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COMPARATIVE REACTIVITIES OF DIOLEPOXIDE METABOLITES OF CARCINOGENIC HYDROCARBONS WITH ØX174 VIRAL DNA

W. T. Hsu*†, E. J. Lin*, P. P. Fu††, R. G. Harvey††, and S. B. Weiss*†

*The Franklin McLean Memorial Research Institute, † The Departments of Biochemistry and Microbiology, and††The Ben May Laboratory for Cancer Research, The University of Chicago, Chicago, Illinois 60637

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SUMMARY

The ØX174 DNA assay system developed earlier is utilized to determine the comparative reactivities with nucleic acid of the diolepoxide metabolites of a series of polycyclic aromatic hydrocarbons varying in carcinogenic potency. The infectious ØX174 viral DNA is exposed to the hydrocarbon derivative for 10 min., then infectivity of the treated DNA is assayed by incubation with E. coli spheroplasts, counting plaque formation on agar plates. The bay region diolepoxides of benzo[a]-pyrene, chrysene, and dibenz[a,h]anthracene, implicated as the ultimately active carcinogenic metabolites of the parent hydrocarbons, exhibit potent viral inhibitory activity. On the other hand no correlation is evident between viral inhibitory activity and either the location of the diolepoxide function in a bay region or the theoretically calculated β-delocalization energies ($\Delta E_{deloc.}$) of the carbonium ion arising from opening the epoxide ring. The significance of these findings with respect to theories of carcinogenesis is discussed.

Diolepoxide metabolites have been implicated as the ultimate carcinogenic metabolites of benzo[a]pyrene (BP)[1] (1), 7,12-dimethylbenz[a]anthracene (DMBA) (2-5), 7-methylbenz[a]anthracene (7-MBA) (6), 3-methylcholanthrene (3-MC) (7-8), and other (9-15) carcinogenic polycyclic aromatic hydrocarbons (PAH). BP has been most intensively studied, and trans-7,8-dihydroxy-anti-9,10-epoxy-7,8,9,10-tetrahydro-BP (anti-BPDE) has been shown to be the principal metabolite of BP which binds covalently to nucleic acids in mammalian cells (1). It was also demonstrated that anti—

[1]Abbreviations: BP, benzo[a]pyrene; BA, benz[a]anthracene; DMBA, 7,12-dimethyl-BA; 7-MBA, 7-methyl-BA; 3-MC, 3-methylcholanthrene; PAH, polycyclic aromatic hydrocarbon(s); H_4BP, 7,8,9,10-tetrahydro-BP; anti(syn)-BPDE, trans-7,8-dihydroxy-anti (syn)-9,10-epoxy-H_4BP.

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BPDE efficiently alkylates single stranded ØX174 DNA, a single alkylation event sufficing to totally inhibit viral replication in Escherichia coli spheroplasts (16,17).

Since alkylation of nucleic acids is generally assumed to be a critical event in carcinogenesis, it is of interest to compare the reactivities with DNA and/or RNA of diolepoxide derivatives of PAH differing in their carcinogenic potency. The phage assay system developed earlier (16,17) provides a simple and convenient experimental method adaptable to this purpose. In the Method B assay procedure the infectious viral nucleic acid is pretreated with the hydrocarbon derivative, unreacted PAH is removed, and infectivity of the treated DNA is assayed by incubation with E. coli spheroplasts, and counting viral plaque formation on agar plates. Utilizing this assay method, we have investigated the comparative activities of a relatively large series of diolepoxide derivatives of PAH ranging widely in their carcinogenic activities. We find that many of these compounds are potent inhibitors of ØX174 DNA viral infectivity, and this inhibitory activity correlates qualitatively with carcinogenic potency. On the other hand, no correlation is evident between viral inhibitory activity and the location of the diolepoxide function in a bay region[2] or the calculated theoretical β-delocalization energies ($\Delta E_{deloc.}$) of the carbonium ions arising from opening the epoxide ring. The significance of these findings with respect to theories of PAH carcinogenesis is discussed.

