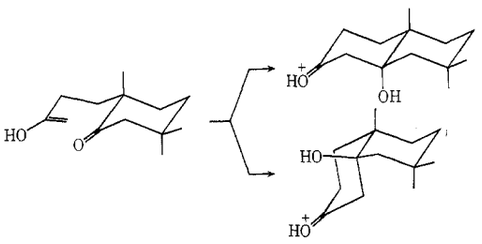


0.55 M in dione I and containing 8 ml of concentrated H_2SO_4 per liter of benzene for 70 h.

The extreme slowness of this aldol condensation is probably due to the fact that a 1,3-diaxial relationship is created in either of the two modes of cyclization shown.

We,[15] and others,[16] have recently found that the acid-catalyzed method of accomplishing Robinson annelations is facilitated in such cases by refluxing the initially formed dione with ethanolic KOH to accomplish the aldol stage of the annelation.

(15) R. D. Clark, E. G. DelMar, and C. H. Heathcock, unpublished observations.

(16) P. M. Worster, Ph.D. Thesis, University of British Columbia, 1975.

Charge Localization in the Carbonium Ions of Methylbenzanthracenes

Ercole Cavalieri* and Melvin Calvin

Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, Nebraska 68105, and Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720

Received March 31, 1976

Covalent binding of aromatic hydrocarbons to cellular macromolecules, the first probable step in the tumor-initiating process, requires metabolic activation by monooxygenase enzyme systems. Acid-catalyzed proton–deuterium exchange was used as a model to simulate the electrophilic oxygen atom activated by such enzymes. Kinetics of exchange with deuterium ion for a series of carcinogenic and noncarcinogenic methylbenzanthracenes were studied by NMR in two sets of conditions, i.e., CCl_4–CF_3COOD (85:15 v/v and 50:50 v/v). Deuteration of the potent carcinogen 7,12-dimethylbenz[*a*]anthracene at the most basic position C-12 generated a carbonium ion with charge localized at the complementary 7 position, resulting in the specific deuteration of the attached methyl group. Similarly, selective attack of deuterium ion on C-6 in 3-methylcholanthrene produced a carbonium ion with a high degree of charge localization at C-12b and, consequently, specific deuteration at the adjacent methylene group. This study has revealed that charge localization in the carbonium ion renders this intermediate chemically reactive; such a distinctive property might play a role in the bioactivation of these compounds.

The common feature unifying the wide variety of structures of chemical carcinogens is the electrophilic character[1,2] of the reactive species responsible for binding to cellular macromolecules. Reaction of these electrophiles with biological nucleophilic targets constitutes the first essential step that is critical in the succession of events leading to neoplasia. In the case of the inert polycyclic aromatic hydrocarbons, binding activation is supplied by monooxygenase enzymes.[3,4]

From a chemical standpoint the hydroxylation reaction catalyzed by these nonspecific enzyme systems points to an oxygen atom transfer reaction, the reactive species being an oxygen atom with six electrons in its outer shell.[5,6]

Although an enzymic mechanism may be different from any known chemical mechanism, it still must fall within the framework of basic chemical laws, and the use of a chemical model might provide fruitful information on the complex mechanism of metabolic activation of these compounds. Following this line of reasoning, without pretense of simulating the "oxenoid" character of the enzymically activated oxygen but solely its electron-deficient properties, the kinetics of acid-catalyzed proton–deuterium exchange by NMR were studied for a series of carcinogenic and noncarcinogenic methylbenzanthracenes. These experiments compared the relative reactivities of the most basic positions and the relative basicities of different sites in the same molecule.

The purpose of this approach was to evaluate whether it was possible to generalize the proposed mechanism of hydrocarbon activation[7] to an extensive series of carcinogenic hydrocarbons. In such a mechanism, attack of the enzymically catalyzed oxygen atom at the most reactive substituting positions would form electrophilic centers at sites complementary to the points of activation, and such centers may react with cellular targets.

