energy participation of non-Koopmans states.

Considering the above data, the experimental spectrum is satisfyingly interpreted. The first two bands observed at 8.46 and 9.2 eV are associated with the ionization of the b_{1g} and b_{2u} combinations of nonbonding pairs of oxygen. We note a similar intensity for these two bands.

As seen above, the b_{2u} orbital presents a clear localization on the ethylene carbons, explaining why the band is shifted toward lower energies after a monomethylation: a first broad band centered at 8.6 eV is observed, covering the two ionizations. The first band presents a clear vibrational structure of about 1200 cm^{-1}, showing the slight geometric reorganization of the ion. In the case of the second less structured band, the vibrational space is lower, and, in addition to valence vibrations $\nu_{C=O}$, may correspond to vibrations associated with the $\nu_{C=C}$ group. The third dimethylenecyclobutanedione band more intense at 10.60 eV corresponds to the $^2B_{1u}$ and $^2B_{3g}$ ionic states. The calculated energies are very close and the two bands overlap. After methylation, however, the B_{1u} state, more delocalized on C=O bonds, is associated with the band at 10.48 eV, while the $^2B_{3g}$ state corresponds to the band at 10.01 eV.

In the 11 eV region of both spectra, there is a low-intensity band as a shoulder of the broad band of the nonsubstituted derivative and as a distinct band for the methylated derivative. This band is probably associated with the non-Koopmans $^4B_{1g}$ and $^2B_{1g}$ states.

Conclusion

The photoelectron spectrum of dimethylenecyclobutane-1,3-dione was obtained by using the technique of spectrometer-coupled flash pyrolysis. We show the participation of a low-energy shake-up structure for this compound. The excellent agreement between experimental results and theoretical predictions illustrates the usefulness of the CIPSI approach for the calculation of ionic states. The electronic structure of this molecule is characterized by a clear circumannular interaction, inducing a particularly low-energy position for the first vacant orbital, the basis of the high reactivity towards 1,3-dienes. Destabilizing through bond interactions, however, apparently cannot explain its instability, probably more related to polar effects.

Acknowledgment. The authors thank the Centre de Calcul Vectoriel pour la Recherche France for generous allotments of computer time (ATP Cray 1984).

Registry No. **1a**, 71028-83-6; **1b**, 78507-07-0; **2a**, 15506-53-3; **3**, 2045-78-5; **4**, 71028-82-5.

Supplementary Material Available: Charges and overlap populations for **1a** (Table II) and bond distances (Å) and angles calculated for **1a**, **2a**, and **3** (Figure 2) (1 page). Ordering information is given on any current masthead page.

Reactivity Effects on Site Selectivity in Nucleoside Aralkylation: A Model for the Factors Influencing the Sites of Carcinogen-Nucleic Acid Interactions

Robert C. Moschel,* W. Robert Hudgins, and Anthony Dipple

BRI-Basic Research Program, Laboratory of Chemical and Physical Carcinogenesis, NCI-Frederick Cancer Research Facility, Frederick, Maryland 21701

Received July 9, 1986

Product distributions are described for 15 reactions between guanosine (1) and a series of *p*-Y-benzyl bromides (**2a–e**), *p*-Y-benzyl chlorides (**3a–e**), and *N*-nitroso-*N*-(*p*-Y-benzyl)ureas (**4a–e**) where Y = **a**, O_2N; **b**, Cl; **c**, H; **d**, CH_3; **e**, CH_3O. The yields of products from reaction at the 7-position of guanosine to produce 7-(*p*-Y-benzyl)guanosines (**5a–e**), at N^2 to produce N^2-(*p*-Y-benzyl)guanosines (**6a–e**), at the O^6-position to produce O^6-(*p*-Y-benzyl)guanosines (**7a–e**), and at the 5-position to produce 4-(*p*-Y-benzyl)-5-guanidino-1-β-D-ribofuranosylimidazoles (**8a–e**) are correlated with the mechanism of the reaction (i.e., the S_N2 or S_N1 character) imposed by the para substituent and/or leaving group and the nature of the incipient charge density (i.e., the "hardness" or "softness") at the reaction center. These observations, coupled with the literature on sites of reaction of carcinogens with nucleic acid components, are used to rationalize the site selectivity differences exhibited by the alkylating and aralkylating classes of carcinogens in their nucleic acid reactions.

Organic chemical carcinogens are electrophilic species that are either directly reactive from the outset or are produced by metabolism of a precarcinogenic and nonchemically reactive form.[1-3] Once these reactive species are produced, their rates, extents, and sites of reaction on cellular macromolecules, such as DNA, are governed by their intrinsic electrophilic reactivity. This reactivity leads to substitution at a variety of sites on the multidentate heterocyclic nucleic acid base components of DNA. For example, the weakly carcinogenic alkylating agents (e.g., methyl methanesulfonate) primarily modify the pyridine-type ring nitrogen sites (e.g., the 7-position of guanine residues)[4] while the more potent carcinogenic alkylating agents (e.g., the *N*-alkyl-*N*-nitroso compounds) modify exocyclic oxygen centers (e.g., O^6 of guanine residues) in addition to ring nitrogen sites.[5,6] In contrast, the aralkylating 7-(bromomethyl)benz[*a*]anthracenes,[7,8] the di-

(1) Miller, E. C.; Miller, J. A. *Pharmacol. Rev.* **1966**, *18*, 805–838.
(2) Dipple, A.; Lawley, P. D.; Brookes, P. *Eur. J. Cancer* **1968**, *4*, 493–506.
(3) Miller, J. A.; Miller, E. C. *J. Natl. Cancer Inst.* **1971**, *47*, 5–14.
(4) Lawley, P. D. *Prog. Nucleic Acid Res. Mol. Biol.* **1966**, *5*, 89–131.
(5) Loveless, A. *Nature (London)* **1969**, *223*, 206–207.
(6) Singer, B. *Prog. Nucleic Acid Res. Mol. Biol.* **1975**, *15*, 219–332.
(7) Dipple, A.; Brookes, P.; Mackintosh, D. S.; Rayman, M. P. *Biochemistry* **1971**, *10*, 4323–4330.