MATERIALS AND METHODS

Assay Procedure: The preparation of ØX174 DNA and the assay of its infectivity were as previously described (16). Modification of ØX DNA infectivity by binding with the PAH diolepoxide derivatives was conducted by the Method B experimental procedure (16) using 10 µg of ØX DNA and 0.5 µg of the PAH compound in 0.1 ml of buffer (pH 7.5). After 10 min incubation at 25°, the DNA was precipitated with ethanol, washed with acetone, dried, and dissolved in Tris-EDTA. Infectivity of the treated DNA was assayed by incubation with E. coli spheroplasts and plating on agar plates with E. coli HF4714 used as indicator for plaque formation. The values of percent inhibition represent an average for triplicate plates.

Materials: The compounds were synthesized by the methods in the references cited: 1, 2, 5, 19, 20 (1); 3, 7, 15, 21, (19); 4 (20); 6, 11, (1,21); 8, 9, (22); 10 (12,23); 12, 13, 16, 17 (1,24); 14 (1,25); 18 (23).

[2] A bay region of a PAH is a molecular region between adjacent fused aromatic rings, such as between the 1- and 4-positions of phenanthrene.

RESULTS AND DISCUSSION

The inhibitory response resulting from treatment of the phage ØX174 DNA virus with a series of PAH diolepoxide derivatives (structural formulae in Fig. 1) are summarized in Table I. Anti-BPDE, 1, was the most potent compound tested, inhibiting viral replication in E. coli spheroplasts essentially quantitatively at the dosage employed. The syn isomer, 2, exhibited lower activity (46%), in line with its reported lower mutagenic activity (1). Both 9,10-epoxy-H_4BP, 3, and 1-oxiranylpyrene, 4, were equally as active as anti-BPDE. Since 3 lacks the two hydroxyl groups of anti-BPDE and 4 lacks also the 7,8-carbon atoms, these structural features apparently do not contribute significantly to the activity of anti-BPDE. The two cis isomeric analogs of anti-BPDE having the hydroxyl groups cis rather than trans (i.e., 5) were only slightly less active (90%). Compounds 19 and 20, the reverse anti and syn diolepoxides of BP having the epoxide function in the 7,8-position, exhibited 72% and 48% inhibitory activity, respectively. The related 7,8-epoxy-H_4BP, 21, also showed substantial activity (78%). Evidently, it is not essential for the epoxide ring to be in a bay region for relatively efficient alkylation of ØX174 DNA to take place.

A relatively similar pattern of activity was observed for the series of BA derivatives. Again the anti isomers were more active than the syn isomers (78% vs 7% for 12 and 13; 32% vs 18% for 16 and 17). The most active compound was the terminal ring BA anti-diolepoxide 12, whose activity (78%) exceeded that of the bay region BA diolepoxide 6 (46%) and the remaining two isomeric BA anti-diolepoxides 11 (55%) and 16 (32%). The bay region isomer is reported to be the most mutagenic and carcinogenic of the isomeric BA diolepoxides (9,10).

In the chrysene series, the bay region diolepoxide 10 showed strong inhibitory activity (91%), while the reverse isomeric chrysene diolepoxide 18 proved essentially inactive. This is consistent with the observed relative mutagenicities (26) and carcinogenecities (11) of these two isomers. The bay region anti-diolepoxides of both benzo[e]pyrene and triphenylene also showed no detectable activity. The latter findings are consistent with triphenylene's lack of carcinogenic activity and the weak borderline activity of benzo[e]pyrene in this respect (27). Finally, the relative