Results and Discussion

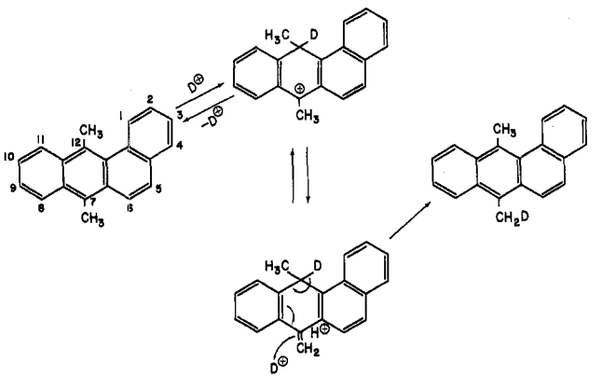
Charge Localization in the Carbonium Ion of 7,12-Dimethylbenz[*a*]anthracene and 3-Methylcholanthrene by NMR. The protonation of 7,12-dimethylbenz[*a*]anthracene (7,12-DMBA) on C-12 in acid medium was previously proposed[8] and charge localization on C-7 in the corresponding arenonium ion was suggested by MO calculations. We have determined the structure of the arenonium ion by comparing the NMR spectra of 7,12-DMBA in neutral and protonated or deuterated acidic solvents (see paragraph at end of paper regarding supplementary material). The ratio of the integrated intensities of the proton peaks in the spectrum of 7,12-DMBA in CCl_4 is, from high to low field, 3:3:5:1:1:2:1. Assignment of the 7-CH_3 (δ 3.00) and 12-CH_3 (δ 3.29) resonances was made by comparison with the spectra of 7-methylbenz[*a*]anthracene (7-MBA) (7-CH_3, δ 3.00) and 12-methylbenz[*a*]anthracene (12-MBA) (12-CH_3, δ 3.32) under the same conditions. A good general correlation exists between the methyl chemical shifts of the 12 monosubstituted methylbenzanthracenes[9] and the corresponding aryl protons of benzanthracene.[10]

The spectrum of 7,12-DMBA in CCl_4–CF_3COOH–H_2SO_4 (volume ratios 50:46.67:3.33) appears generally shifted to lower field with respect to the spectrum in CCl_4. This is attributable to the presence of the positive charge, as well as to the increased polarity of the solvent system. However, the 12-CH_3 group (details of assignment given below) is shifted toward the aliphatic region (δ 1.72) and occurs as a doublet (J = 7.6 Hz). A new quartet at δ 5.61 corresponding to the proton added at the basic 12 position (see below) shows the same coupling constant (J = 7.6 Hz) as the 12-CH_3 doublet. The original compound could be recovered unchanged by dilution with cold water and extraction into CCl_4.

When 7,12-DMBA was dissolved in a mixture of CCl_4–CF_3COOD–D_2SO_4 (volume ratios 50:46.67:3.33), the spectrum showed that the quartet was absent, the 12-CH_3 (δ 1.72) appeared as a singlet, and the 7-CH_3 was entirely exchanged with deuterium when the spectrum was recorded 1 h after dissolution. The solution of 7,12-DMBA in deuterated acid was quenched in D_2O after 1.75 h, and extracted with CCl_4, in which solvent the spectrum was recorded. A comparison of this spectrum with the original 7,12-DMBA spectrum in CCl_4 (see above) demonstrates unequivocally that the 7-CH_3 has been exchanged and the upfield shifted CH_3 group in protonated and deuterated acidic solvents (see above) must necessarily be the one substituted in 12 position.

The mechanism of deuteration of 7,12-DMBA at 7-CH_3 is shown in Scheme I. The selective attack of deuterium ion on

Scheme I



C-12 produces a carbonium ion with a high degree of charge localization at C-7 resulting in an appreciable acidity of the attached methyl group. As a consequence of this effect, the exchange of the proton in the 7-CH_3 is observed. Further stabilization leaves the 7-CH_3 selectively deuterated.

In the case of 3-methylcholanthrene (3-MC), the spectrum was recorded in $CDCl_3$ (see paragraph at end of paper regarding supplementary material) and the assignment of the aliphatic protons was made following the rules described above. The aromatic protons were assigned by comparing the 3-MC spectrum with that of benzanthracene[10] and by applying Martin's empirical rules,[11] which suggest spectral regions for each type of aromatic proton.

