
</tr>
<tr>
<td>monophosphate</td>
<td>9.5</td>
<td>1.7</td>
<td>1.75</td>
</tr>
</tbody>
</table>
Table 9 (Cont'd)

<table>
<thead>
<tr>
<th>Nucleophile</th>
<th>pH</th>
<th>$k_{\text{obs}}$ (s$^{-1}$) x 10$^2$</th>
<th>$k_{\text{obs}}$ w/nucleophile</th>
<th>$k_{\text{obs}}$ w/o nucleophile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guanosine monophosphate</td>
<td>7.0</td>
<td>insol.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.5</td>
<td>3.2</td>
<td>3.30</td>
<td></td>
</tr>
<tr>
<td>Deoxyguanosine</td>
<td>7.0</td>
<td>1.5</td>
<td>1.55</td>
<td></td>
</tr>
<tr>
<td>monophosphate</td>
<td>9.5</td>
<td>3.3</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>Guanosine</td>
<td>7.0</td>
<td>1.3</td>
<td>1.34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.5</td>
<td>5.2</td>
<td>5.36</td>
<td></td>
</tr>
<tr>
<td>Deoxyguanosine</td>
<td>7.0</td>
<td>1.4</td>
<td>1.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.5</td>
<td>5.8</td>
<td>6.0</td>
<td></td>
</tr>
</tbody>
</table>

extended to any of the other nucleosides or nucleotides. The pH was adjusted by dropwise addition of mM sodium hydroxide in 50% aqueous acetone, 0.5 M lithium chloride solution.

Very small rate enhancements cannot be taken as significant in this case because hydroxide ion is a nucleophile, even though a poor one and because of the difficulties of adjusting the pH of an unbuffered aqueous-organic solution.

The following conclusions can be drawn from the data in Table 9. No systematic increase in nucleophilicity is due to the presence or absence of the phosphate group or the 2'-hydroxyl group of ribose. Nucleophilicity is most dependent on the presence of guanine or adenine bases at pH 7.

In order to obtain more quantitative data on the nucleophilicities of the bases, the three nucleosides, adenosine, cytidine and uridine and the deoxynucleoside thymidine were tested at 50 mM concentrations. The results are shown in Table 10. Since guanosine was
Table 10

Enhancement of the rate of solvolysis of 6-chloromethylbenzo[a]-pyrene in 50% aqueous acetone with 0.5 M LiCl at 25°C by nucleosides at 50 mM concentrations.

<table>
<thead>
<tr>
<th>Nucleophile</th>
<th>( k_{\text{observed}} ) (s(^{-1}) x (10^2))</th>
<th>( k_{\text{obs}} ) w/nucleophile</th>
<th>( k_{\text{obs}} ) w/o nucleophile</th>
<th>( \frac{k_{\text{nuc}}}{k_2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uridine</td>
<td>0.99</td>
<td>1.02</td>
<td>1.1 x (10^1)</td>
<td></td>
</tr>
<tr>
<td>Thymidine</td>
<td>1.0</td>
<td>1.03</td>
<td>1.7 x (10^1)</td>
<td></td>
</tr>
<tr>
<td>Cytosine</td>
<td>1.4</td>
<td>1.4</td>
<td>2.2 x (10^2)</td>
<td></td>
</tr>
<tr>
<td>Adenosine</td>
<td>2.4</td>
<td>2.5</td>
<td>8.4 x (10^2)</td>
<td></td>
</tr>
<tr>
<td>Guanosine*</td>
<td>2.6</td>
<td>2.7</td>
<td>9.5 x (10^2)</td>
<td></td>
</tr>
</tbody>
</table>

*extrapolated from 10 mM

not sufficiently soluble, the result is an extrapolation from a concentration of 10 mM. The results show that guanosine and adenosine are better nucleophiles than cytosine, which is still definitely a nucleophile, and uridine and thymidine do not show any rate enhancement beyond the limits of experimental error. They are either very poor nucleophiles or do not react at all.

Guanosine at High pH. In order to further investigate the relationship between pH and the nucleophilicity of guanine residues, solvolyses were run at several pH values from 7.0 to 10.4 in the presence of 0.01 M guanosine. The reactions were run in 50% aqueous dioxane for solubility purposes. A lithium chloride concentration of 0.5 M was again found suitable. Table 11 lists the observed first order rate constants at the pH values used. Figure 20 is a graph of the observed first order rate constants versus the pH of the solution.
<table>
<thead>
<tr>
<th>pH</th>
<th>$k_{\text{obs}} \times 10^{-2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.0</td>
<td>1.6</td>
</tr>
<tr>
<td>7.2</td>
<td>1.7</td>
</tr>
<tr>
<td>7.4</td>
<td>1.7</td>
</tr>
<tr>
<td>7.6</td>
<td>1.8</td>
</tr>
<tr>
<td>7.8</td>
<td>2.0</td>
</tr>
<tr>
<td>8.1</td>
<td>2.0</td>
</tr>
<tr>
<td>8.3</td>
<td>2.2</td>
</tr>
<tr>
<td>8.5</td>
<td>2.3</td>
</tr>
<tr>
<td>8.7</td>
<td>2.4</td>
</tr>
<tr>
<td>8.9</td>
<td>2.4</td>
</tr>
<tr>
<td>9.0</td>
<td>3.0</td>
</tr>
<tr>
<td>9.1</td>
<td>3.5</td>
</tr>
<tr>
<td>9.2</td>
<td>3.9</td>
</tr>
<tr>
<td>9.4</td>
<td>4.8</td>
</tr>
<tr>
<td>9.5</td>
<td>5.4</td>
</tr>
<tr>
<td>9.6</td>
<td>6.3</td>
</tr>
<tr>
<td>9.7</td>
<td>6.5</td>
</tr>
<tr>
<td>10.0</td>
<td>6.9</td>
</tr>
</tbody>
</table>
Figure 20. The observed first order reaction rates for the solvolysis of 6-chloromethylbenzo[a]pyrene in 50% aqueous dioxane in the presence of 0.5 M lithium chloride and 10 mM guanosine as a function of pH.

