Environmental Mutagenesis 9:183–189 (1987)

Electronic Considerations in the Mutagenesis of Some 4,5-Bridged Chrysenes

E. Lee-Ruff, A.S. Raj, H. Kruk, and M. Katz

Departments of Chemistry (E.L.-R., M.K., H.K.) and Biology (A.S.R.), York University, North York, Ontario M3J 1P3 Canada

The mutagenic activity of four 4,5-bridged chrysene derivatives, benz(a)aceanthrylene, and 5-methylchrysene was examined using histidine auxotrophic strains TA98 and TA100 of *Salmonella typhimurium*. All compounds showed a positive mutagenic response with both TA100 and TA98 in the presence of S-9. A correlation between the electronic character of the bridging group and mutagenic activity for the chrysene derivatives is proposed.

Key words: bridged chrysenes, electronic-structural effects, mutagenesis

INTRODUCTION

Jerina and his co-workers [1976, 1977] have postulated the theory that the primary metabolites and ultimate carcinogens-mutagens of polynuclear aromatic hydrocarbons (PAH) are the diol epoxides and carbonium ions at the "bay region" derived from the epoxides of these compounds. However, recent interest in the mutagenic activity of cyclopenta(cd)pyrene [Gold et al, 1980] has prompted studies in related PAHs which do not possess a bay region [Harvey, 1981]. Recently, Coombs et al [1981] reported that the non-bay region hydrocarbon, 15,16-dihydro-1,11-methanocyclopenta(a)phenanthren-17-one (1), was a moderately strong carcinogen (Fig. 1). This result is surprising in view of metabolic studies of other cyclopenta(a)-phenanthrenones which suggest a bay region 3,4-diol-1,2-epoxide is the ultimate carcinogen. Chrysene 2 possesses two bay region sites, and yet it is only a weakly active mutagen [Salamone et al, 1979; Chang et al, 1983]. Of the methylchrysenes, 5-methylchrysene has been found to be a potent mutagen, the activity being comparable to that of benzo(a)pyrene [Hecht et al, 1978]. It has been suggested that steric and electronic factors are involved in the enhanced activity of 5-methylchrysene, the electronic factor associated with the electron releasing effect of the methyl group contributing to greater electron density at both the C-10 and K regions, and the steric

Received May 20, 1985; revised and accepted October 14, 1986.

Address reprint requests to E. Lee-Ruff, Dept. of Chemistry, Farquharson Building, York University, North York, Ontario M3J 1P3, Canada.

effect associated with the deformation from planarity of the ring system as a result of the interaction between the 5-methyl and 4-H substituents. Indirect evidence obtained from mutagenic studies of 5-methyl-12-fluorochrysenes suggests that metabolic activation takes place at the 11,12 positions with formation of an 11,12-epoxide [Hecht et al, 1978]. More recent evidence [Melikian et al, 1983] suggests that the activation of 5-methylchrysene occurs via the bay region 1,2-diol-3,4-oxide and the 7,8-diol-9,10-oxide. These studies point to the former metabolite as binding more efficiently to DNA. The electron donating effect of the 5-methyl substituent lies in a node of the molecular orbital of the carbocation in the former case and would not be expected to enhance the stability of this ion.

In order to assess independently the electronic effect of C-5 substitution on mutagenic activity, we synthesized and tested 4,5-bridged chrysenes in which the electronic character of the bridging substituent was varied from an electron releasing methano group (3(b)) to an electron withdrawing oxo group (3(a) and 3(d)) (Fig. 1). The nature of the bridging group could affect the electron density at both C-4 and C-10 and the consequent activation at those sites. Compound 3(b) is structurally related to 5-methylchrysene; however, the former maintains the coplanarity of the ring system as the result of bridging [Clayton et al, 1983]. It can also be formally regarded as a congener of cyclopenta(cd)pyrene. Structure 3(c) is isoelectronic with benzo(a)pyrene and as such may be expected to exhibit mutagenic activity. In addition to the above compounds, a related derivative of 4,5-methanochrysene, benz-(a)aceanthrylene (4), was prepared and assayed as an example of a related cyclopentaarene lacking a bay region (Fig. 1).

MATERIALS AND METHODS

Chemicals

The syntheses of the bridged chrysenes 3(a)–(c) have been reported by us [Lee-Ruff et al, 1984]. Benz(a)aceanthrylene (4) was conveniently prepared from the fluorenylketene 1,3-cyclohexadiene adduct 5 using the sequence of steps illustrated in Figure 2.