The meso-anthracene proton H6 is a singlet[12] and, with the angular proton H7, exhibits the largest downfield shift. The spectrum in $CDCl_3$–CF_3COOH–H_2SO_4 (volume ratios 50:46.67:3.33) shows a two-proton singlet in the aliphatic region (δ 4.75) corresponding to the selective protonation of the meso-anthracenic 6-carbon atom.

The 3-MC spectrum in $CDCl_3$–CF_3COOH–D_2SO_4 (volume ratios 50:46.67:3.33) recorded 0.5 h after preparation of the solution displayed complete exchange of H6 and partial deuteration of 12b-CH_2. The acidic deuterated solution was poured into D_2O after 2 h and extracted with $CDCl_3$, in which solvent the spectrum was recorded. A complete deuteration of H6 and 12b-CH_2 was observed. Since the doublet H4 in the original 3-MC spectrum in $CDCl_3$ (see above) was transformed into a singlet, it is clear that the H5 exchange also occurred.

Deuteration of 3-MC at the most basic 6 position generates a carbonium ion with charge localized at the complementary 12b position resulting in the specific deuteration of the adjacent methylene group, in analogy with 7,12-DMBA.

For hydrocarbons of lower basicity, e.g., 7-MBA, a similar study is experimentally impossible since the lifetime of the arenonium ion under the same acidic conditions shortens and no protonation can be observed. In most of the cases, however, it was the decomposition of the compound under such severe conditions that hampered the accomplishment of the experiment.

Kinetics of Proton–Deuterium Ion Exchange in Methylbenzanthracenes. The benzanthracene series has been chosen for the well-studied tumorigenic activity of these compounds[13–16] as well as for their interesting and intriguing structure–activity relationship. In this series the carcinogenic activity ranges from borderline with benzanthracene to potent with some methylbenzanthracenes. It is noteworthy that changes in the position of the methyl group can transform an active compound into a totally inactive one and that in different positions some alkyl substituents elicit different levels of activity. For instance, among the 12 monomethylbenzanthracenes only four display carcinogenic activity, i.e., the 7, 6, 8, and 12 substituted, in decreasing order.

In order to evaluate the relative reactivity of anthracene, benzanthracene, monomethyl-, dimethyl-, and trimethylbenzanthracenes, kinetics of deuterium exchange at the most basic positions were determined. After assignment of the protons in the NMR spectrum of the hydrocarbon, the extent of deuteration was calculated from the ratio between the integrated peak areas corresponding to the partially substituted protons and the integrated peak areas corresponding to the nonsubstituted protons. The angular protons were generally chosen as the nonsubstituted protons since they are localized separately in a downfield spectral region.

Solutions of 0.0352 and 0.0234 M[17] hydrocarbon were made in CCl_4–CF_3COOD (85:15) and CCl_4–CF_3COOD (50:50), respectively. Aliquots were removed periodically, poured into chilled D_2O, extracted with $CHCl_3$, and dried over sodium sulfate. Each extract was then evaporated, the residue was dissolved in a standard amount of CCl_4, and the NMR spectrum was recorded.

The relative rates of deuteration are summarized in Tables I and II. The basicities of the monomethylbenzanthracenes were previously measured by means of competitive extraction experiments[18,19] where the hydrocarbon competed for a limited amount of acid. In the monomethylbenzanthracene series, presented in Table II, 7-MBA and 12-MBA showed the most reactive meso-anthracenic positions. In 6- and 8-MBA the higher reactivity of the 7 position with respect to the corresponding position in BA is caused by the release of steric crowding between the methyl group and the adjacent hydrogen at the 7 position (peri effect)[18] in the tetrahedral conjugated acid. A similar effect on C-12 by the 11-methyl substituent is observed in 11-MBA. The reactivity of the 7 and 12 position in 2-MBA remains unchanged compared to BA, while the presence of a methyl group in the 5 position increased the reactivity of C-7 in 5-MBA.