Figure 20 resembles a titration curve, and presumably corresponds to the loss of a proton from the nitrogen at the 1-position of guanosine. The $pK_a$ for this dissociation has recently been reported to be 9.03 in aqueous solution.\textsuperscript{132} The curve obtained here is centered at a somewhat higher pH, about 9.3, but both the $pK_a$ and the pH measurement are probably altered somewhat by the presence of 50% dioxane. The reactive species under these conditions should be:
CONJUGATES WITH NUCLEOPHILES

The next chapter describes studies of compounds formed by trapping of the benzo[a]pyrenyl-6-methyl carbonium ion. With reference to the kinetic studies described here, it was found, not unexpectedly, that good nucleophiles formed conjugates in amounts corresponding to their nucleophilicity in the kinetic studies. The only exceptions which were found to this rule are guanosine and guanosine-5'-monophosphate at pH values above 9, where only traces of conjugate were formed, and this corresponded to the conjugate formed (in much larger amount) at neutral pH. The reason for this is unknown, and although the connection may seem tenuous, it brings to mind the work of Murray and co-workers who found that polyriboguanylic acid in neutral aqueous ethanol catalyzed the formation of 4-hydroxybenzo[a]pyrene from benzo[a]pyrene 4,5-oxide whereas other polynucleotides did not. Since fairly high pH values are necessary for the apparent hyper-nucleophilicity of guanine residues described here, it might not be significant in biological systems.
EXPERIMENTAL

**Solvents.** The acetone was reagent grade and was used as is. Dimethylacetamide was redistilled and stored over molecular sieve. Dioxane was redistilled immediately before use. Aqueous acetone solutions were made up volume to volume and contraction of the solution was not considered in calculating water concentration.

**Chemicals.** Aniline, n-propylamine, diethylamine and triethylamine were redistilled immediately before use as added nucleophiles. Pyridine, sodium azide and lithium chloride were reagent grade and were used without further purification. The N-acetylcysteine, deoxyribonucleosides, nucleosides, deoxynucleotides and nucleotides were obtained from Sigma Chemical Company and used without further purification.

**Spectra.** Ultraviolet absorption spectra were taken on a Cary-15 spectrophotometer.

**Reaction rate determinations.** Absorbance changes were recorded on a Gilford modified Beckmann DU. Three ml of solvent were placed in a 1 cm cuvette in the spectrophotometer beam and 20 $\lambda$ of 5 mM 6-chloromethylbenzo[a]pyrene or 50 $\lambda$ of 5 mM 9-chloromethylnanthracene in acetone solution was added with a micropipette. The reaction mixture was stirred briefly and the cover was placed on the cuvette compartment. Absorbance changes were recorded versus time using chart speeds appropriate to the reaction rate. Very slow solvolyses were run overnight in a 2 ml glass stoppered cuvette in order to prevent solvent evaporation.
The temperature of the cuvette compartment was maintained at 25°, or as described in the text, by means of a circulating water system, and the temperature of the reaction mixture was checked after each run.

Approximately twelve absorbance/time values, over a period of at least three half lives were fed into the computer for determination of the first order rate constant, half life and coefficient of correlation for each solvolysis. In duplicate runs, rate constant values were always reproducible within ±5% except in cases where the pH was adjusted to 9.5 in which case they were reproducible to within ±20%.
STRUCTURAL STUDIES

ISOLATION OF CONJUGATES

Studies of the conjugates formed when 6-chloromethylbenzo[a]pyrene is solvolyzed in the presence of nucleosides were undertaken in order to determine their number and to obtain information regarding their structure. An additional purpose was to find out to what extent the chemical shift of the labelled arylmethyl carbon would indicate the structure of a conjugate. Reference chemical shifts determined in this way might, in the future, be used to study conjugates with intact nucleic acids using small amounts of material and a minimum of chemical manipulation of the sometimes labile conjugates.

Reactions were run in a manner similar to the kinetic runs in 50% dioxane described previously but with a higher concentration of 6-chloromethylbenzo[a]pyrene and without lithium chloride.

Thin Layer Chromatographic Analysis (TLC). The reaction mixtures were spotted on silica gel and microcrystalline cellulose TLC plates to check for the presence of fluorescent compounds in addition to 6-hydroxymethylbenzo[a]pyrene. The results indicated that nucleosides containing adenine, guanine and cytosine combine with the carbonium ion to form two different conjugates each. Nucleosides containing thymine and uracil did not form any conjugates in detectable amounts.

Except for a few preliminary TLC runs, these studies were done only on nucleosides, not deoxynucleosides or nucleotides, since the sugar and phosphate groups do not appear to be involved in conjugate
formation. The $R_f$ values of the conjugates and other compounds on several TLC systems are listed in Table 12.

**Fluorescence Spectra of Conjugates.** Fluorescent spots representing conjugates were scraped from the TLC plates and eluted into ethanol. The corrected fluorescence excitation and emission spectra of the solution were taken in order to confirm that the spots represented compounds containing the intact benzo[a]pyrene ring system. It was found that, as with ultraviolet absorption spectra, the fluorescence spectra were diagnostic of the ring system and little affected by substituents on the methyl group. The corrected excitation and emission spectra of 6-hydroxymethylbenzo[a]pyrene are shown in Figure 21. Note that the excitation spectrum corresponds closely to the absorption spectrum (Fig. 12) in the same wavelength region.

**Column Chromatography.** Reaction mixtures were chromatographed on a Sephadex LH-20 column using methanol. The procedure used was essentially that of Dipple and Slade. The eluate was collected in one hundred fractions of 5 ml each and assayed at 392 nm. Three absorbing elution peaks were observed when reaction mixtures from the solvolysis of 6-chloromethylbenzo[a]pyrene in the presence of adenosine, guanosine or cytidine were chromatographed. The second peak in each case had an elution volume identical to that of an authentic sample of 6-hydroxymethylbenzo[a]pyrene and the material in this peak was inseparable from 6-hydroxymethylbenzo[a]pyrene on several thin layer chromatographic systems. Material from the other two elution peaks, when chromatographed on TLC plates correspond to the fluores-
Table 12

$R_f$ values for methyl substituted 6-methylbenzo[a]pyrenes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Silica Gel</th>
<th>Microcrystalline Cellulose</th>
<th>Solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>6-Chloromethylbenzo[a]pyrene</td>
<td>0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-Hydroxymethylbenzo[a]pyrene</td>
<td>0.70</td>
<td>0.79</td>
<td>.96</td>
</tr>
<tr>
<td>6-n-Propylaminomethylbenzo[a]pyrene</td>
<td>0.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzo[a]pyrenyl-6-methyltriethyl ammonium chloride</td>
<td>0</td>
<td></td>
<td>.81</td>
</tr>
<tr>
<td>5-(Benzo[a]pyrenyl-6-methyl)-N-acetylcysteine</td>
<td>0.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzo[a]pyrenyl-6-methyl pyridinium chloride</td>
<td>0</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>6-Azidomethylbenzo[a]pyrene</td>
<td>0.81</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-Anilinomethylbenzo[a]pyrene</td>
<td>0.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenosine: conjugate A</td>
<td>0.18</td>
<td></td>
<td>.47</td>
</tr>
<tr>
<td>conjugate B</td>
<td>0.46</td>
<td></td>
<td>.70</td>
</tr>
<tr>
<td>Cytidine: conjugate A</td>
<td>0.51</td>
<td></td>
<td>.49</td>
</tr>
<tr>
<td>conjugate B</td>
<td>0.80</td>
<td></td>
<td>.95</td>
</tr>
<tr>
<td>Guanosine: conjugate A</td>
<td>0.10</td>
<td></td>
<td>.39</td>
</tr>
<tr>
<td>conjugate B</td>
<td>0.30</td>
<td></td>
<td>.74</td>
</tr>
</tbody>
</table>

Figure 21. The corrected fluorescence excitation (a) and emission (b) spectra of 6-hydroxymethylbenzo[a]pyrene.
cent spots obtained by TLC of the whole reaction mixture. This was true for each of the three nucleosides. Thus, it appears that all the conjugates observed by TLC were eluted in the first 500 ml. Each of the 6 conjugates was distinguishable from all others on at least one TLC system.

