EPOXIDES AS MICROSOMAL METABOLITES OF POLYCYCLIC HYDROCARBONS

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1. Introduction

The main metabolites of polycyclic hydrocarbons are hydroxylated derivatives [1]. These could theoretically arise from epoxide intermediates [2] formed by the NADPH-dependent microsomal mixed function oxidase. K-region epoxides of polycyclic hydrocarbons that have been prepared synthetically [3] have been shown to be alkylating agents that react covalently with nucleic acids [4] and with the constituents of cells in culture [5]. These K-region epoxides are also mutagenic to T_2 bacteriophage [6], to bacteria [7], to Drosophila [8] and to Chinese hamster cells [9] and are active in producing malignant transformation of cells in culture [10]. Consequently, it seemed important to establish that epoxides are in fact involved in the metabolism of polycyclic hydrocarbons. In the experiments described here, direct evidence has been obtained that epoxides are formed as microsomal metabolites of phenanthrene, benz[a]anthracene and dibenz[a,h]anthracene.

2. Materials and methods

Glucose-6-phosphate dehydrogenase and cofactors were purchased from Boehringer (Mannheim) and ^{3}H-labelled hydrocarbons (specific activities: phenanthrene 470 mCi/mmole, benz[a]anthrecene 450 mCi/mmole, dibenz[a,h]anthracene 400 mCi/mmole) from the Radiochemical Centre, Amersham, Bucks. Washed rat liver microsomes were prepared from animals pretreated with 3-methylcholanthrene [1]. Incubation mixtures consisting of microsomes (≡ 10 g liver) resuspended in pyrophosphate buffer (0.1 M, pH 8.0, 80 ml) containing glucose-6-phosphate (1 mmole), glucose-6-phosphate dehydrogenase (96 units), NADPH (48 μmole), nicotinamide (2 mmole), $MgCl_2$ (1 mmole) and styrene oxide (8.3 μmole) were preincubated for 10 min at 30°. ^{3}H-Labelled hydrocarbon (400 μg) was added in DMSO (0.2 ml) and after incubation for 3 min at 30°, the mixture was extracted with ether (1 vol.). The appropriate unlabelled K-region epoxide (3 mg) was added to the ether extract which was then dried, concentrated and chromatographed on an alumina column as described in the legend to fig. 1. Fractions containing the eluted epoxide peaks were pooled, concentrated and used to confirm the presence of radioactively labelled epoxide.

3. Results

Figs. 1 and 2 show the elution profiles obtained with extracts from microsomal incubations of ^{3}H-phenanthrene and ^{3}H-benz[a]anthracene. In each case, a radioactive peak was eluted, between those of the unchanged hydrocarbon and the dihydrodiol, that was coincident with the UV absorbent peak of the unlabelled carrier epoxide. Table 1 shows the crystallisation to constant specific activity of these radioactive epoxides using additional unlabelled samples of phenanthrene 9,10 oxide and benz[a]anthracene 5,6 oxide as carriers.

Fig. 3 shows the results obtained when radioactive epoxide, obtained from a ^{3}H-dibenz[a,h]anthracene incubation, was treated with acid to rearrange the epoxide to the corresponding phenolic compound. The products, when examined by thin layer chromatography, included a radioactive spot with an R_f