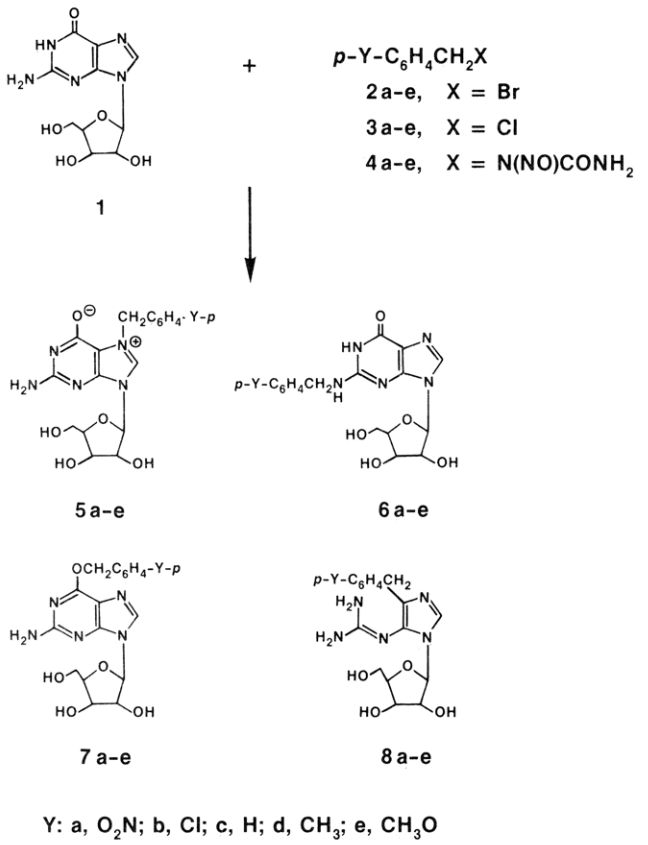
0022-3263/86/1951-4180$01.50/0

hydrodiol epoxides of the polycyclic aromatic hydrocarbons (reviewed in ref 9), and the carcinogenic arylamines[10] modify the exocyclic amino groups of the nucleic acid bases (e.g., N^2 of guanine residues). The arylamines also react with C-8 on guanine.[10] It appears that, with the exception of aflatoxin B_1-DNA interactions,[11-13] carcinogenic potency is reflected in an agent's ability to modify sites other than the pyridine-type ring nitrogen sites.[14] Consequently, a clearer understanding of the mechanisms responsible for directing an agent to react at specific sites will provide insight into the chemical factors which determine the relative carcinogenicity of a reactive electrophile.

Because the important DNA-reactive metabolites of carcinogens are often unknown or synthetically inaccessible, mechanistic information must be sought through studies of model reactions between nucleosides and more readily available electrophiles that can interact with the range of reaction sites involved in carcinogen–nucleic acid interactions. In this regard, we have demonstrated that reactions between nucleosides and benzylic electrophiles constitute a very useful model system.[14-20] Certainly, the solvolytic reactivity of benzylic electrophiles has been widely studied.[21-34] From several investigations of the effects of para substituent, solvent, leaving group, and α-deuterium substitution on the rates of solvolysis of benzyl derivatives, it is generally agreed that, under similar conditions of solvent ionizing power, for the series of *p*-Y-benzyl-X bearing the same leaving group (e.g., Br, Cl, *p*-toluenesulfonate (OTs)), the S_N2 character for solvolysis decreases with para substituent in the order p-NO_2 > p-Cl > p-H > p-CH_3 > p-CH_3O. Carbon leaving group bond breakage becomes more advanced, and the structure of the transition state becomes progressively "looser" with these substituent changes. As a corollary, participation by solvent to exert an S_N2-type "push" for leaving group expulsion decreases as the substituent is made more electron donating by resonance. At the extreme, *p*-methoxybenzyl derivatives exhibit limiting S_N1 solvolytic behavior.[21-34] Additionally, the leaving group also influences the structure of the solvolysis transition state. Again, under similar solvent conditions for solvolysis of *p*-Y-benzyl-X bearing the same *p*-substituent, carbon leaving group bond breakage becomes more advanced in the series I < Br < Cl < leaving groups attached through oxygen (e.g., OTs or nitrate). These changes impart higher positive charge density or carbonium ion character to the benzylic carbon. Nucleophilic participation by solvent in the transition state decreases in the series I > Br > Cl > OTs.[22,26,31,33-35] This is the series of increasing electronegativity as well as decreasing size and polarizability of the departing atom (i.e., the sequence of increasing leaving group "hardness").[36]

In earlier studies, we examined solvent and leaving group effects on sites of benzylation on adenosine and guanosine.[14-16] We demonstrated that increasing ionizing power of the reaction solvent and increasing leaving group "hardness" brought about higher extents of reaction at exocyclic sites relative to ring nitrogen sites on both nucleosides. This suggested that reaction at exocyclic sites, as opposed to ring nitrogen sites, was favored by changes in reaction medium or leaving group that increased the S_N1 character in the reactivity of the benzylating agent. For guanosine reactions, the O^6/N^2 product ratio was fairly insensitive to solvent changes with a particular benzylating agent, but this ratio increased significantly with leaving group in the sequence Br < Cl < OTs < *N*-nitrosourea suggesting that reaction at O^6 relative to N^2 was favored by increasing "hardness" of the benzylic carbon reaction center. In this report, we test these conclusions by investigating the effects of changes in para substituent as well as leaving group on sites of aralkylation on guanosine under largely aqueous conditions. Specifically, we describe product distributions from reactions between guanosine (**1**) and a series of *p*-Y-benzyl bromides (**2a–e**), *p*-Y-benzyl chlorides (**3a–e**), and *N*-nitroso-*N*-(*p*-Y-benzyl)ureas (**4a–e**) to produce 7-(*p*-Y-benzyl)guanosines (**5a–e**), N^2-(*p*-Y-benzyl)guanosines (**6a–e**), O^6-(*p*-Y-benzyl)guanosines (**7a–e**), and 4-(*p*-Y-benzyl)-5-guanidino-1-β-D-ribofuranosylimidazoles (**8a–e**) where Y = **a**, O_2N; **b**, Cl; **c**, H; **d**, CH_3; **e**, CH_3O. With this model system we can compare