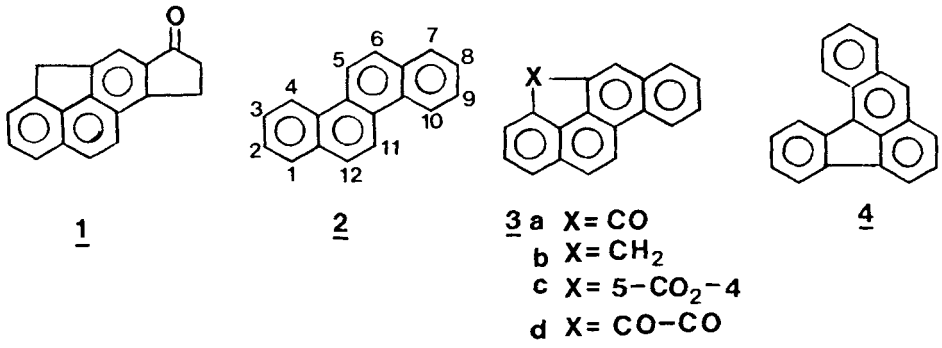


Fig. 1. Structurally related PAHs.

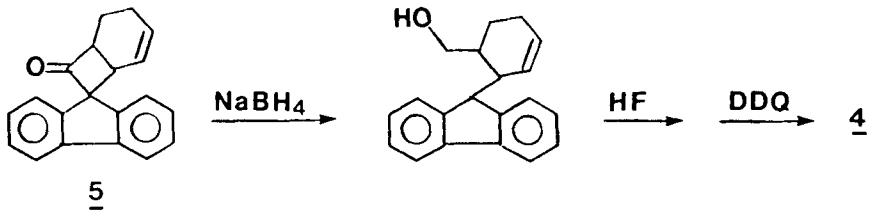


Fig. 2. Preparation of benz(a)aceanthrylene (4) from the fluorenylketene 1,3-cyclohexadiene adduct.

Biological Assay

Mutagenic activity was assayed for the six compounds using histidine auxotroph strains TA98 and TA100 of *Salmonella typhimurium*, performed either by Biomutatech Inc. of Toronto or by our group. These strains were kindly supplied to both laboratories by Dr. B.N. Ames, University of California at Berkeley, and were maintained as described by Ames et al [1975].

All compounds were dissolved in dimethyl sulfoxide (DMSO) and tested according to the procedure described by Maron and Ames [1983]. Using TA98, the dose range was from as low as 0.001 μg per plate to as high as 1,000 μg per plate, with and without S-9 mix. TA100 was tested only with S-9 mix, and the dose range was from 0.1 to 100 μg per plate. For both strains, the final S-9 mix consisted of a 10% liver homogenate (Arochlor 1254-induced; male Sprague-Dawley rats). For TA98, tests for mutagenic potential of a compound included the positive control benzo[a]pyrene (BaP), along with solvent control DMSO. An additional positive control, 2-anthramine, was used with TA100. For both TA98 and TA100, three plates per dose were used, and the averages of the revertants per plate and standard errors were calculated.

RESULTS AND DISCUSSION

The mutagenic activity of the 4,5-bridged compounds varied with the nature of the 4,5-bridging substituent. The details of the chemicals, dose used, presence or absence of S-9, and the average number of revertants per plate, along with the standard error of the means are tabulated in Table I for TA100 and Table II for TA98. Mutagenicity in units of revertants/μg was calculated from the initial linear portion of the dose (μg/plate) versus revertants per plate plot. Positive response was seen for all chemicals using both strains TA100 and TA98. Although the absolute magnitude of the response varied between strains, similar trends in mutagenic activity of the 4,5-bridged compounds were seen in both instances.

Using TA100 with S-9 activation, 5H-chryseno[4,5bcd]pyran-5-one was the most mutagenic while benzo(a)pyrene-4,5-dione was least mutagenic of the 4,5-bridged compounds. The activities of 4,5-oxochrysene and 4,5-methanochrysene were of similar order of magnitude and fell in between the activities of 5H-chryseno[4,5bcd]pyran-5-one and benzo(a)pyrene-4,5-dione. Using TA98 with S-9 activation, 5H-chryseno[4,5bcd]pyran-5-one was again most active of the 4,5-bridged compounds. 4,5-Methanochrysene showed positive response while 4,5-oxochrysene showed minimal response using TA98 with S-9 activation. It would appear from our data that mutagenic activity of these compounds is more pronounced with the TA100 strain.