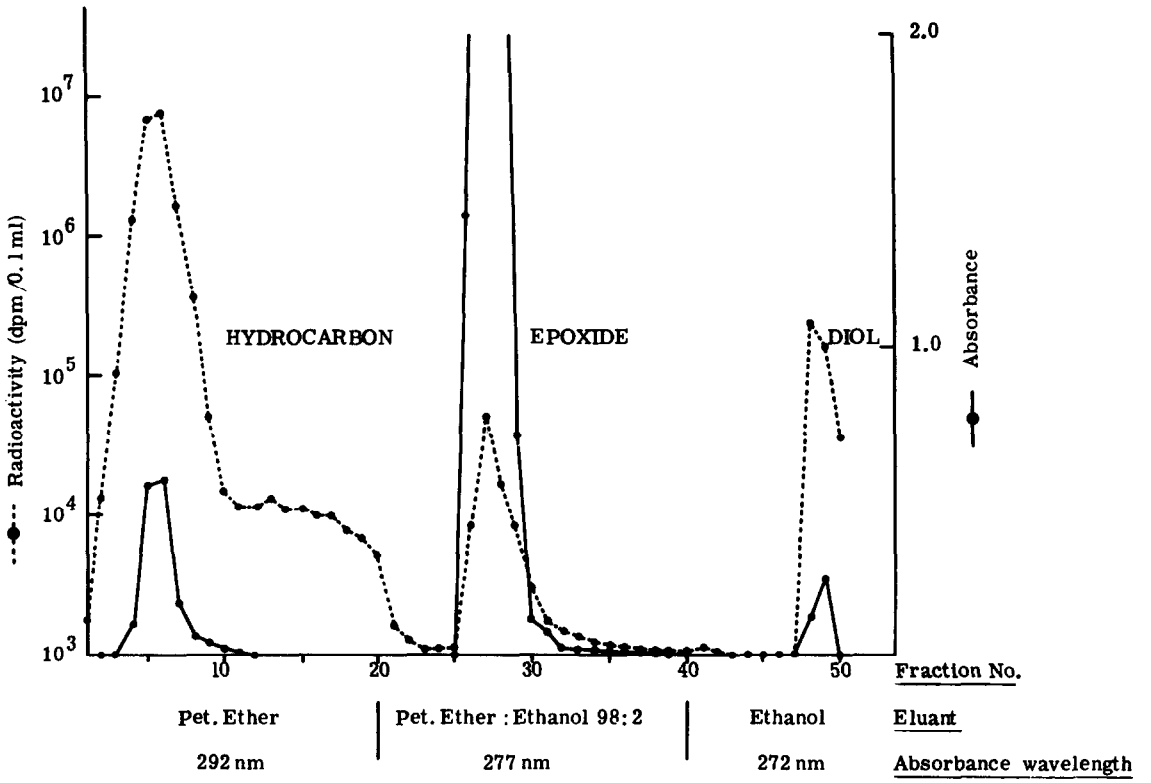

Fig. 1. Column chromatography of ^{3}H-phenanthrene metabolites. The concentrated ether extract from a rat liver microsomal incubation (see text) was applied to a column (1.3 × 4 cm) of activated alumina (100–200 mesh, Type H) and eluted with solvent. 100-drop fractions were collected and radioactivity and UV absorbance measured.

Table 1

Recrystallisation of phenanthrene 9,10-oxide and benz[a]anthracene 5,6-oxide containing radioactive epoxide formed from the corresponding ^{3}H-hydrocarbon.

Phenanthrene 9,10-oxide			Benz[a]anthracene 5,6-oxide		
Crystallisation	Yield (mg)	dpm/mg ($\times 10^{-4}$)	Crystallisation	Yield (mg)	dpm/mg ($\times 10^{-4}$)
1	42.8	6.08	1	39.6	8.75
2	36.2	5.10	2	33.2	6.55
3	29.3	5.22	3	26.4	8.45
4	23.8	4.99	4	17.3	7.70

Samples (50 mg) of unlabelled phenanthrene 9,10-oxide or benz[a]anthracene 5,6-oxide were added to pooled fractions containing the radioactive epoxide peaks obtained by alumina column chromatography of ether-extracts of ^{3}H-phenanthrene or ^{3}H-benz[a]anthracene incubations. The solutions were evaporated to dryness and the epoxide repeatedly crystallised from cyclohexane.