(8) Rayman, M. P.; Dipple, A. *Biochemistry* **1973**, *12*, 1202–1207.
(9) Dipple, A.; Moschel, R. C.; Bigger, C. A. H. "Polynuclear Aromatic Carcinogens" In *Chemical Carcinogens*, 2nd ed.; Searle, C.E., Ed.; ACS Monograph 182; American Chemical Society: Washington, DC, 1984; pp 41–163.
(10) Kriek, E.; Westra, J. C. "Metabolic Activation of Aromatic Amines and Amides and Interactions with Nucleic Acids" In *Chemical Carcinogens and DNA*; Grover, P. L., Ed.; CRC: Boca Raton, FL, 1979; pp 1–28.
(11) Miller, E. C.; Miller, J. A. "Searches for Ultimate Chemical Carcinogens and Their Reactions with Cellular Macromolecules" in *Accomplishments in Cancer Research*; Fortner, J. G., Rhoads, J. E., Eds.; Lippincott: Philadelphia, PA, 1981; pp 63–98.
(12) Essigman, J. M.; Croy, R. G.; Bennett, R. A.; Wogan, G. N. *Drug Metab. Rev.* **1982**, *13*, 581–602.
(13) Hertzog, P. J.; Smith, J. R. L.; Garner, R. C. *Carcinogenesis* **1982**, *3*, 723–725.
(14) Dipple, A.; Moschel, R. C.; Hudgins, W. R. *Drug. Metab. Rev.* **1982**, *13*, 249–268.
(15) Moschel, R. C.; Hudgins, W. R.; Dipple, A. *J. Org. Chem.* **1979**, *44*, 3324–3328.
(16) Moschel, R. C.; Hudgins, W. R.; Dipple, A. *J. Org. Chem.* **1980**, *45*, 533–535.
(17) Moschel, R. C.; Hudgins, W. R.; Dipple, A. *Tetrahedron Lett.* **1981**, *22*, 2427–2430.
(18) Moschel, R. C.; Hudgins, W. R.; Dipple, A. *J. Am. Chem. Soc.* **1981**, *103*, 5489–5494.
(19) Carrell, H. L.; Zacharias, D. E.; Glusker, J. P.; Moschel, R. C.; Hudgins, W. R.; Dipple, A. *Carcinogenesis* **1982**, *3*, 641–645.
(20) Moschel, R. C.; Hudgins, W. R.; Dipple, A. *J. Org. Chem.* **1984**, *49*, 363–372.
(21) Robertson, R. E.; Scott, J. M. W. *J. Chem. Soc.* **1961**, 1596–1604.
(22) Koshy, K. M.; Robertson, R. E.; Strachan, W. M. J. *Can. J. Chem.* **1973**, *51*, 2958–2962.
(23) Kochi, J. K.; Hammond, G. S. *J. Am. Chem. Soc.* **1953**, *75*, 3445–3451.
(24) Okamoto, Y.; Brown, H. C. *J. Org. Chem.* **1957**, *22*, 485–494.
(25) Swain, C. G.; Langsdorf, W. P. *J. Am. Chem. Soc.* **1951**, *73*, 2813–2819.
(26) Shiner, V. J. "Deuterium Isotope Effects in Solvolytic Substitution at Saturated Carbon" In *Isotope Effects in Chemical Reactions*; Collins, C. J., Bowman, N. S., Eds.; ACS Monograph 167; Van Nostrand Reinhold: New York, 1970; pp 90–159.
(27) Streitwieser, A.; Hammond, H. A.; Jagow, R. H.; Williams, R. M.; Jesaitis, R. G.; Chang, C. J.; Wolf, R. *J. Am. Chem. Soc.* **1970**, *92*, 5141–5150.
(28) daRoza, D. A.; Andrews, L. J.; Keefer, R. M. *J. Am. Chem. Soc.* **1973**, *95*, 7003–7009.
(29) Harris, J. M.; Mount, D. L.; Smith, M. R.; Neal, W. C.; Dukes, M. D.; Raber, D. J. *J. Am. Chem. Soc.* **1978**, *100*, 8147–8156.
(30) Young, P. R.; Jencks, W. P. *J. Am. Chem. Soc.* **1979**, *101*, 3288–3294.
(31) Harris, J. M.; Shafer, S. G.; Moffatt, J. R.; Becker, A. R. *J. Am. Chem. Soc.* **1979**, *101*, 3295–3300.
(32) Vitullo, V. P.; Grabowski, J.; Sridharan, S. *J. Chem. Soc., Chem. Commun.* **1981**, 737–738.
(33) Bentley, M. D.; Dewar, M. J. S. *J. Am. Chem. Soc.* **1970**, *92*, 3991–3996.
(34) Koshy, K. M.; Robertson, R. E. *Can. J. Chem.* **1974**, *52*, 2485–2490.
(35) Bentley, T. W.; Schleyer, P. v. R. *Adv. Phys. Org. Chem.* **1977**, *14*, 1–67.
(36) Pearson, R. G. "The Influence of the Reagent on Organic Reactivity" In *Advances in Linear Free Energy Relationships*; Chapman, N. B., Shorter, J., Eds.; Plenum: London, U.K., 1972; pp 281–319.