TABLE I. Mutagenic Activity of Bridged Chrysenes*

	Chemical	Dose (μg/plate)	Revertants[a] per plate S.E. (with S-9)	Mutagenicity (revertants/μg)
3a	4,5-Oxochrysene	0.0	81.3 ± 8.74	
		0.1	81.0 ± 8.89	
		1.0	112 ± 13.1	
		5.0	293 ± 17.8	41
		10.0	342 ± 5.8	
		20.0	422 ± 60.0	
		50.0	358 ± 136	
		100.0	424 ± 106	
3b	4,5-Methanochrysene[b]	0.0	95.7 ± 10.6	
		0.1	112 ± 6.51	
		1.0	136 ± 10.6	
		5.0	364 ± 42.0	53
		10.0	517 ± 55.8	
		20.0	565 ± 74.8	
		50.0	719 ± 50.5	
		100.0	757 ± 11.4	
3c	5H-chryseno[4,5bcd]-pyran-5-one	0.0	82.7 ± 3.21	
		1.0	666 ± 143	
		5.0	234 ± 72.1	
		10.0	95.7 ± 54.9	583
		20.0	78.3 ± 25.8	
		50.0	66.0 ± 6.24	
		100.0	64.3 ± 26.2	
3d	Benzo(a)pyrene-4,5-dione	0.0	75.7 ± 15.0	
		0.1	79.0 ± 13.5	
		1.0	84.3 ± 12.5	
		5.0	98.3 ± 17.2	6.4
		10.0	140 ± 14.6	
		20.0	165 ± 12.1	
		50.0	91.3 ± 20.5	
		100.0	106 ± 1.53	
4	Benz(a)aceanthrylene	0.0	71.0 ± 8.89	
		0.1	80.3 ± 21.1	
		1.0	186 ± 19.2	
		5.0	911 ± 173	168
		10.0	608 ± 203	
		20.0	100 ± 52.1	
		50.0	87 ± 21.3	
		100.0	107 ± 25.5	
	5-Methylchrysene[b]	0.0	118 ± 7.37	
		0.1	110 ± 21.0	
		1.0	190 ± 31.7	
		5.0	910 ± 226	160
		10.0	1243 ± 122	
		20.0	1272 ± 122	
		50.0	1085 ± 87.9	
		100.0	1036 ± 205	

*Ames' salmonella assay, using TA-100 with S-9 activation.

[a]Average from three plates. Positive controls using 10 μg benzo(a)pyrene per plate gave 363 ± 56.9 revertants and with 8 μg of 2-anthramine gave 1,005 ± 87.8 revertants per plate. Negative controls were obtained from value of revertants per plate with 0.0 dose of compound.

[b]In this case, the positive controls with 10 μg of benzo(a)pyrene gave 794 ± 63.0 revertants per plate. The mutagenicity values for these compounds are not corrected since their response may not correlate linearly with that of the positive control.

TABLE II. Mutagenic Activity of Bridged Chrysenes

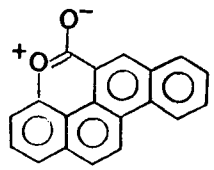
	Chemical	Dose μg/plate	Revertants[a] per plate S.E. (with S-9)	Mutagenicity revertants/μg	Revertants[a] per plate S.E. (without S-9)
3a	4,5-Oxochrysene	0.1	58.5 ± 3.3		47.0 ± 1.0
		1.0	66.0 ± 5.0		39.5 ± 4.75
		10.0	68.0 ± 5.0	8	46.5 ± 2.75
		100.0	49.0 ± 3.5		45.5 ± 2.75
		1,000.0	34.0 ± 0.0		38.0 ± 0.5
3b	4,5-Methanochrysene	0.1	63.5 ± 2.0		59.0 ± 2.0
		1.0	99.0 ± 0.5		62.5 ± 4.75
		10.0	133.0 ± 8.5	39	89.0 ± 3.5
		100.0	170.0 ± 3.0		58.3 ± 2.4
		1,000.0	167.0 ± 0.0		90.0 ± 0.0
3c	5H-chryseno[4,5bcd]-	0.001	51.0 ± 4.0		33.0 ± 4.0
	pyran-5-one	0.01	82.0 ± 2.5		32.6 ± 2.6
		0.1	135.0 ± 0.0	800	45.5 ± 2.8
		1.0	178.0 ± 2.0		34.5 ± 1.3
		10.0	176.5 ± 5.3		55.0 ± 6.0
		100.0	155.5 ± 4.8		47.5 ± 0.25
		1,000.0	90.0 ± 0.0		34.0 ± 0.0
4	Benz(a)aceanthrylene	0.001	77.6 ± 3.7		51.3 ± 7.1
		0.01	84.0 ± 3.5		56.5 ± 3.4
		0.1	115.5 ± 3.75	380	43.5 ± 3.3
		1.0	85.5 ± 6.3		35.0 ± 0.0
		10.0	74.0 ± 4.5		45.5 ± 2.3
		100.0	63.5 ± 5.8		27.5 ± 1.8
		1,000.0	54.0 ± 5.5		25.0 ± 2.0
	5-Methylchrysene	0.0	19.3 ± 1.53		
		0.1	23.0 ± 1.00		
		1.0	24.3 ± 3.21		
		5.0	27.7 ± 9.07		
		10.0	33.3 ± 11.0		
		20.0	38.0 ± 1.73		
		50.0	44.0 ± 7.00		
		100.0	37.0 ± 5.29		
Controls					
BaP		10.0	374.0 ± 15.6		—
DMSO		0.1ml	58.0 ± 1.4		57.3 ± 1.6