Nearly all the dimethylbenzanthracenes studied are very potent carcinogens,[16] including the well-known 7,12-DMBA. The addition of a methyl group in the 6 or 8 position of 7-MBA doubles the rate of deuteration on C-12 (Table I). This observation is very curious in view of the fact that the 6- or 8-methyl substituent on BA provide no acceleration whatsoever on C-12. This effect is further increased 30 times when the methyl groups in 7 and 8 positions form a five-membered ring, as in 3-MC.[12] It seems likely that most of this rate enhancement can be attributed to increased hyperconjugative stabilization of the 3-MC arenonium ion resulting from the quasi-coplanarity of the C_1–H bonds and the p orbitals of the aromatic ring. Since canonical forms of 6-protonated 3-MC, in which the positive charge is localized at C-3, cannot be written, it is not reasonable to attribute a major portion of this rate enhancement to the additional 3-methyl substituent.

In 6,12-DMBA and 8,12-DMBA C-7 shows about twice the reactivity as in 12-MBA (Table II). The 7,12-DMBA is characterized by steric repulsion between the 12-CH_3 and the

TABLE I RATES OF DEUTERODEPROTONATION IN CCl_4 - CF_3COOD (85:15) AT 23 ± 1° C

HYDROCARBON	POSITION OF SUBSTITUTION	REACTION TIME (MIN.) 0.5	1	1.5	2	15	20	30	45	60	90	120	180	240	360	480	960	1680	2340	3660
ANTHRACENE	9						0.05			0.07	0.11	0.13		0.24						
	10						0.05			0.07	0.11	0.13		0.24						
BA	7															0.12	0.20	0.45	0.52	0.71
	12															0.09	0.18	0.43	0.50	0.69
7-MBA	12					0.19		0.31	0.43	0.50	0.65	0.86								
12-MBA	7							0.33	0.45	0.52	0.65	0.90								
6,7-DMBA	12					0.37		0.55												
7,8-DMBA	12					0.50		0.68												
8,12-DMBA	7					0.36		0.58												
7,12-DMBA	7-CH_3							0.36		0.73		1.41		2.07	2.52	2.79				
3-MC	5									0.28		0.48	0.59							
	6	0.50	0.75	0.86	0.91															
	12b -CH_2									0.39		0.70	0.89	1.11	1.30	1.45				
6,7,12-TMBA	7-CH_3							1.19				2.26								
	12-CH_3							1.50				2.58								
6,8,12-TMBA	7					0.50		0.68												

TABLE II RATES OF DEUTERODEPROTONATION IN CCl_4 - CF_3COOD (50:50) AT 23 ± 1° C

HYDROCARBON	POSITION OF SUBSTITUTION	REACTION TIME (MIN.) 1	2.5	5	10	20	30	45	60	90	120	180	240	360	480
ANTHRACENE	9				0.19	0.31	0.42								
	10				0.19	0.31	0.42								
BA	7						0.32		0.50			0.77	0.83	0.93	
	12						0.32		0.50			0.76	0.82	0.92	
2-MBA	7						0.36	0.51	0.63	0.78	0.86				
	12						0.31	0.45	0.56	0.73	0.82				
5-MBA	7				0.39		0.79	0.90	1.00						
	12				0.14		0.36	0.51	0.62	0.77	0.85				
6-MBA	7				0.22		0.44	0.55	0.71	0.82	0.89				
	12				0.16		0.28	0.39	0.50	0.62	0.71				
7-MBA	12		0.39	0.56	0.79										
8-MBA	7				0.22		0.45	0.57	0.68						
	12				0.17		0.33	0.43	0.52						
11-MBA	7				0.14		0.33	0.45	0.54	0.67	0.76				
	12				0.25		0.44	0.55	0.65	0.77	0.85				
12-MBA	7		0.38	0.57	0.81										
6,7-DMBA	5												1.00		
	12	0.38	0.77												
	7-CH_3												0.17		0.29
6,12-DMBA	7	0.41	0.74	0.92											
8,12-DMBA	7	0.41	0.71	0.91											
7,12-DMBA	7-CH_3				0.61	1.21	1.66		2.40						
3-MC	5			0.28	0.45	0.68									
	12b -CH_2			0.26	0.42	0.68	1.00								
6,8,12-TMBA	5												1.00		
	7	0.58	0.79												
	12-CH_3												0.27		0.43