REFERENCE CHEMICAL SHIFTS

From the preceding chapters, it can be seen that a number of compounds of the type

\[ \text{and} \]

\[ \text{CH}_2X \]

\[ \text{CH}_2XR \]

were available by direct synthesis or by carbonium ion trapping under solvolytic conditions. In cases where \(-XR\) is a simple nucleophile, there can be little doubt as to the structure of the conjugate; and in any case, the structure can be confirmed by the chemical shift of the labelled carbon. This chemical shift can in turn be used as a reference for comparison with those of the nucleoside conjugates. The compounds of relatively simple structure whose chemical shifts were determined and used in this way are listed in Table 13. All chemical shifts in this table and elsewhere are given in parts per million downfield from TMS. Chemical shift values determined here
Table 13

Chemical shifts, in ppm downfield from TMS of the labelled arylmethyl carbons in some benzo[a]pyrenyl-6-methyl-\(^{13}\)C derivatives.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-Methyl-(^{13})C-benzo[a]pyrene</td>
<td>14.3</td>
</tr>
<tr>
<td>6-Chloromethyl-(^{13})C-benzo[a]pyrene</td>
<td>39.5</td>
</tr>
<tr>
<td>6-n-Propylaminomethyl-(^{13})C-benzo[a]pyrene</td>
<td>45.6</td>
</tr>
<tr>
<td>6-Hydroxymethyl-(^{13})C-benzo[a]pyrene</td>
<td>55.8</td>
</tr>
<tr>
<td>N-acetyl-s-(Benzo[a]pyrenyl-6-methyl-(^{13})C)-cysteine</td>
<td>29.6</td>
</tr>
<tr>
<td>6-Anilinomethyl-(^{13})C-benzo[a]pyrene</td>
<td>40.2</td>
</tr>
<tr>
<td>6-Azidomethyl-(^{13})C-benzo[a]pyrene</td>
<td>46.5</td>
</tr>
<tr>
<td>1-(Benzo[a]pyrenyl-6-methyl-(^{13})C)-pyridinium chloride</td>
<td>55.2</td>
</tr>
<tr>
<td>Benzo[a]pyrenyl-6-methyl-(^{13})C-triethylammonium chloride</td>
<td>56.8</td>
</tr>
</tbody>
</table>

were measured relative to solvent DMSO-\(d_6\) (with two exceptions) and chemical shift values calculated using the formula \(\delta_{\text{TMS}} = \delta_{\text{DMSO-}d_6} + 39.6\).\(^{113}\) The chemical shift of 6-anilinomethylbenzo[a]pyrene was measured relative to added dioxane using \(\delta_{\text{TMS}} = \delta_{\text{dioxane}} + 66.2\).\(^{134}\) The chemical shift of 1-benzo[a]pyrenyl-6-methylpyridinium chloride was measured relative to internal methanol using \(\delta_{\text{TMS}} = \delta_{\text{MeOH}} + 48.0\).\(^{135}\)

A correlation diagram similar to Shapiro's\(^{114}\) was drawn up for the chemical shifts of substituted 6-methylbenzo[a]pyrenes plotted against those for the 1-substituted propanes listed in Table 14. This diagram, which resulted in a good straight line, is shown in Figure 22. By means of this correlation, the chemical shifts of
Figure 22. Correlation between the chemical shifts of substituted 1-propyl carbons and similarly substituted benzo[a]pyrenyl-6-methyl carbons.

1-substituted propanes and other substituted alkanes from the literature can be used as references.

It is well known that predictions of chemical shift values based on substituent effects do not work as well for substituted methanes and ethanes as they do for larger alkanes. It would be
Table 14

Chemical shifts of some 1-propyl carbons in ppm downfield from TMS.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propane</td>
<td>15.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1-Chloropropane</td>
<td>46.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dipropylamine</td>
<td>54.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>1-Propanol</td>
<td>63.6&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ethyl propyl sulfide</td>
<td>36.5&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>N-propylaniline</td>
<td>51.6&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ref. 136
<sup>b</sup> Estimated: 15.5(δ propane) + 31(substituent effect for chloride)
<sup>c</sup> Estimated: 44.1(δ diethylamine) + 10 ppm(substituent effect for β methyl)
<sup>d</sup> Ref. 137
<sup>e</sup> Estimated: 26.5(δ diethyl sulfide)<sup>138</sup> + 10 ppm (substituent effect for β methyl)
<sup>f</sup> Estimated: 5.3 ppm downfield of n-propyl chloride in analogy with the corresponding methyl derivatives.

expected that the correlation described above might not be as good either. There are, however, few chemical shifts yet reported in the literature for alkylated heterocyclic rings of the kind in question where the alkyl group is larger than methyl. For this reason, a correlation diagram to compare the chemical shifts of substituted methanes (Table 15) with methyl substituted 6-methylbenzo[a]pyrenes was attempted (Fig. 23).
Figure 23. Correlation between the chemical shifts of substituted methyl carbons and similarly substituted benzo[a]pyrenyl-6-methyl carbons.
Table 15
Chemical shifts of some methyl carbons in ppm downfield from TMS.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane</td>
<td>-2.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chloromethane</td>
<td>24.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Methylpropylamine</td>
<td>33.8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Methanol</td>
<td>48.0&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dimethyl sulfide</td>
<td>19.5&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>N-methylanaline</td>
<td>30.2&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ref. 136  
<sup>b</sup> Ref. 138  
<sup>c</sup> Estimated to be equal to methylisobutylamine<sup>139</sup>  
<sup>d</sup> Ref. 135  
<sup>e</sup> Ref. 140  
<sup>f</sup> Ref. 139

CONJUGATES WITH ADENOSINE

A solvolysis of 6-chloromethyl-<sup>13</sup>C-benzo[a]pyrene in 50% aqueous dioxane with 25 mM adenosine was run as described in the experimental section. The solvolysis was run in dioxane instead of acetone for the purpose of standardization, since guanosine is not sufficiently soluble in aqueous acetone and had to be run in aqueous dioxane. In each case, solvolyses were also run in aqueous acetone in order to confirm the fact that the same conjugates are produced. The concentration of guanosine was, of necessity, lower in the aqueous acetone solvolysis.