*Ames' salmonella assay, using TA-98, with and without S-9 activation.
[a]Average from three plates.

The stability of a carbocationic intermediate from hydrolytic ring opening of an arene oxide necessary for covalent binding with nucleophilic sites in cellular macromolecules [Weinstein et al, 1976] may be influenced by the nature of the 4,5-bridging substituent. In the case of 4,5-methanochrysene, the mutagenic activity found with both TA98 and TA100 is consistent with the relative electron releasing property of the CH_2 group, thus enhancing the stability of a carbocationic intermediate. The low activity of benzo(a)pyrene-4,5-dione (3(d)) on strain TA100 is consistent with the electron withdrawing nature of the oxo groups which would tend to destabilize any carbocationic intermediate. The observed activity of 4,5-oxochrysene (3(a)), on TA100 only, is unexpected. It is possible that destabilization of the carbocationic

intermediate does indeed occur but not to the extent needed to prevent mutagenic activity. Alternatively the bridging of the bay region by a one-carbon center may impart in-plane angular distortions [Kashino et al, 1986] of the arene system so as to render the ring system more strained and hence more susceptible to molecular activation. This effect would be diminished in the two-carbon bridge in the dioxo derivative 3d, which exhibits minimal activity in strain TA100. The lactone bridged species 3(c) exhibits activity consistent with the greater electron releasing nature of the ester function relative to ketones but inconsistent when compared to the methano bridged species. The σ values for CH_3, $-CO_2CH_3$, and $-CO-CH_3$ are -0.170, $+0.31$ and $+0.502$ respectively [Hammett, 1970]. A similar trend would be expected for the σ values of the bridging substituents $-CH_2-$, $-CO_2-$, and C=O. These correlations are only qualitative. The activity exhibited by the 4,5-pyranochrysene 3c was much higher than expected on the basis of its electron withdrawing nature alone. This activity may be rationalized in terms of a greater contribution of the zwitterion form 6 and consequent decrease in electron demand (Fig. 3). Such a species is isoelectronic with the π-electron framework of benzo(a)pyrene. Furthermore, the 4,5-bridging substituent may also affect the nature of C-4, thus modifying the stability of the corresponding carbocation derived from the 1,2-diol-3,4-epoxide.

These results suggest that the increased mutagenic activity of 5-methylchrysene compared to chrysene is partly due to electronic effects and partly due to in-plane angular distortions brought about by pinching the bay region carbons, imparting a certain amount of ring strain to the arene system. The 4,5-bridging substituents may also affect the electronic nature C-4 and modify the stability of the corresponding carbocation for subsequent binding to target sites. However, since 4-methylchrysene has been shown to exhibit greatly reduced mutagenic activity when compared with 5-methylchrysene, the C-5 site probably contributes a greater amount than the C-4 site. These substituent effects may intervene at the metabolic activation stage and/or at the nonenzymatic covalent binding stage of the oxidized metabolite. Not much is known concerning the electronic demand of the cytochrome P-450 monooxygenase enzyme system, although an Fe^{+3} oxene has been proposed as the reactive species. On the other hand, the correlation of covalent binding ability with electrophilic character of benzylic carbon associated with ring opening of the epoxide function has been well established [Jerina and Lehr, 1977]. The mutagenic activity of benz(a)aceanthrylene 4 is surprising in light of the absence of a bay and K region in the molecular structure. Its structural similarity to cyclopenta(cd)-pyrene is noteworthy, and the possible activation of the 1,2 positions in the form of an epoxide would result in a stable benzylic carbocation upon hydrolytic ring opening



6

Fig. 3. Zwitterion contributor of 5H-chryseno [4,5 bed]-pyran-5-one.