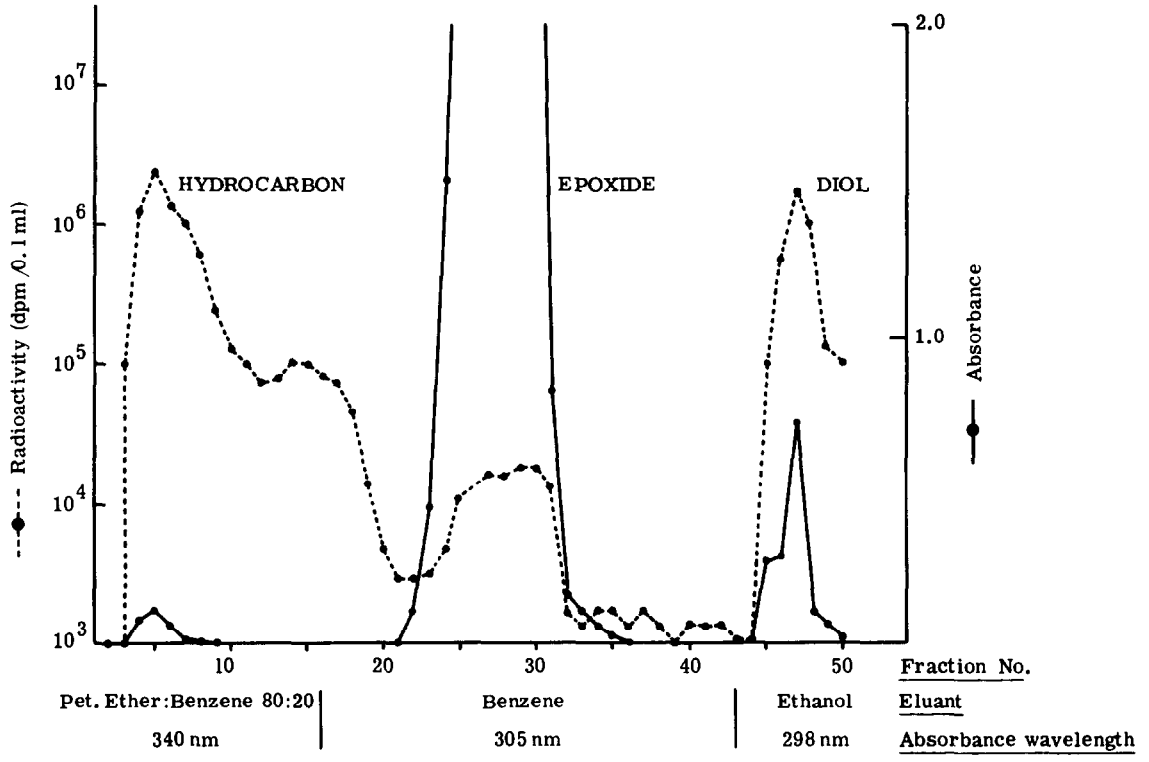

Fig. 2. Column chromatography of ^{3}H-benz[a]anthracene metabolites. (For details, see legend to fig. 1.)

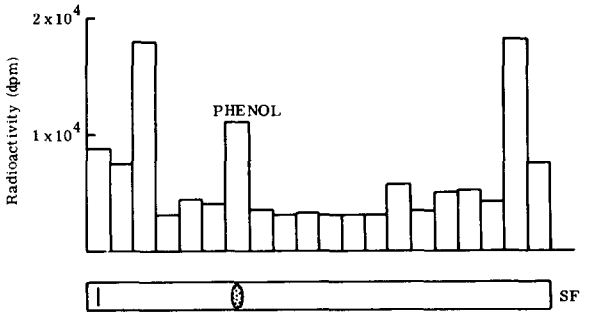

Fig. 3. Acid-catalysed rearrangement of radioactive epoxide from a ^{3}H-dibenz[a,h]anthracene incubation. Fractions containing the carrier epoxide peak from an alumina column were pooled and evaporated and the residue redissolved in methanol. The solution was acidified and the rearrangement products examined as previously described [11] using thin-layer chromatography on silica gel G developed with benzene:ethanol, 19:1.

similar to that of authentic 5-hydroxydibenz[a,h]anthracene [1].

When incubated with microsomal epoxide hydrase [12], radioactive epoxide, formed metabolically from ^{3}H-phenanthrene, was converted into products that included one with the thin layer chromatographic characteristics of *trans*-9,10-dihydro-9,10-dihydroxyphenanthrene (fig. 4) [13]. In addition, when radioactive epoxide formed metabolically from ^{3}H-benz[a]anthracene, was refluxed with glutathione at alkaline pH, a radioactive product was formed that possessed the paper chromatographic properties of the acid-labile glutathione conjugate formed from authentic benz[a]-anthracene 5,6-oxide [11] (fig. 5).