The reaction mixture was chromatographed on a column of Sephadex
LH-20 with methanol. Figure 24 is an elution profile obtained by assaying the absorbance of each fraction at 392 nanometers. The

![Absorbance vs Fraction No.](image)

Figure 24. Elution profile from Sephadex LH-20 of a reaction mixture from the solvolysis of 6-chloromethylbenzo[a]pyrene in the presence of adenosine.

fractions constituting conjugate peak A were pooled, the solvent removed, and the residue taken up in 5 ml of DMSO-\(_d_6\) as a CMR sample. Since the high absorbance values obtained in the original assay precluded an accurate estimation of the amount of material in peak A, a 20 µl aliquot of this sample was placed in 3 ml of acetone. The absorbance of this solution was 0.08 at 392 nm. Making the estimate that the extinction coefficient of the conjugate is the same as that of 6-hydroxymethylbenzo[a]pyrene at 392 nm, the concentration of the
5 ml sample was 0.44 mM and a total of 2.2 μmol of material were produced. A similar calculation for conjugate B indicates a total of 2.4 μmol of material. Since 25 μmol of starting material were solvolyzed, conjugate A, conjugate B and the alcohol represent approximately 9%, 10% and 81% of the product respectively. The CMR sample of peak B material was obtained in a different manner as described below.

The chemical shifts of the labelled carbons in the two conjugates are 47.5 and 46.0 ppm downfield from TMS respectively. The chemical shifts are good indications that in both cases, the labelled carbon is bonded to nitrogen. Before discussing these shifts further, however, some chemical evidence bearing on the structures will be mentioned and the Dimroth rearrangement will be discussed briefly because of its possible relevance to the adenosine, cytidine and guanosine conjugates.

Chemical Conversion. The literature concerning the alkylation of adenosine, discussed in the background chapter, suggests that relatively stable carbonium ions might alkylate the amino nitrogen as well as the nitrogen at the 1-position of adenosine. Thus, the structures of the conjugates might be XXV and XXVI as shown in Figure 25.

When 1-alkyladenosines are heated with base, they often rearrange to form N\(^6\)-alkyladenosines. Jones and Robbins\(^93\) heated 1-methyladenosine with dilute sodium hydroxide on a steam bath and obtained N\(^6\)-methyladenosine. They identified the product by comparing
Figure 25. 1-(Benzo[a]pyrenyl-6-methyl)adenosine hydrochloride (XXV) and N6-(benzo[a]pyrenyl-6-methyl)adenosine (XXVI). The structural assignments for conjugates A and B with adenosine.

it to an authentic sample prepared by the reaction of methylamine with 6-chloro-9-β-D-ribofuranosylpurine, a synthesis established by Johnson et al. 141

Fleysher et al. 142 synthesized numerous N6-alkyladenosines in nearly quantitative yield by heating the corresponding 1-alkyladenosines in aqueous ammonia.

The conjugates from peak A and B were both treated with base under two sets of conditions. In one procedure the conjugate was heated in a boiling water bath in a sealed tube with concentrated
ammonia for one hour. In the other, it was maintained at 50° for 24 hours in 50% aqueous methanol with 50 mM sodium hydroxide.

Conjugate B was unaffected by either treatment. Conjugate A was changed by both treatments to a product which appears to be identical to conjugate B. It has the same elution volume from Sephadex LH-20, the same ultraviolet absorption spectrum and is inseparable from conjugate B by TLC.

Dipple et al. accomplished a closely analogous transformation from 1-(benzanthryl-7-methyl)adenosine to N\(^6\)-(benzanthryl-7-methyl)adenosine. They identified the N\(^6\)-alkylated derivative by synthesizing it by an unambiguous route.

A rearrangement of this type, which involves the real or apparent migration of an alkyl group from a heterocyclic nitrogen to an extracyclic amino or imino group was first reported by Rathke but is sometimes named after Dimroth who studied a similar reaction at a later date. Brown studied the mechanism of another, similar reaction and determined by nitrogen-15 labelling studies that the alkylated ring nitrogen and extracyclic nitrogen atoms exchange places. The rearrangement has also been observed with a 1,3-disubstituted cytosine by Mizuno et al. who refer to it as a Dimroth rearrangement. The specific rearrangements studied by Dimroth, Brown and Mizuno et al are indicated in Figure 26. The mechanism of the second example has been studied but not entirely delineated, and in addition, these three examples are not precisely analogous, so the mechanisms are obscure. Despite their similarity to these, the rearrangements discussed above involving
alkylated adenosines were not referred to as Dimroth rearrangements by the respective authors. In any case, observation of the shift has been considered solid evidence for structural assignment, hence, the assignment of structure XXV to conjugate A and XXVI to conjugate B.

The chemical shift of the labelled carbon in elution peak B material was obtained from a sample of rearranged material from
elution peak A along with genuine material from elution peak B.

$^{13}$C Chemical Shift Values for Labelled Carbons in Conjugates with Adenosine. The chemical shifts of the labelled carbons in the conjugates XXV and XXVI are 47.5 and 46.0 ppm downfield from TMS respectively, both within the range expected for the labelled carbon attached to nitrogen.

As a first estimate, it might seem that the chemical shifts observed for the two conjugates, being shifts for carbons attached to secondary and primary amines, should be separated by several ppm more than they are. The few examples available in the literature of methylated heterocyclics of the kind in question, however, indicate that carbons attached to ring nitrogens do have chemical shifts upfield of those bonded to secondary amines. The methyl C-13 chemical shifts of the compounds in Figure 27, for example, compare with the methyl shifts of methylcyclohexylamine (33.5) and N-methylaniline (30.2) rather than with those of dimethyl-3-aminopropylamine (45.4) or methyldibenzyamine (42.1).

Figure 27. Chemical shift values for the methyl carbons in a) 1-methyl-9-(β-D-ribofuranosyl)hypoxanthine and b) 7-methylpurine in ppm downfield from TMS.
The structure of a 1-alkyladenosine, as originally formed, is sometimes written as XXVII which can easily form the neutral structure XXVIII by losing a proton from the amino group as indicated in Figure 28.