Y: a, O_2N; b, Cl; c, H; d, CH_3; e, CH_3O

product distributions resulting from reaction between guanosine and a series of benzylating agents that react through known mechanisms and relate possible changes in product distribution to the known changes in the reactivity of the benzylating agent imparted by the para substituent and/or leaving group as summarized above.

Results

In these studies, radiolabeled guanosine was used throughout and its concentration was held very low in all cases. We demonstrated previously[15,16] that product distributions were independent of guanosine concentration in reactions with benzyl bromide, benzyl chloride, benzyl tosylate, and *N*-nitroso-*N*-benzylurea over a wide range of guanosine concentrations (albeit low concentration due to its limited solubility). Furthermore, for benzyl bromide and benzyl chloride reactions, increased rates of disappearance of the benzylating agents in the presence of guanosine could not be detected.[15] Irrespective of mechanistic considerations, this finding reflects the very weak nucleophilicity of guanosine (relative to water) toward electrophiles more complex than the simplest methylating agents. The low guanosine concentration ($\sim 10^{-7}$ M) employed in the present study eliminates the possibility that guanosine could influence either the mechanism or the overall rate of disappearance of any of the benzylating agents. The latter were used in large excess (1.7×10^{-2} M in every case) to ensure that measurable amounts of product were produced in each reaction even though only a minute fraction of the benzylating agent is consumed in reaction with guanosine relative to water. The high specific radioactivity of the guanosine employed made it possible to accurately quantify formation of all reported products. Product yields were determined when the benzylating agents had totally hydrolyzed, i.e., after 4–5 half times for solvolysis of these agents in H_2O.[21,22] All reactions were carried out in *N*,*N*-dimethylformamide (DMF)–0.1 M aqueous phosphate buffer (pH 6.9) (1:99) or totally aqueous phosphate buffer pH 6.9, 40 °C.

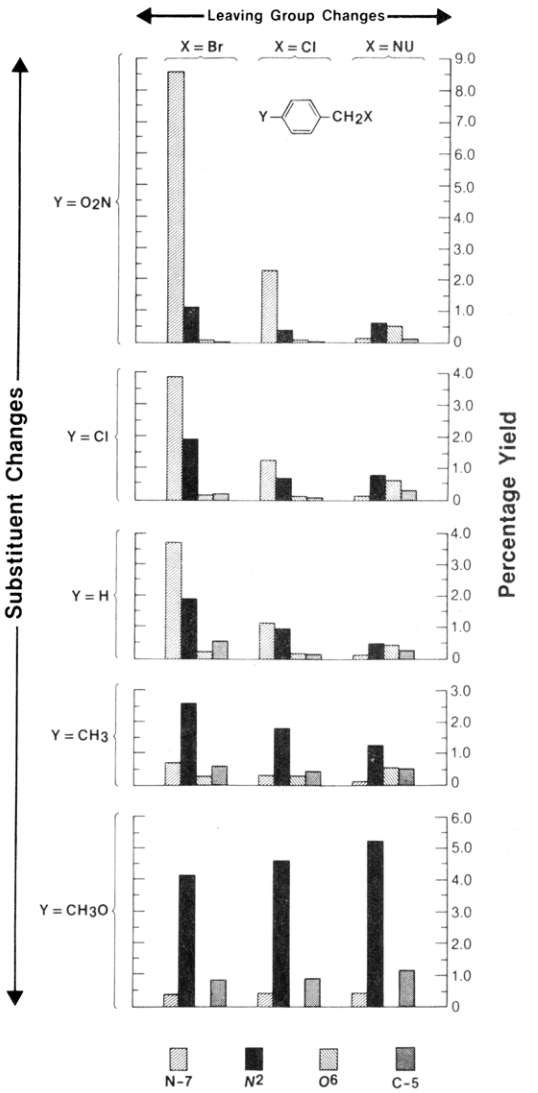


Figure 1. Histogram of the percentage yield of benzylation at various sites on guanosine as a function of para substituent (Y) and leaving group (X) on the benzylating agent. The leaving group abbreviation NU represents the *N*-nitrosourea functional group.

In Figure 1 we present, in histogram format, the yields of guanosine products derived from benzylation at the 7-position (abbreviated N-7, i.e., products **5a–e**), at the N^2-position (abbreviated N^2, i.e., products **6a–e**), at the O^6-position (abbreviated O^6, i.e., products **7a–e**), and at the 5-position (abbreviated C-5, affording 4-(*p*-Y-benzyl)-5-guanidino-1-β-D-ribofuranosylimidazole products **8a–e**) as a function of both the para substituent (Y) and the leaving group (X) on the benzylating agent (**2a–e**, **3a–e**, and **4a–e**). A tabular summary of these percentages is presented in Table I. Yields are reported as percentage guanosine converted to the respective benzylated product, and they are the average of triplicate determinations. In the slowest of these reactions (i.e., those involving **3a** and **3b**) imidazole ring opening of the 7-substituted products **5a** and **5b** was extensive.[20] The yields reported for **5a** and **5b** (Figure 1 and Table I) are the sum of the respective yields for the ring-opened and intact 7-substituted product.