ACKNOWLEDGMENTS

We thank the Natural Science and Engineering Research Council (Strategic grants program), the Ministry of Environment, Province of Ontario Air Resources Branch, and the Provincial Lottery Corporation (project No. 81-005-33) for their generous support.

REFERENCES

Ames BN, McCann J, Yamasaki E (1975): Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian microsome mutagenicity test. Mutat Res 31:347–364.

Chang RL, Levin W, Wood AW, Yagi H, Tada M, Vyas KP, Jerina DM, Conney AH (1983): Tumorigenicity of enantiomers of chrysene 1,2-dihydrodiol and of diastereomeric bay-region chrysene 1,2-diol-3,4-epoxides on mouse skin and in newborn mice. Cancer Res 43:192–196.

Clayton AF, Coombs MM, Henrick K, McPartlin M, Trotter J (1983): X-ray structural studies and molecular orbital calculations (CNDO/2) in a series of cyclopenta (a)phenanthrenes: Attempts at correlation with carcinogenicity. Carcinogenesis 4:1569–1576.

Coombs MM, Bhatt TS, Livingston DC, Fisher SW, Abbott PJ (1981): Chemical structure, metabolism and carcinogenicity in the cyclopenta (a) phenanthrene series. In Cooke M, Dennis AJ (eds): "Chemical Analysis and Biological Fate: Polynuclear Aromatic Hydrocarbons." Columbus: Battelle Press, pp 63-74.

Gold A, Nesnow S, Moore M, Garland H, Curtis G, Barry H, Graham D, Eisenstadt E (1980): Mutagenesis and morphological transformation of mammalian cells by a non-bay region polycyclic cyclopenta(cd)pyrene and its 3,4-oxide. Cancer Res 40:4482-4484.

Hammett LP (1970): "Physical Organic Chemistry. Reaction Rates, Equilibria and Mechanisms," 2nd Ed. New York: McGraw-Hill, pp 356.

Harvey RG (1981): Activated metabolites of carcinogenic hydrocarbons. Acc Chem Res 14:218-226.

Hecht SS, Loy M, Mazzarese R, Hoffman D (1978): On the carcinogenicity of 5-methylchrysene: Structure-activity studies and metabolism. In Gelboin HV, Tso POP (eds): "Polycyclic Hydrocarbons and Cancer," Vol 1. New York: Academic Press, pp 119-130.

Jerina DM, Lehr RE (1977): The Bay-Region theory: A quantum mechanical aproach to aromatic hydrocarbon-induced carcinogenicity. In Ullrich V, Roots I, Hilderbrandt AG, Estabrook RW, Conney AH (eds): "Microsomes and Drug Oxidation." Oxford, UK: Pergamon Press, pp 709–720.

Jerina DM, Lehr RE, Yagi H, Hernandez O, Dansette PM, Wislocki PG, Wood AW, Chang RL, Levin W, Conney AH (1976): Mutagenicity of benzo(a)pyrene derivatives and the description of a quantum mechanical model which predicts the ease of carbonium ion formation from diol epoxides. In de Serres FJ, Fouts JR, Bend JR, Philpot RM (eds): "In Vitro Metabolic Activation in Mutagenesis Testing." Amsterdam: Elsevier Biomedical Press, pp 159–177.

Kashino S, Zacharias DE, Peck RM, Glusker JP, Bhatt TS, Coombs MM (1986): Bay region distortions in Cyclopenta, (a)phenanthrenes. Cancer Res 46:1817–1829.

Lee-Ruff E, Kruk H, Katz M (1984): A short synthesis of 4,5-methanochrysene and 6-oxo-7-oxabenzo(a)pyrene, two benzo(a)pyrene analogues. J Org Chem 49:553-555.

Maron DM, Ames BN (1983): Revised methods for the *Salmonella* mutagenicity test. Mutat Res 113:173–215.

Melikian A, Lavoie EJ, Hecht SS, Hoffmann D (1983): In Cooke M, Dennis AJ (eds); "Polynuclear Aromatic Hydrocarbons: Formation, metabolism and measurement." Columbus: Battelle Press, p 861.

Salamone MF, Heddle JA, Katz M (1979): The mutagenic activity of thirty polycyclic aromatic hydrocarbons (PAH) and oxides in urban airborne particulates. Env Int 2:37-43.

Weinstein IB, Jeffrey AM, Jennette KW, Blobstein SH, Harvey RG, Harris C, Autrup H, Kasai H, Nakanizhi K (1976): Benzo(a)pyrene diol epoxides as intermediates in nucleic acid binding in vitro and in vivo. Science 193:592-594.