![Chemical Structures](image)

**Figure 28.** Deprotonation of 1-substituted adenosine.

Due to the imino group, XXVIII is more basic than unsubstituted adenosine which becomes protonated at pH 3.59.\(^{132}\)

Conjugate XXV appears to lose a proton at roughly neutral pH, an event which is indicated by a change in the absorption spectrum and is entirely reversed by lowering the pH again. The spectra of the low and high pH forms are shown in Figure 29.

Deprotonation of the conjugate does not, within the limits of experimental error, change the chemical shift of the arylmethyl carbon. This suggests that the structure of the protonated form might best be written as the protonated imine XXV rather than as a quaternary ammonium salt like XXVII. The chemical shift of the arylmethyl
Figure 29. The absorption spectra of conjugate A with adenosine at low pH (a) and high pH (b).

carbon in a simple quaternary ammonium salt is well downfield of the observed chemical shift in this adenosine compound (Table 12). Protonation of an amine would be expected to have a negligible effect on a carbon three bonds away,\textsuperscript{149} although the effects of an intervening double bond and nitrogen atom are not well documented.

The compound from peak B, with probable structure XXVI did not
exhibit the enhanced basicity of XXV. When dilute sodium hydroxide was added to material off the column, the spectrum was unchanged.

CONJUGATES WITH CYTIDINE

An example of an elution profile of a reaction mixture in which 6-chloromethylbenzo[a]pyrene was solvolyzed in 50% aqueous dioxane with 25 mM cytidine as shown in figure 30. The reaction was run as described in the experimental section and 4 ml of the solution were applied to the column. This experiment was run on a small scale in order to obtain maximum separation for quantitation of the products. A CMR sample of peak A material was obtained by the reaction of 6-bromomethyl-13C-benzo[a]pyrene with cytidine in dimethylacetamide. In this way a larger sample of the conjugate was obtained with respect to the amount of starting material used because the bulk of

Figure 30. Elution profile from Sephadex LH-20 of a reaction mixture from the solvolysis of 6-chloromethylbenzo[a]pyrene in the presence of cytidine.
the starting labelled bromide was not converted to alcohol. The identity of the product with that produced under solvolytic conditions was confirmed by TLC. A CMR sample of peak B material was produced by scaling up the solvolysis experiment and using 6-chloromethyl-\textsuperscript{13}C-benzo[a]pyrene. The quantity of conjugate B produced in duplicate experiments was surprisingly variable.

Assuming that the extinction coefficients of the conjugates at 392 nm are nearly the same as that of 6-hydroxymethylbenzo[a]pyrene, the total amount of material in each peak was estimated by adding up the amounts in each fraction in Figure 30. The results of these calculations indicate that 0.8 \( \mu \)mol of conjugate A and 1.0 \( \mu \)mol of conjugate B were produced. Since 10 \( \mu \)mol of starting material was used, conjugates A, B and the alcohol represent 8\%, 10\% and 82\% of the product respectively, in this particular run.

**Chemical Shift Values for the Labelled Carbons in Conjugates with Cytidine.** The chemical shifts of the labelled carbons in conjugates A and B are 44.1 and 40.8 ppm downfield from TMS which indicates that they are probably bonded to nitrogen. Cytidine contains three nonequivalent nitrogens. Attack at the 1-position appears to be unknown and would produce a quaternary ammonium salt which the chemical shifts do not indicate. Therefore, the structures of the two conjugates are probably XXIX and XXX (Fig. 31).

By analogy with the adenosine conjugates and guanosine conjugates discussed below, the compounds in peaks A and B are probably the 3 and \( N^4 \)-substituted cytidines respectively. The amino substituted nucleosides are eluted later from Sephadex LH-20 and have
Figure 31. 3-(Benzo[a]pyrenyl)-6-methyl)cytidine hydrochloride (XXIX) and N^4-(benzo[a]pyrenyl)-6-methyl)cytidine (XXX). The structural assignments for conjugates A and B with cytidine.

higher $R_f$ values on silica gel and microcrystalline cellulose TLC plates, with the solvent systems used here, than the ring substituted nucleosides. Also, the chemical shifts of the labelled carbons in the amino substituted compounds are upfield of those in the respective ring substituted compounds. Finally, the ring substituted conjugates are eluted from the Sephadex LH-20 column in the protonated form (as the hydrochloride or hydrobromide salt) as indicated by a spectral change upon addition of base. The reaction
mixtures were at approximately pH 5.5 when placed on the column. As with the adenosine product, the material in peak A is deprotonated at a pH well above the pK of cytidine which is 4.1. The loss of a proton occurs at roughly neutral pH and the event is indicated by a change in the absorption spectrum as indicated in Figure 32. The spectral change is smaller than that for the adenosine compound.

![Absorbance vs Wavelength Graph]

Figure 32. The absorption spectra of conjugate A with cytidine at low pH (a) and high pH (b).
Chemical Conversion. Some attempts were made to convert the conjugate in peak A to that in peak B by way of the Dimroth rearrangement. Treatment of peak A material with sodium hydroxide gave a compound of unknown structure with a chemical shift 37.3 ppm downfield from TMS. This compound has low $R_f$ values in the TLC systems used here, unlike the peak B conjugates. Neither Brookes and co-workers$^{105}$ nor Shapiro and Shiuey$^{107}$ who studied benzylated cytidines have reported a Dimroth rearrangement for 3-benzylcytidine, although such a conversion would be a valuable confirmation of structure. The Dimroth rearrangement of 3-arylmethylcytidines might not be as easily accomplished as the reported rearrangement of simpler 1,3-disubstituted cytosines.

CONJUGATES WITH GUANOSINE

A solvolysis of 6-chloromethyl-$^{13}$C-benzo[a]pyrene in 50% aqueous dioxane with 25 mM guanosine was run in the same manner as the one described for adenosine except that the guanosine had to be dissolved by heating the mixture to 70°C. The mixture was then cooled in a water bath and the solution of labelled chloride was added to this supersaturated solution. The reaction mixture was chromatographed on a column of Sephadex LH-20 with methanol. Figure 33 is an elution profile obtained by assaying the absorbance of each 5 ml fraction at 392 nm. The material in peak A was used as a CMR sample. A 20 μl aliquot of this 5 ml sample was placed in 3 ml of acetone and the absorbance of this solution at 392 nm was 0.11. Assuming the compound has the same extinction coefficient
Figure 33. Elution profile from Sephadex LH-20 of a reaction mixture from the solvolysis of 6-chloromethylbenzo[a]pyrene in the presence of guanosine.

as 6-hydroxymethylbenzo[a]pyrene, about 3.0 µmol were produced. By making the same assumption for the material in peak B, and adding up the amount of material in each fraction it was estimated that 1.0 µmol were produced. Thus, conjugate A, conjugate B and the alcohol represent approximately 12%, 5% and 83% of the product, respectively.