Different leaving groups, i.e., bromide, chloride, and *N*-nitrosourea, bring about dramatic changes in product distributions for each para-substituted benzylating agent except the *p*-methoxybenzyl derivatives **2e**, **3e**, and **4e** (Figure 1, Table I). For example, with the *p*-nitrobenzyl derivatives, **2a**, **3a**, and **4a**, these leaving group changes

Table I. Percentage Yield of Benzylated Product at Various Sites on Guanosine as a Function of Para Substituent (Y) and Leaving Group (X) on the Benzylating Agent[a]

p-Y-Bzl-X					
Y	X	7-(p-Y-Bzl)Guo, products 5	N^2-(p-Y-Bzl)-Guo, products 6	O^6-(p-Y-Bzl)Guo, products 7	4-(p-Y-Bzl)-5-guanidino-imidazole nucleosides, products 8
O_2N	Br	8.57 ± 0.45	1.13 ± 0.03	0.075 ± 0.003	0.018 ± 0.0
O_2N	Cl	2.30 ± 0.30	0.39 ± 0.06	0.070 ± 0.0	0.010 ± 0.0
O_2N	NU[b]	0.13 ± 0.01	0.60 ± 0.01	0.53 ± 0.01	0.12 ± 0.02
Cl	Br	3.87 ± 0.10	1.92 ± 0.03	0.16 ± 0.01	0.20 ± 0.01
Cl	Cl	1.27 ± 0.10	0.70 ± 0.07	0.12 ± 0.02	0.07 ± 0.0
Cl	NU	0.15 ± 0.0	0.80 ± 0.03	0.64 ± 0.02	0.33 ± 0.01
H	Br	3.67 ± 0.10	1.88 ± 0.04	0.23 ± 0.01	0.57 ± 0.02
H	Cl	1.15 ± 0.13	0.97 ± 0.13	0.19 ± 0.04	0.18 ± 0.0
H	NU	0.15 ± 0.01	0.50 ± 0.02	0.48 ± 0.01	0.27 ± 0.01
H_3C	Br	0.72 ± 0.01	2.61 ± 0.07	0.29 ± 0.01	0.62 ± 0.01
H_3C	Cl	0.32 ± 0.02	1.82 ± 0.04	0.30 ± 0.02	0.46 ± 0.02
H_3C	NU	0.14 ± 0.01	1.26 ± 0.02	0.56 ± 0.01	0.54 ± 0.03
H_3CO	Br	0.39 ± 0.04	4.16 ± 0.20	nd[c]	0.82 ± 0.05
H_3CO	Cl	0.43 ± 0.05	4.61 ± 0.20	nd[c]	0.88 ± 0.03
H_3CO	NU	0.42 ± 0.04	5.23 ± 0.10	nd[c]	1.17 ± 0.05

[a] Yields are reported as the average % guanosine converted ± the range of % values. [b] The abbreviation NU represents the nitrosourea functional group. [c] Not detected at reaction completion; see text.

bring about a significant reduction in the extent of formation of the 7-substituted product **5a** and an increase in the formation of the O^6-substituted product **7a**. The extents of reaction at the N^2- and 5-position are less sensitive to these leaving group changes on the *p*-nitrobenzyl residue. With the *p*-chlorobenzyl derivatives **2b–4b** and the benzyl derivatives **2c–4c**, the same leaving group changes bring about similar, although progressively less pronounced changes, in product distribution (Figure 1). In all these cases, the reaction at the 7-position decreases significantly. To a lesser extent reaction at the N^2-position also decreases while reaction at O^6 and, in most cases, C-5 increases. Clearly, the net result of these leaving group changes is to shift the major site of reaction away from the 7- and N^2-positions to solvent, the O^6- and 5-position. Leaving group effects with the *p*-methylbenzylating agents **2d–4d** are similar although the extents of reaction at the 7-position are relatively low with these agents in all cases. For guanosine reactions with the *p*-methoxybenzylating agents **2e–4e**, leaving group effects on product distribution are virtually absent.

Different para substituents, i.e., nitro through methoxy, bring about substantial changes in product distributions for the benzyl halides but not for the nitrosoureas. Changes from electron-withdrawing to resonance-electron-donating para substituents on the benzylating agent direct reaction away from the 7-position but, unlike the leaving group effects described above, a dramatic diminution in total products does not accompany these changes. Instead, the decreased reaction at the 7-position tends to be offset by increased reaction with other sites, most notably with the exocylic N^2-position. For reactions involving the nitrosoureas **4a–c**, substituent changes exert little influence on product distributions although increased reaction at N^2 is readily observed with derivatives **4d** and **4e**. Thus, para-substituent and leaving-group changes exhibit widely different effects on product distributions.

The O^6/N^2 product ratio for the reactions involving **2a–d**, **3a–d**, and **4a–c**, is essentially constant as a function of the para substituent, but it is markedly dependent on leaving group (Figure 1, Table I). Thus, the average O^6/N^2 ratio produced in the reactions with the bromides **2a–d** is 0.09, while for the chlorides **3a–d** it is 0.2, and for the *N*-nitrosoureas **4a–c** the ratio is 0.9. In the reaction of guanosine with *N*-nitroso-*N*-(*p*-methylbenzyl)urea (**4d**), the ratio falls to 0.4. As a result of its instability, O^6-(*p*-methoxybenzyl)guanosine (**7e**) is not detected after extended times in reactions with any *p*-methoxybenzylating agent,[18] but it can be detected as a minor product in the reactions of *p*-methoxybenzyl chloride and *N*-nitroso-*N*-(*p*-methoxybenzyl)urea with guanosine at very early stages of reaction.[37] This indicates that *p*-methoxybenzylating agents clearly favor reaction at the exocyclic N^2- rather than O^6-position of guanosine.

The yields for the 4-(*p*-Y-benzyl)-5-guanidino-1-β-D-ribofuranosylimidazole products 8 are generally increased by changes in leaving group or para substituent which favor reaction at either the O^6- or N^2-position relative to the 7-position. Under the conditions of temperature and pH in these experiments, the formation of products 8 by a dissociation/reassociation mechanism from a preformed O^6-substituted product is possible only with O^6-(*p*-methoxybenzyl)guanosine (**7e**).[18] However, since **7e** formation is disfavored in reactions with the *p*-methoxybenzylating agents, the yields for **8e** are largely a result of direct reaction at carbon-5 of guanosine. In the other reactions, products **8a–d** can be formed only by direct attack at position 5. Since products 8 require more steps for their formation than the 7-, N^2-, or O^6-substituted products and since rates for any or all of these steps may be substituent dependent,[18] the final yields for these products may well depend on more than just the ability of the benzylating agent to react initially at carbon-5.