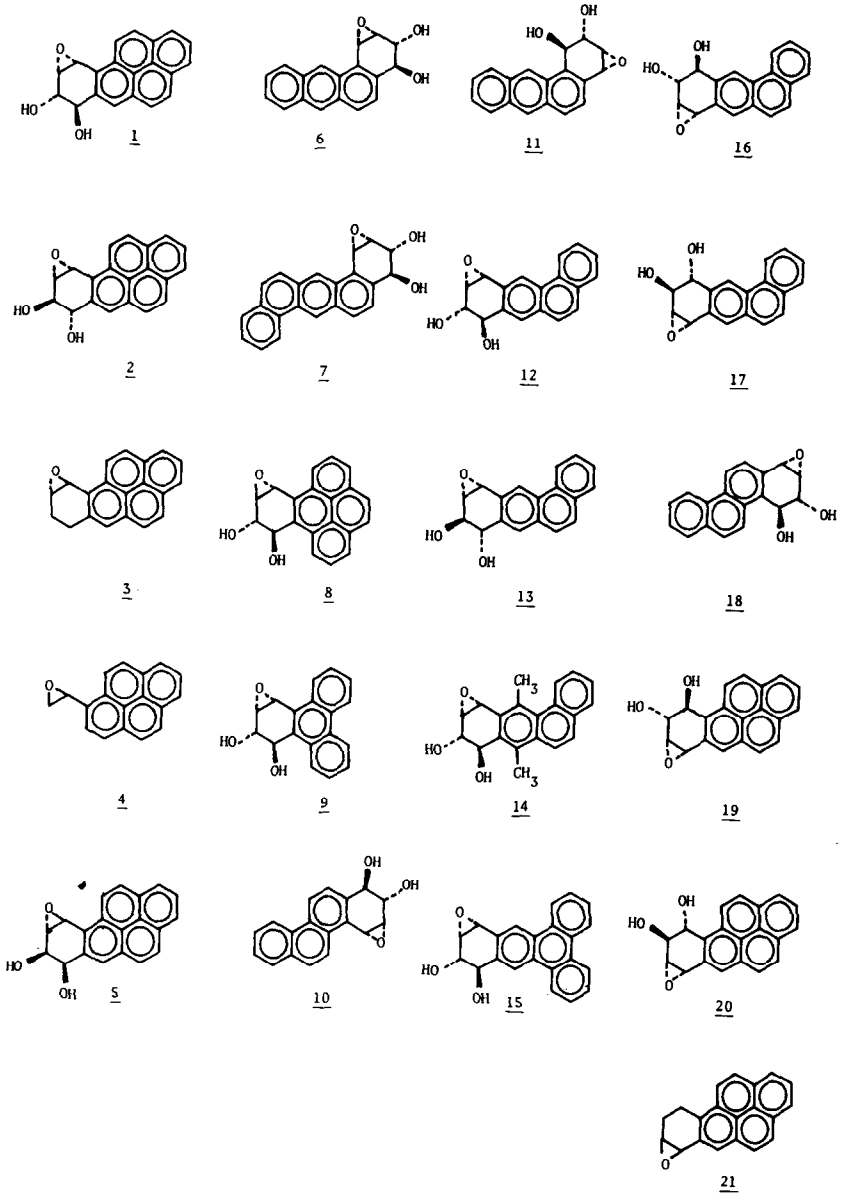

Fig. 1. Structures of PAH diolepoxides and related compounds. 1: anti-BPDE; 2: syn-BPDE; 3: 9,10-epoxy-H_4BP; 4: 1-oxiranylpyrene; 5: cis-7,8-dihydroxy-anti (syn)-9,10-epoxy-H_4BP; 6: trans-3,4-dihydroxy-anti-1,2-epoxy-1,2,3,4-H_4BA; 7: trans-3,4-dihydroxy-anti-1,2-epoxy-1,2,3,4-H_4-dibenz[a,h]anthracene; 8: trans-9,10-dihydroxy-anti-11,12-epoxy-9,10,11,12-H_4-benzo[e]pyrene; 9: trans-9,10-dihydroxy-anti-11,12-epoxy-9,10,11,12-H_4-triphenylene; 10: trans-1,2-dihydroxy-anti-3,4-epoxy-1,2,3,4-H_4-chrysene; 11: trans-1,2-dihydroxy-anti-3,4-epoxy-1,2,3,4-H_4-BA; 12: trans-8,9-dihydroxy-anti-10,11-epoxy-8,9,10,11-H_4-BA; 13: trans-8,9-dihydroxy-syn-10,11-epoxy-8,9,10,11-H_4BA; 14: trans-8,9-dihydroxy-anti-10,11-epoxy-8,9,10,11-H_4DMBA; 15: trans-10,11-dihydroxy-anti-12,13-epoxy-H_4-dibenz[a,c]anthracene; 16: trans-10,11-dihydroxy-anti-8,9-epoxy-8,9,10,11-H_4BA; 17: trans-10,11-dihydroxy-syn-8,9-epoxy-8,9,10,11-H_4BA; 18: trans-3,4-dihydroxy-anti-1,2-epoxy-1,2,3,4-H_4-chrysene; 19: trans-9,10-dihydroxy-anti-7,8-epoxy-H_4BP; 20: trans-9,10-dihydroxy-syn-7,8-epoxy-H_4BP; 21: 7,8-epoxy-H_4BP.