Sufficient peak B material for a CMR sample was produced by reaction of 6-bromomethyl-¹³C-benzo[a]pyrene with guanosine in DMA. As usual, the identity of this product with the peak B material produced under solvolytic conditions was checked chromatographically.

The ¹³C Chemical Shift Values for Labelled Carbons in Conjugates with Guanosine. The chemical shifts of the labelled carbons in the conjugates from elution peaks A and B are 45.7 and 41.2 ppm down-field from TMS. These shifts indicate that the labelled carbons are bonded to nitrogen in each case. Reports of previous studies
concerning the alkylation of guanosine by relatively stable carbonium ions suggest XXXI and XXXII as possible structures for the two conjugates (Fig. 34). The structures of 7-alkylguanosines are

![Structures XXXI and XXXII](image)

Figure 34. 7-(Benzo[a]pyrenyl-6-methyl)guanosine hydrochloride (XXXI) and N°-(benzo[a]pyrenyl-6-methyl)guanosine (XXXII). The structural assignments for conjugates A and B with guanosine.

sometimes shown as XXXIII, but the positive charge can be placed on the nitrogen at the 9-position to give XXXIV (Fig. 35), so the best representation for the electronic structure is probably XXXI. Because the nitrogen at the 7-position has a partial positive charge, it would seem that the resonance of the carbon bonded to it would be shifted downfield, toward the resonance of carbons in
Figure 35. Resonance structures for 7-substituted guanosine.

Quaternary ammonium salts. Reference shifts in the literature which are applicable to this structure are not numerous. Consider, however, the shift of the methyl carbons in compound XXXV, 35.1 ppm downfield from TMS. This shift is very near that of the methyl carbons in some similar neutral compounds such as XXXVI and XXXVII\(^{150}\) (Fig. 36).

Figure 36. A salt (XXXV) which is analogous to that formed by substitution at the 7-position of guanosine. The chemical shift of the methyl carbon is well upfield of the shift expected for methyl carbons bonded to quaternary nitrogens.\(^{150}\)
Chemical shift values in the range of those determined for the guanosine conjugates are compatible with structure XXXI.

In contrast to 1-substituted adenosines and 3-substituted cytidines, compounds of structure XXXI cannot easily lose a proton to form a neutral compound. Instead, they probably lose a proton from the 1-position to form a betaine as shown in Figure 37.

![Chemical structure diagram](image)

Figure 37. Formation of a betaine by deprotonation of a 7-substituted guanosine.

The guanosine conjugate from peak A was dissolved in 5 ml of 50% aqueous methanol. The pH of the solution was approximately 5.5. The pH was raised by dropwise addition of dilute sodium hydroxide. At pH 8.5 a yellow solid precipitated from solution. Figure 38 is an absorption spectrum of the precipitate redissolved in methanol as well as a spectrum of the material before adding base. The spectral change is entirely reversible and depends on the pH of the solution. Although ultraviolet absorption spectra do not appear to be good indications of structure in these systems, it does appear from these spectra that the protonation and deprotonation of the
Figure 38. The absorption spectra of conjugate A with guanosine at low pH (a) and high pH (b).

Quanosine conjugate is quite different from that of the adenosine conjugate, causing opposite effects in the spectra.

A sample of labelled conjugate A was prepared, precipitated from aqueous solution by addition of base, and dissolved in DMSO-d6 as a CMR sample. The chemical shift of the deprotonated conjugate A is 44.6 ppm downfield of TMS or 1.1 ppm upfield of the protonated form. Takeuchi and Dennis have recently reported the chemical
shifts of some 1-methylpyridinium-3-oxides and their betaines which may be applicable as references. Figure 39 shows two such systems and the shifts of the methyl carbons in each form.

![Chemical structures](image)

Figure 39. Formation of betaines from 1-methylpyridinium-3-oxides causes an upfield shift of about 2 ppm for the N-methyl carbon.\textsuperscript{151}

Deprotonation of the 1-methylpyridinium-3-oxides moves the methyl carbon resonance upfield by approximately 2 ppm. These examples are particularly significant because the effect of deprotonation on the chemical shift in this type of compound is opposite that to be expected in simple amine-amine hydrochloride equilibria. The resonance of each carbon affected would be expected to shift downfield upon going from an amine hydrochloride to a free amine.
Conjugate B, because of its chromatographic properties appears to be the amino group alkylation product XXXII. The chemical shift is very near that of the corresponding cytidine conjugate. This compound does not appear to have any enhanced basicity compared to guanosine itself, which probably rules out the 3-substituted conjugate. The possibility that conjugate B is the 1-substituted guanosine cannot be ruled out entirely from the data presented here, but seems unlikely.

**Chemical Conversion.** 7-Alkyladenosines are very subject to the loss of the ribose group as compared to other substituted nucleosides. They are known to lose the ribose merely upon heating in neutral aqueous solution. When conjugate A with guanosine was heated in aqueous solution in a boiling water bath, the only fluorescent compound left was 6-hydroxymethylbenzo[a]pyrene. Apparently, in compound XXXI, the benzo[a]pyrenyl-6-methyl bond to guanosine is more labile than the bond between ribose and guanine.

**REACTIONS IN DMA.**

These were done as an alternative to scaling up solvolysis reactions in cases where sufficient conjugate for a CMR sample was not produced. The results of these reactions differ from those of Dipple et al. with 7-bromomethylbenzantracene. They found that mainly the 7-substituted guanosine, 1-substituted adenosine and 3-substituted cytidine were formed. In the present experiments, however, both of the conjugates formed (with the chloride) under solvolytic conditions with adenosine and guanosine were also formed.
with the bromide in DMA and in roughly equal amounts, although only conjugate A with cytidine was produced in any quantity. Attempts at precise quantitation were not made for two reasons. First, the extinction coefficients of the conjugates were uncertain and second, the product distribution was not precisely reproducible from experiment to experiment, especially with cytidine.

CONCLUSIONS REGARDING THE USE OF CMR IN THESE EXPERIMENTS

An easily distinguishable peak was obtained from a 5 ml sample of 6-hydroxymethyl-\textsuperscript{13}C-benzo[\textit{a}]pyrene (enriched to 90\% \textsuperscript{13}C) with an accurately measured concentration of 8.5 x 10\textsuperscript{-4} M, using 100,000 pulses. Some of the CMR samples of unknown conjugates were also run at this concentration or somewhat lower. CMR signals from \textsuperscript{13}C labelled compounds can be obtained from such dilute samples that CMR is a useful technique for studying the reaction products of activated carcinogens with isolated biomolecules. This is true even when experiments, for the purpose of biochemical relevancy, are run in aqueous solution where most of the activated carcinogen might become deactivated by water instead of reacting with the biomolecule. Structural information can be obtained from the products without scaling up reactions unreasonably.