Discussion

As a result of the reactivity effects imparted to **2a** by the *p*-nitro substituent and bromide leaving group, the S_N2 character in the reactivity of *p*-nitrobenzyl bromide should be the greatest of the 15 reactions studied here.[21-34] Reaction at the 7-position of guanosine clearly predominantes with **2a** indicating that 7-substitution is associated with agents that react by the more or less classical S_N2 mechanism. This is consistent with the observations that other agents that also react through the S_N2 mechanism (e.g.,

(37) After either a 1.5- or 3-min reaction between radiolabeled guanosine and *p*-methoxybenzyl chloride, product mixtures were diluted with 0.5 volume of methanol (to retard solvolysis of product **7e**),[18] and the resulting solutions were chromatographed on Sephadex LH-20 essentially as described for preparation of the marker nucleoside **7e**.[18] From the radioactivity associated with the resulting O^6- and N^2-substituted products, an average **7e**/**6e** ratio of 0.05 was calculated. Similar studies of reactions between guanosine and *N*-nitroso-*N*-(*p*-methoxybenzyl)urea indicated a **7e**/**6e** ratio of 0.19 at early times. In both cases, the O^6/N^2 product ratio is markedly lower than that which might be anticipated from the apparent constancy of this ratio obtained with the other chlorides or the *N*-nitrosoureas.

simple methylating agents) also react extensively with the 7-position of guanine residues.[4,38] In contrast, the *p*-methoxybenzylating derivatives **2e–4e** react by the limiting S_N1 mechanism through the intermediacy of the resonance stabilized *p*-methoxybenzyl carbonium ion.[21-34] Reactions with such stabilized ions exhibit constant selectivity for competing nucleophiles regardless of leaving group.[39-41] For guanosine reactions with **2e–4e**, the constant site selectivity order (i.e., $N^2 \gg$ C-5 > 7) indicates that the stabilized *p*-methoxybenzyl carbonium ion exhibits a marked selectivity for reaction at the exocyclic amino group of guanosine. Such selectivity is reminiscent of the reactions with nucleic acid components of the more complex polycyclic aromatic hydrocarbon carcinogens.[7-9] The reactions of the *N*-(*p*-Y-benzyl)-*N*-nitrosoureas **4a–c** also exhibit nearly constant, albeit low, selectivity. These agents probably also react by a mechanism near the S_N1 extreme through an intermediate that closely resembles the respective carbonium ion (i.e., the aralkyldiazonium ion).[42] However, the low yields of guanosine products they produce and the fairly even distribution of the benzyl residues from **4a–c** over the various receptor sites indicate that these ionic intermediates are of low stability. Only the *p*-methyl and *p*-methoxy substituent can stabilize the intermediate sufficiently to bring about increased selectivity for reaction at the exocyclic amino group on guanosine. The reactivity of the remaining bromides (**2b–d**) and chlorides (**3a–d**) are regarded as more "borderline" in nature. Kinetic probes of their solvolytic reactions indicate that they reflect reactions on a continuum of decreasing S_N2 character with increasing electron donating ability of the attached para substituent.[21-34] This continuum of decreasing S_N2 character is in evidence in the reactions of **2b–d** and **3a–d** with guanosine through the gradual decrease in the extents of reaction with the 7-position and increased reaction at the other three reaction sites. Overall, it appears that multidentate guanosine may be a very sensitive probe for mechanism in solvolysis reactions.

Both the substituent and leaving group changes that increase the S_N1 character in the reactivity of the various benzylating agents increase the rate of reaction at the exocyclic N^2- and O^6-positions relative to the 7-position. This indicates that some degree of S_N1 character is required for reaction at sites other than the 7-position. However, the para substituent and leaving group differ substantially in their ability to influence the relative rates of reaction of the O^6 and N^2 sites. For reactions involving the benzyl halides **2a–d** and **3a–d**, substituents have little influence on the O^6/N^2 ratio, but this ratio is higher for the chlorides than the bromides and is highest in the reactions of the nitrosoureas **4a–c**. Clearly, the increasing O^6/N^2 ratio follows the order of increasing leaving group "hardness"[36] which indicates that higher charge density at the benzylic carbon reaction center directs a greater proportion of total reaction to the exocyclic oxygen center (the "harder" Lewis base of the two exocyclic sites on guanosine). Conversely, reaction at the exocyclic amino group on guanosine is favored with the "softer" ionic species derived from the benzylic bromides rather than the chlorides. When the leaving group is not involved in the transition state, the "hardness" or "softness" of the reaction center is governed by the extent to which charge can be delocalized through resonance. The *p*-methoxy substituent will create a relatively "soft" benzylic carbon reaction center while electron-withdrawing para substituents will create a relatively "hard" reaction center.

With respect to the broader issue of site selectivity determination in carcinogen nucleic acid interactions, we argue, therefore, that at least two aspects of chemical reactivity need to be considered in order to rationalize the sites for electrophilic attack on nucleic acid components: the mechanism of reaction, and the charge character at the reaction center.[14-18] The mechanism of reaction (i.e., where the mechanism resides on the continuum between the S_N2 and S_N1 extremes) will determine the extent of reaction at pyridine type ring nitrogen sites relative to reaction at exocyclic or other reaction sites and discrimination between the exocyclic oxo and amino sites will be governed by the charge density at the electrophilic reaction center. When the center is "hard" (as with the alkyl or branched-chain alkyldiazonium ions), reaction at exocyclic oxygen is favored. When the center is "soft" (as with the resonance stabilized ions derived from polycyclic aromatic hydrocarbon carcinogens), reaction is favored with the exocyclic amino groups.