hydrogen at C-1. Release of the strain upon protonation at C-12 renders this position the most basic. This effect can be visualized by the selective deuteration of the 7-CH_3 group (Table I), which occurs twice as fast as in the corresponding 12b-CH_2 of 3-MC. Finally, the steric crowding between the 6- and 7-methyl group in 6,7,12-TMBA renders C-7 more basic than C-12 (Table I), as can be observed by the relative deuteration of the methyl groups substituted at the positions complementary to the points of electrophilic attack.

Conclusion

This investigation has primarily revealed that charge lo-

calization in the carbonium ion of 7,12-DMBA, 3-MC, and 6,7,12-TMBA renders this intermediate chemically reactive on the methyl group(s) attached to the carbon atoms at which the charge is localized. Unfortunately, under the acidic conditions sufficiently mild to avoid decomposition, no exchange of the methyl group of other hydrocarbons could be observed. Nevertheless, from the kinetic study of deuterium ion exchange in the methylbenzanthracene series it can be inferred that a general mechanism of hydrocarbon activation by attack of the enzymically catalyzed oxygen species at the most reactive substituting positions with simultaneous formation of electrophilic centers at positions complementary to the points of activation[7] seems to be rather unlikely.

It will be seen in the accompanying paper how the one-electron oxidation of the hydrocarbon, which represents a more plausible mechanism of biological activation, generates radical cations with a significant degree of positive charge localization. Such an effect plays a decisive role in determining the reactivity toward nucleophilic trapping of these intermediates.

Acknowledgments. We wish to express our thanks to Dr. Melvin Newman for the monomethylbenzanthracene compounds and Dr. John Pataki for the dimethylbenzanthracene and trimethylbenzanthracene samples. The valuable comments of Dr. Robert Roth are much appreciated. The financial support of the Cancer Research Funds of the University of California, Berkeley, of the U.S. Atomic Energy Commission, and of the NCI-NIH PH-43-68-959 contract are gratefully acknowledged.

Registry No.—Anthracene, 120-12-7; BA, 56-55-3; 7-MBA, 2541-69-7; 12-MBA, 2422-79-9; 6,7-DMBA, 20627-28-5; 7,8-DMBA, 604-81-9; 8,12-DMBA, 20627-31-0; 7,12-DMBA, 57-97-6; 3-MC, 56-49-5; 6,7,12-TMBA, 20627-33-2; 6,8,12-TMBA, 20627-34-3; 2-MBA, 2498-76-2; 5-MBA, 2319-96-2; 6-MBA, 316-14-3; 8-MBA, 2381-31-9; 11-MBA, 6111-78-0; 6,12-DMBA, 568-81-0; 7,12-DMBA$^+$, 59230-87-4; 7,12-DMBA$^+$-d_4, 59230-88-5; 7,12-DMBA-d_3, 59230-67-0; 3-MC$^+$, 59230-89-6; 3-MC-d_2, 59230-90-9.

Supplementary Material Available. The 220-MHz proton NMR spectra of 7,12-DMBA and 3-MC in different solvent systems (2 pages). Ordering information is given on any current masthead page.