The chemical shifts of carbons attached to nitrogen atoms in nucleosides are fairly close together for the most nucleophilic nitrogens, and therefore, structural assignments based on shifts alone would have to be made cautiously. Carbons attached to the nucleophilic nitrogens in nucleosides have shifts which would be
expected to be well separated from carbons bonded to oxygen, however, and this distinction could be made rather unequivocally.

EXPERIMENTAL

Thin layer chromatography. Silica gel and microcrystalline cellulose TLC plates were 5 x 20 cm precoated on glass and were purchases from Applied Science Laboratories Inc. Chromatograms were developed in covered jars in an atmosphere saturated with the developing solvent. The chromatograms were developed in the dark. The solvents used are listed under Table 12. The fluorescent spots were viewed under ultraviolet light. Unreacted nucleosides were visible as absorbing spots. The three conjugates from elution peak A of guanosine, adenosine and cytidine formed streaks at low $R_f$ rather than spots on silica gel, but the two conjugates with each nucleoside are well separated from each other.

Fluorescence Spectra. Emission and excitation spectra were determined with a Perkin Elmer MPF-44A fluorescence spectrophotometer equipped with a corrected spectra accessory and were recorded with a Houston Instrument Omnigraphic 2000 X-Y recorder. Spectra of several fluorescent TLC spots including the nucleoside conjugates were determined by scraping the spot off the TLC plate and eluting with 3 ml of ethanol. Each fluorescent spot thus tested proved to contain a compound with an intact benzo[a]pyrene ring system.

Column Chromatography. A 90 cm glass column with an internal diameter of 15 mm was packed to a height of 75 cm with Sephadex LH-20, purchased from Pharmacia Fine Chemicals, Inc. The Sephadex
LH-20 was swelled for at least four hours in methanol before use as column packing. The column was wrapped with aluminum foil. Samples were placed on the column and eluted with methanol; this operation was done in a cold room at a temperature of approximately 4°C. Fractions were collected with an LKB Ultrorac fraction collector. Under the conditions used, two hundred drops gave 5 ml fractions. The ultraviolet absorption of the eluate was monitored, as it came off the column, at 280 nm with an LKB Uvicord detector-recorder. Each fraction was then assayed at 392 nm on a Gilford modified Beckmann DU. Elution peaks for acetone and unreacted nucleosides in addition to compounds containing the BAP moiety were detected at 280 nm and the peaks containing the BAP moiety were picked out from the others in the assay at 392 nm. The fractions containing each conjugate were pooled and the methanol was taken off on a rotary evaporator. Each sample was stored in DMSO-d₆ in the absence of light prior to running CMR spectra.

In cases where reactions were scaled up to use more than 25 μmol of 6-chloromethylbenzo[a]pyrene (7.5 mg) or 6-bromomethylbenzo[a]-pyrene (8.7 mg) the reaction mixture was chromatographed on a 45 cm glass column with an internal diameter of 3 cm, packed to a height of 30 cm.

**CMR Spectra.** Spectra were obtained either with a Varian XL-100 CW/FT spectrometer on FT mode or with a Varian CFT-20 spectrometer. Up to 100,000 pulses were used, depending on the concentration of the sample. The solvent was DMSO-d₆ except in the case of the pyridinium salt where D₂O was used. Spectra were taken at ambient temperature.
CMR Samples of Synthetic Products. Samples of 6-benzo[a]pyrene-carboxaldehyde-formyl-\textsuperscript{13}C, 6-methyl-\textsuperscript{13}C-benzo[a]pyrene, 6-hydroxy-methyl-\textsuperscript{13}C-benzo[a]pyrene and 6-chloromethyl-\textsuperscript{13}C-benzo[a]pyrene were made up 6 mg/ml in DMSO-d\textsubscript{6}.

Sensitivity Experiment. The 6-hydroxymethylbenzo[a]pyrene sample was diluted 1/25 by placing .2 ml of the sample in 4.8 ml of DMSO-d\textsubscript{6}. A satisfactory signal was obtained from the labelled carbon on the XL-100 using 100,000 pulses. The amount of material in the sample was 1.2 mg (4.2 \textmu mol). This sample size is probably approaching the minimum amount of material necessary for routine spectra.

CMR Samples of Conjugates With Simple Nucleophiles.

N-Acetyl-S-(benzo[a]pyrenyl-6-methyl)cysteine. N-Acetylcysteine (0.17 g, 1 mmol) was dissolved in 5 ml of 80% acetone 20% water and a solution of 6-chloromethyl-\textsuperscript{13}C-benzo[a]pyrene (XXII) (7.5 mg, 25 \textmu mol) in 5 ml of acetone was added with stirring. After 1 h, most of the acetone was stripped off on a rotary evaporator. The remaining mixture was centrifuged, the supernatant decanted and the residue taken up in DMSO-d\textsubscript{6}.

6-n-Propylnaminomethylbenzo[a]pyrene. n-Propylamine (0.3 g, 5 mmol) was dissolved in 5 ml of 80% acetone 20% water and XXII (7.5 mg, 25 \textmu mol) in 5 ml of acetone was added with stirring. After 1 h, 1 ml of 0.1 M NaOH was added and most of the acetone was stripped off on a rotary evaporator. The remaining mixture was centrifuged, the supernatant was decanted and the residue taken up in DMSO-d\textsubscript{6}.

N-(benzo[a]pyrenyl-6-methyl)aniline. Aniline (0.1 ml, 1.1 mmol) was dissolved in 5 ml of acetone along with XXII (7.5 mg, 25 \textmu mol).
One ml of water was added with stirring. After 1 h, 1 ml of 0.1 M NaOH was added and most of the acetone was removed on a rotary evaporator. The solid was filtered off, washed with water and taken up in DMSO-d$_6$.

6-Azidomethylbenzo[a]pyrene. Sodium azide (33 mg, 0.51 mmol) was dissolved in 5 ml of 80% acetone 20% water and a solution of XXII (7.5 mg, 25 µmol) in 5 ml of acetone was added with stirring. After 1 h, most of the acetone was removed on a rotary evaporator. The precipitate was filtered off, washed with water and taken up in DMSO-d$_6$.

Benzo[a]pyrenyl-6-methyltriethylammonium Chloride. Triethylamine (0.51 g, 5 mmol) was dissolved in 5 ml of 80% acetone 20% water and a solution of XXII (7.5 mg, 25 µmol) in 5 ml of acetone was added with stirring. After 1 h, the solvent was removed entirely on a rotary evaporator and the residue was taken up in DMSO-d$_6$.