Experimental Section

[5′-^{3}H]Guanosine (**1**) (specific radioactivity 21 Ci/mmol) was obtained from Amersham Searle, Arlington Heights, IL. Substituted benzylamines and the majority of substituted benzyl halides were obtained from Aldrich Chemical Co., Milwaukee, WI. *p*-Chlorobenzyl bromide as well as *p*-methoxybenzyl chloride and bromide were synthesized by treating the appropriate alcohol with HBr or HCl gas, and the product was distilled under vacuum. The preparation and properties of nucleoside products **5a–e** through **8a–e** have been described.[15-20] Elemental analyses were by Galbraith Laboratories, Inc., Knoxville, TN.

General Method for the Preparation of *p*-Y-Benzylureas. To a solution of 0.1 mol of *p*-Y-benzylamine hydrochloride in 300 mL of H_2O was added 0.15 mol of KNCO in 100 mL of H_2O, and the solutions were stirred for 18 h at room temperature. The precipitated ureas were collected by filtration and crystallized from EtOH/H_2O (1:1), affording analytically pure products in overall yields of 60–80%. *p*-Methoxybenzylurea: mp (corrected) 163 °C. Anal. Calcd for $C_9H_{12}N_2O_2$: C, 59.98; H, 6.71; N, 15.55. Found: C, 60.18; H, 6.95; N, 15.49. *p*-Methylbenzylurea: mp 185 °C. Anal. Calcd for $C_9H_{12}N_2O$: C, 65.83; H, 7.37; N, 17.06. Found: C, 65.73; H, 7.50; N, 16.98. Benzylurea: mp 148 °C (lit.[43] mp 146–150 °C). *p*-Chlorobenzylurea: mp 193 °C. Anal. Calcd for $C_8H_9N_2OCl$: C, 52.04; H, 4.91; N, 15.17. Found: C, 52.15; H, 5.04; N, 15.14. *p*-Nitrobenzylurea: mp 201 °C. Anal. Calcd. for $C_8H_9N_3O_3$: C, 49.23; H, 4.65; N, 21.53. Found: C, 49.52; H, 4.82; N, 21.59.

General Method for the Preparation of *N*-Nitroso-*N*-(*p*-Y-benzyl)ureas. The appropriate *p*-Y-benzylurea (0.01 mol) in 25 mL of glacial acetic acid/H_2O (4:1) was treated with 0.04 mol of $NaNO_2$ overnight at room temperature. Crude product was precipitated by pouring the reaction solution into approximately 200 mL of ice/H_2O. Recovered solids were collected by filtration, dried in air, and crystallized from a small volume of benzene to afford crystalline *N*-nitroso-*N*-(*p*-Y-benzyl)ureas in overall yields of 30–50%. *N*-Nitroso-*N*-(*p*-nitrobenzyl)urea (**4a**): mp 117 °C. Anal. Calcd for $C_8H_8N_4O_4$: C, 42.86; H, 3.60; N, 25.00. Found: C, 43.00; H, 3.71; N, 24.91. *N*-Nitroso-*N*-(*p*-chlorobenzyl)urea (**4b**): mp 121 °C. Anal. Calcd for $C_8H_8N_3O_2Cl$: C, 44.98; H, 3.77; N, 19.68. Found: C, 45.15; H, 3.89; N, 19.75. *N*-Nitroso-*N*-benzylurea (**4c**): mp 100 °C (lit.[44] mp 100.5 °C). *N*-Nitroso-*N*-(*p*-methylbenzyl)urea (**4d**): mp 119 °C (lit.[45] mp 115 °C). Anal. Calcd for $C_9H_{11}N_3O_2$: C, 55.95; H, 5.74; N, 21.75. Found: C, 56.06;

(38) Lawley, P. D. "Carcinogenesis by Alkylating Agents" In *Chemical Carcinogens*, 2nd ed.; Searle, C. E., Ed.; ACS Monograph 182; American Chemical Society: Washington, DC, 1984; pp 325–484.
(39) Jencks, W. P. *Acc. Chem. Res.* **1980**, *13*, 161–169.
(40) Jencks, W. P. *Chem. Soc. Rev.* **1981**, *10*, 345–375.
(41) Ritchie, C. D. *Acc. Chem. Res.* **1972**, *5*, 348–354.
(42) Moss, R. A. *Acc. Chem. Res.* **1974**, *7*, 421–427.
(43) Argabright, P. A.; Phillips, B. L.; Sinkey, V. J. *J. Org. Chem.* **1967**, *32*, 3261–3262.
(44) Garrett, E. R.; Goto, S.; Stubbins, J. F. *J. Pharm. Sci.* **1965**, *54*, 119–123.
(45) Wiessler, M.; Romruen, K.; Pool, B. L. *Carcinogenesis* **1983**, *4*, 867–871.

H, 5.86; N, 21.73. *N*-Nitroso-*N*-(*p*-methoxybenzyl)urea (**4e**): mp 118–120 °C. Anal. Calcd for $C_9H_{11}N_3O_3$: C, 51.67; H, 5.30; N, 20.09. Found: C, 51.71; H, 5.39; N, 20.11.

Kinetics of Solvolysis of *N*-Nitroso-*N*-(*p*-Y-benzyl)ureas. An aliquot (30 μL) from an appropriately concentrated stock solution of the individual *N*-nitroso-*N*-(*p*-Y-benzyl)urea dissolved in ethanol was added to 3 mL of aqueous 0.1 M phosphate buffer (pH 6.9) 40 °C to arrive at final concentrations of the nitroso compound in the range of 10^{-4} M. Rates of disappearance of the *N*-nitroso-*N*-(*p*-Y-benzyl)ureas were determined from the time-dependent decrease in absorbance of these solutions at 235 nm. Observed first-order rate constants (k_{obsd}) were calculated from the slopes of plots of ln ($OD_t - OD_\infty$) vs. time, and the reported k_{obsd} values are the average of three determinations. Values of k_{obsd} (min^{-1}) for the *N*-nitroso-*N*-(*p*-Y-benzyl)ureas (**4a–e**) under these conditions are as follows: **4a**, 22.7×10^{-2}; **4b**, 13.4×10^{-2}; **4c**, 9.93×10^{-2}; **4d**, 9.07×10^{-2}; **4e**, 9.32×10^{-2}.