References and Notes

(1) J. A. Miller, *Cancer Res.*, **30**, 559–576 (1970).
(2) J. A. Miller, and E. C. Miller, *J. Natl. Cancer Inst.*, **47**, 5–14 (1971).
(3) P. L. Grover, and P. Sims, *Biochem. J.*, **110**, 159–160 (1968).
(4) H. V. Gelboin, *Cancer Res.*, **29**, 1272–1276 (1969).
(5) V. Ullrich, *Angew. Chem., Int. Ed. Engl.*, **11**, 702–712 (1972).
(6) G. A. Hamilton, "Molecular Mechanism of Oxygen Activation", O. Hayaishi, Ed., Academic Press, New York, N.Y., 1974, pp 405–451.
(7) E. Cavalieri and M. Calvin, *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 1251–1253 (1971).
(8) C. MacLean, J. H. van der Waals, and E. L. Mackor, *Mol. Phys.*, **1**, 247–256 (1958).
(9) L. K. Keefer, L. Wallcave, J. Loo, and R. S. Peterson, *Anal. Chem.*, **43**, 1411–1416 (1971).
(10) T. J. Batterham, L. Tsai, and H. Ziffer, *Aust. J. Chem.*, **18**, 1959–1966 (1965).
(11) (a) R. H. Martin, *Tetrahedron*, **20**, 897–902 (1964); (b) R. H. Martin, N. Defay, F. Geerts-Evrard, and S. Delavarenne, *Tetrahedron*, **20**, 1073–1090 (1964).
(12) In the 3-MC nomenclature C-6, C-7, 12b-CH_2, and 2a-CH_2 correspond to C-12, C-1, 7-CH_3, and 8-CH_3 in the benzanthracene series, respectively.
(13) W. F. Dunning and M. R. Curtis, *J. Natl. Cancer Inst.*, **25**, 387–391 (1960).
(14) J. L. Stevenson and E. Von Haam, *Am. Ind. Hyg. Assoc. J.*, **26**, 475–478 (1965).
(15) C. B. Huggins, J. Pataki, and R. G. Harvey, *Proc. Natl. Acad. Sci. U.S.A.*, **58**, 2253–2260 (1967).
(16) J. Pataki and C. Huggins, *Cancer Res.*, **29**, 506–509 (1969).
(17) Anthracene and 3-MC were 0.0117 M in CCl_4–CF_3COOD (50:50).
(18) E. L. Mackor, G. Dallinga, J. H. Kruizinga, and A. Hofstra, *Recl. Trav. Chim. Pays-Bas*, **75**, 836–844 (1956).
(19) D. M. Brouwer, E. L. Mackor, and C. MacLean, "Carbonium Ions", Vol. II, G. Olah and P. v. R. Schleyer, Ed., Wiley-Interscience, New York, N.Y., 1970, pp 852–853.

Reaction of Methylbenzanthracenes and Pyridine by One-Electron Oxidation. A Model for Metabolic Activation and Binding of Carcinogenic Aromatic Hydrocarbons

Ercole Cavalieri* and Robert Roth

Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, Nebraska 68105

Received March 31, 1976

A simple procedure for generation and trapping of polycyclic aromatic hydrocarbon radical cations in homogeneous solutions of pyridine and iodine is described. Radical cations of benz[*a*]anthracene and its alkyl derivatives are trapped by nucleophilic attack of pyridine on the aromatic nucleus in the order C-7 > C-12 > C-5. When positions 7 or 7 and 12 are blocked by a methyl group, pyridine substitution on the alkyl group competes with ring substitution. Mechanisms for the two types of substitution are proposed and trapping specificity is discussed in terms of charge density and steric factors in the radical ions.

Recently, there has been increasing speculation that radical cations might be the critical intermediates in carcinogenesis by polycyclic aromatic hydrocarbons. Following the original suggestion by Wilk[1] that these intermediates might be important, the conversion of aromatic hydrocarbons to carcinogenic metabolites via one-electron oxidation was demonstrated.[2] More recently Wilk and Girke[3] have reported that the benzo[*a*]pyrene (B[*a*]P) radical cation reacts with nucleic acid bases. Based on the capacity of Fe^{3+} to effect one-electron oxidation of aromatic hydrocarbons,[1,4] it was suggested that hexacoordinated Fe^{3+} in the form of cytochrome P-450 present in microsomes[5] and nuclei[6–8] might act as the cellular oxidant.

Despite the potential biological significance, most studies of aromatic hydrocarbon radical cations have been limited to ESR properties or the mechanistic details of electron transfer steps. While interest in reactions of radical cations with nucleophiles has increased, factors governing the site of nucleophilic attack have received little attention. Among biologically interesting molecules, well-characterized products from nucleophiles and radical cations have been reported only for B[*a*]P.[4,9–11] We have therefore undertaken a study of nu-