Table 1. Direct Inactivation of ØX174 DNA Infectivity by PAH Diolepoxides and Related Compounds in E. Coli Spheroplasts[a]

Compound	% Inhibition of Infectivity[b]	$\Delta E_{deloc.}$[c]	Compound	% Inhibition of Infectivity	$\Delta E_{deloc.}$[c]
1	>99	0.794	12	78	0.572
2	46	0.794	13	7	0.572
3	>99	0.794	14	64	0.572
4	>99	0.794	15	38	0.544
5[d]	90	0.794	16	32	0.526
6	46	0.766	17	18	0.526
7	66	0.738	18	0	0.526
8	0	0.714	19	72	0.488
9	12	0.644	20	46	0.488
10	91	0.640	21	78	0.488
11	55	0.628			

[a] Ø174 DNA (10 µg) and 0.5 µg of PAH compound were incubated together in 0.1 ml of buffer (pH 7.5) for 10 min. at 25°. The DNA was reisolated as previously described for Method B (16) and assayed in triplicate for infectivity of E. coli spheroplasts.

[b] Differences of < 20% in plaque titer are not cinsidered significant (16).

[c] $\Delta E_{deloc.}$ is the difference between the calculated delocalization energies of the epoxide structure and its ring-opened ionized form in β units (18).

[d] The syn and anti isomers were present in 1:1 ratio.

inhibitory activities of the two dibenzanthracene isomers 7 and 15 were 66% and 38%, respectively. While the former value is consistent with the relative oncogenicity of the parent PAH, the latter is somewhat higher than anticipated on the basis of the weak carcinogenicity of the parent PAH (27).

On the basis of these results, there appears to be an approximate correlation between inhibitory activity in the phage assay system and the reported mutagenicities and carcinogenicities of these PAH metabolites. The only notable exception is the observed somewhat higher activity (78%) of the terminal ring diolepoxide of BA 12 than the bay region isomer 6 (46%). Whether this difference is a consequence of differences in stability or some other factor will require further experimental study to determine.

If it is assumed that the phage assay system provides a valid measure of the relative reactivities of PAH metabolites with nucleic acids, it is of interest to compare the experimental findings with the predicted reactivities calculated by MO theoretical methods. Available evidence indicates anti-BPDE reacts by an SN_1 mechanism involving initial ionization of the epoxide ring (1a,b). Assuming analogous PAH metabolites react by a similar mechanism, the overall rates of reaction may be predicted to be a function of the calculated β-delocalization energies ($\Delta E_{deloc.}$) of the resulting carbonium ion intermediates (28). Although the highest value of ΔE_{deloc} is indeed found for anti-BPDE and related compounds which also exhibit maximum extent of reaction with viral DNA, no particular correlation is evident throughout the remainder of Table I. Thus, the benzo[e]pyrene and triphenylene compounds which fail to inactivate viral DNA to a significant extent have higher values of ΔE_{deloc} (0.714 and 0.644, respectively) than the chrysene derivative (0.640) one of the most active compounds tested. Also, compounds 19, 20, and 21 predicted theoretically to be least reactive (ΔE_{deloc} = 0.488) are all highly active.

We tentatively conclude that ΔE_{deloc} does not provide a reliable index of reactivity of PAH diolepoxides with nucleic acids, at least for single stranded DNA. The "bay region theory" of carcinogenesis (28) which has this assumption as it central premise must, therefore, be seriously questioned.

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