4. Discussion

These investigations demonstrate that epoxides are formed when the polycyclic hydrocarbons phenanthrene, benz[a]anthracene or dibenz[a,h]anthracene are metabolished *in vitro* by the NADPH-dependent

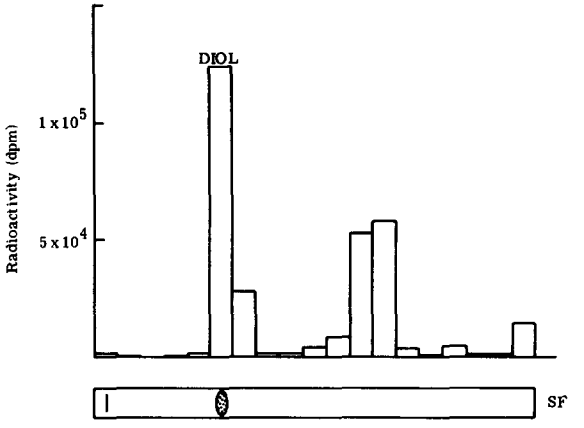

Fig. 4. Enzyme-catalysed conversion of radioactive phenanthrene epoxide to the corresponding dihydrodiol. Fractions containing the carrier epoxide peak, obtained by column chromatography of an ether extract from a ^{3}H-phenanthrene incubation, were pooled and evaporated to dryness. The residue was redissolved in acetone (0.2 ml) and incubated for 1 hr at 37° with rat liver microsomes (≡ 10 g liver) resuspended in Tris buffer (pH 7.4, 0.1 M, 50 ml). The ether-soluble products were examined as previously described [13], using thin layer chromatography on silica gel G developed with benzene: ethanol, 19:1.

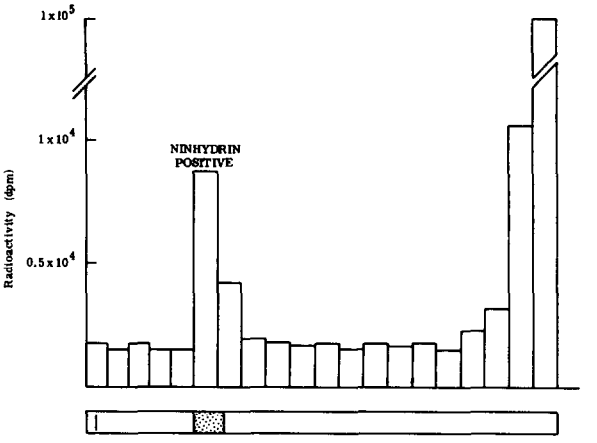

Fig. 5. Formation of acid-labile glutathione conjugate of radioactive benz[a]anthracene epoxide. Fractions containing the carrier epoxide peak, obtained by column chromatography of an ether-extract from a ^{3}H-benz[a]anthracene incubation were pooled and evaporated to dryness. The residue was redissolved in acetone (5 ml) and refluxed for 18 hr with an aqueous solution (5 ml) containing glutathione (2.5 mg) and sodium bicarbonate (5 mg). The products were examined, as previously described [11], using paper chromatography on Whatman 3 MM developed with butan-1-ol:propan-1-ol:aq. 2 M NH_3 (2:1:1 by vol.).

microsomal mixed function oxidase of rat liver and that these radioactive epoxides can be converted, under appropriate conditions, into the corresponding dihydrodiol, phenol or glutathione derivatives (figs. 3–5).

The specific activities of the radioactive epoxides isolated from microsomal incubations of phenanthrene or benz[a]anthracene did not fall on repeated recrystallisation with the respective carrier K-region epoxides. This indicates that with these two hydrocarbons, the epoxides formed are most probably the K-region derivatives. The site of oxidation of dibenz[a,h]anthracene in these experiments has not been determined to date; the formation of uncharacterised epoxide derivatives of this hydrocarbon in microsomal incubation mixtures has also been described by Selkirk et al. [14].

The evidence that has accumulated from work on benzene [12], on naphthalene [15] and on the three polycyclic hydrocarbons examined here, clearly shows that the initial step in the microsomal oxidation of aromatic double bonds is the formation of an epoxide. This conclusion supports the working hypothesis that polycyclic hydrocarbon carcinogenesis results from somatic mutations produced by epoxide intermediates [4–10]. However, it does not explain why, in a range of hydrocarbons metabolised by similar pathways, some are potent carcinogens whilst others are not; this problem is currently under investigation.

Acknowledgements

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