1-(Benzo[a]pyrenyl-6-methyl)pyridinium Chloride. In 1 ml of pyridine was dissolved XXII (7.5 mg, 25 µmol). The mixture was allowed to stand for 1 h, then 1 ml of water was added and acetone acetone was added dropwise until the product began to crystallize. The reaction mixture was chilled and centrifuged and the supernatant decanted. The crystals were dissolved in D$_2$O and 20 µL of methanol were added as a CMR reference.

CMR Samples of Conjugates with Nucleosides.

Solvolytic Reactions. Reactions run under solvolytic conditions for the purpose of producing conjugates with nucleosides for CMR.
samples were run according to the following general procedure:

In 5 ml of distilled, deionized water and 2.5 ml of dioxane was dissolved enough nucleoside to make 10 ml of a 25 mM solution. To this solution was added, dropwise and with stirring 5 ml of 5 mM XXII (7.5 mg, 25 μmol) in dioxane. The addition was accomplished over a period of about ten minutes. In this way, the composition of the solvent was averaged out to 50% aqueous dioxane to correspond to kinetic experiments described in the previous section where 50% aqueous/organic solvents were used. Column chromatography of the resulting reaction mixtures provided sufficient material for a CMR sample of the early eluting conjugates with adenosine, cytosine and guanosine. Each reaction was also run using acetone instead of dioxane, and chromatographed in order to compare the product distribution with that in dioxane. Guanosine was run as a saturated solution (about 5 mM) in the 50% acetone solvolysis and the conjugates corresponded to those formed in aqueous dioxane. Solvolyses were run in the presence of adenosine and cytidine at concentrations of 25 mM but only on a 2 ml scale. The reactions run to produce conjugates for purposes of comparison and attempted chemical conversion used unlabelled 6-chloromethylbenzo[a]pyrene.

In order to produce a sufficient amount of the late eluting cytidine conjugate for a CMR sample, the amounts of XXII and cytidine were increased fourfold and the amount of the respective solvents were doubled. The reaction mixture was chromatographed on the larger column described above.
Reactions of 6-Bromomethylbenzo[a]pyrene with Nucleosides in Dimethylacetamide. In order to produce a sufficient amount of the late eluting conjugate with guanosine for a CMR sample, 6-bromomethyl-\(^{13}\)C-benzo[a]pyrene (8.5 mg, 25 μmol) was dissolved in 5 ml of dimethylacetamide containing 17 mg of guanosine and allowed to stand for 16 h. Column chromatography of the reaction mixture and thin layer chromatography of material from the elution peaks indicated that the same two conjugates were produced in this experiment as in the solvolysis described above. Only a small amount of 6-hydroxymethyl-\(^{13}\)C-benzo[a]pyrene was produced. This was probably due to the water of crystallization present in the guanosine. Thus, a larger amount of conjugate with respect to starting material was produced than in solvolyses. Somewhat surprisingly, the relative amounts of the two were similar to the relative amounts produced under solvolytic conditions.

Unlabelled 6-bromomethylbenzo[a]pyrene was allowed to react with adenosine and cytidine in order to compare the product distribution in each case with that produced under solvolytic conditions. These reactions were run as described for guanosine.

Conversion of 1-(Benzo[a]pyrenyl-6-methyl)adenosine to N\(^{6}\)-(Benzo[a]pyrenyl-6-methyl)adenosine. Five ml of elution peak A from a solvolysis of 6-chloromethylbenzo[a]pyrene in the presence of adenosine were concentrated to 2 ml. One ml was placed in a test tube with 1 ml of water and 0.1 ml of 1 M NaOH and heated at 50° for 25 h with stirring. The other ml of peak A was placed in a test tube with
1 ml of concentrated ammonia. The test tube was sealed and heated in a boiling water bath for 1 h. The contents of both test tubes were neutralized with dilute HCl and spotted on TLC plates. Only one major fluorescent spot appeared in each case and this corresponded to untreated material from elution peak B. Treatment of peak A material with base under the same conditions but without heating left it unchanged. Treatment of peak B material with base, as expected, left it unchanged.

A CMR sample of conjugate B was prepared by treating an entire reaction mixture produced by the solvolysis of XXII in the presence of adenosine in 50% acetone with NaOH. The solution was neutralized, the solvent taken off on a rotary evaporator, and the residue taken up in DMSO-d₆.

The cytidine conjugate from peak A was treated in the same manner with both NaOH and ammonia. In the first case, a new compound was produced in good yield, but in neither case was peak B material produced.
CONCLUSION

The carcinogenic activity of electrophiles such as 6-chloro-
methylbenzo[a]pyrene might be related to their selectivity for
reaction with certain critical biological nucleophiles or critical
nucleophilic sites on biomolecules. The critical cellular targets
might be nucleic acids.

In aqueous-organic solution, 6-chloromethylbenzo[a]pyrene is
solvolyzed by an $S_{N}1$ process, and the intermediate benzo[a]pyrenyl-
6-methyl carbonium ion reacts selectively with nucleophiles present
in solution. The solvolysis can be followed spectrophotometrically
and, since the BAP ring system is a good chromophore, this is a
sensitive method for determining first order rate constants. By
using the inhibition of the common ion effect, observed first order
rate constants can be used as an indication of the extent of reac-
tion of the carbonium ion with good nucleophile in the solution.
Thus, the method is useful for studying the selectivity of the
carbonium ion and is complementary to isolation and structural
studies of reaction products.

In conjunction with carbon-13 labelling, CMR was useful in
obtaining information about the structures of conjugates formed
between 6-chloromethylbenzo[a]pyrene and nucleosides and sensitive
enough to be used in this type of experiment without scaling up the
solvolysis reactions unreasonably. Both kinetic and CMR studies
using nucleosides as potential nucleophiles indicated that the
benzo[a]pyrenyl-6-methyl carbonium ion reacts selectively with adenosine, guanosine and cytidine, but not with uridine or thymidine. Two conjugates each were formed with adenosine, guanosine and cytidine. The chemical shift values of the labelled arylmethyl carbons in each of the conjugates indicate that the carbon is bonded to nitrogen. Also, these chemical shifts along with other data indicate that the conjugates result from reaction of the carbonium ion with the nitrogens at the 1-position of adenine, the 7-position of guanine and the 3-position of cytosine as well as the extracyclic amino groups of all three bases.

This study provides evidence that those carcinogens which react by way of a relatively stable carbonium ion differ from many non-carcinogenic alkylating agents in that they react with the extracyclic amino groups of the nucleic acid bases. It also shows that CMR in conjunction with carbon-13 labelling might be useful in further studies of this type, including those involving intact nucleic acids.
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