Aralkylation of [5′-^{3}H]Guanosine. An aliquot (10 μL) from an aqueous stock solution of [5′-^{3}H]guanosine (**1**) (1×10^{-5} M) was added to 1 mL of 0.1 M phosphate buffer, pH 6.9, 40 °C. An aliquot (10 μL) from a freshly prepared stock solution of the *p*-Y-benzyl halide (**2a–e** or **3a–e**) in DMF was added to achieve final reaction solutions which were 1.7×10^{-2} M in aralkyl halide. The appropriate amount of *N*-nitroso-*N*-(*p*-Y-benzyl)urea (**4a–e**) was added as a solid. The resulting suspensions were incubated at 40 °C with constant stirring until reactions were complete, at which time the appropriate mixture of marker nucleosides in methanol was added and the product mixtures were loaded on a 0.78 × 30 cm Aminex A-5 (Bio-Rad Laboratories, Richmond, CA) column. Elution with acidic ammonium formate solutions was as previously described.[18,20] Imidazole-ring-opened products derived from the 7-substituted guanosines (**5a–e**) elute after guanosine during elution with the first 100 mL of buffered solvent.[20] Because O^6-(*p*-methylbenzyl)guanosine (**7d**) decomposes under the acidic column conditions,[20] product mixtures derived from reactions of guanosine with **2d–4d** were first loaded on the Aminex A-5 column equilibrated with 1 M $NH_4^+HCO_2^-$, pH 9.3, 45 °C. UV absorption was continuously monitored at 254 nm and fractions (1 mL) were collected and mixed with 10 mL of PCS (Amersham/Searle) for scintillation counting. Unmodified guanosine elutes in fractions 17–21 under these conditions. When 60 mL of this solvent had passed through the column, elution was carried out with 0.85 M $NH_4^+HCO_2^-$ in DMF/H_2O (12:88), pH 9.3, 45 °C. N^2-(*p*-Methylbenzyl)guanosine (**6d**) elutes in fractions 100–110, O^6-(*p*-methylbenzyl)guanosine (**7d**) elutes in fractions 120–130, and 4-(*p*-methylbenzyl)-5-guanidino-1-β-D-ribofuranosylimidazole (**8d**) elutes in fractions 150–160. The amount of 7-substituted guanosine (**5d**) produced in these reactions was quantified by eluting the column with the acidic buffers described previously.[20]

A tabular summary of the percent yield for products **5–8** is presented in Table I.

Acknowledgment. Research was sponsored by the National Cancer Institute, DHHS, under Contract No. N01-CO-23909 with Bionetics Research, Inc. The contents of this publication do not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Electrochemical Reduction of Trivalent Organophosphorus Compounds: Mechanism and Products from Phosphorus–Heteroatom Bond Cleavage

T. J. Hall and J. H. Hargis*

Department of Chemistry, Auburn University, Auburn University, Alabama 36849

Received February 24, 1986

The cathodic cleavage at platinum electrodes of phosphorus–heteroatom bonds in fluoro-, chloro-, and bromodiphenylphosphine and in phenyl diphenylphosphinite in dry acetonitrile solution has been accomplished with the formation of tetraphenyldiphosphine as the exclusive product. A mechanism that involves the intermediacy of diphenylphosphinyl anions is suggested on the basis of cyclic voltammetry and controlled potential coulometry studies.

Introduction

The application of electrochemical techniques to the synthesis of organic compounds is an area which is rapidly developing. Relatively little attention, however, has been devoted to the study of organophosphorus compounds. We have undertaken an investigation of the electrochemical cleavage of phosphorus–heteroatom bonds in trivalent phosphorus compounds in anticipation that either the radicals II or the anions III (eq 1) which are potential intermediates might be synthetically useful.

$$\underset{\text{Ia, X = Cl}\\\text{Ib, X = Br}\\\text{Ic, X = F}\\\text{Id, X = OPh}}{Ph_2PX} + e^- \rightarrow Ph_2PX^{\bullet -} \rightarrow \underset{\text{II}}{Ph_2P^\bullet} + X^- \xrightarrow{e^-} \underset{\text{III}}{Ph_2P^-} \quad (1)$$

Dessy et al.[1] have reported that reduction of chlorodiphenylphosphine, Ia, at a mercury cathode at −3.4 V vs. $Ag|AgClO_4$ in glyme resulted in the formation of diphenylphosphine (eq 2). Dessy interpreted this to result

$$Ph_2PCl + e^- \rightarrow Ph_2P^\bullet + Cl^- \xrightarrow{S\text{-}H} Ph_2PH \quad (2)$$

from a one-electron reduction forming the diphenylphosphinyl radical, II, which subsequently abstracted hydrogen from the solvent forming product (eq 2). These authors reported that no reversibility was detected by cyclic voltammetry using sweep rates of up to 100 V/s.

Results

We have investigated the cyclic voltammetry of Ia–d at a platinum disk electrode in painstakingly dried acetonitrile containing tetra-*n*-butylammonium perchlorate as an electrolyte. We have observed rather complex concentration and sweep rate dependent behavior, and the peaks observed are broad and poorly defined. Additional scouting studies of the cyclic voltammograms at glassy carbon and at mercury gave completely analogous results, and the results obtained were identical with and without use of *iR* compensation. Figure 1 shows cyclic voltam-

(1) Dessy, R. E.; Kitching, W. *J. Am. Chem. Soc.* **1966**, *88*, 467.

0022-3263/86/1951-4185$01.50/0