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# Different pathways involved in the metabolism of the 7,8- and 9,10-dihydrodiols of benzo(a)pyrene

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The conversion of aromatic hydrocarbons into dihydrodiols is an established route of metabolism. Recently it has become evident that some of the dihydrodiols are themselves further metabolized by the oxidation of the adjacent olefinic bond, as in the conversion of 8,9-dihydro-8,9-dihydroxybenz(a)anthracene into 8,9-dihydro-8,9-dihydroxybenz(a)anthracene 10,11-oxide [1], which may be the metabolite that reacts with the DNA of hamster embryo cells that are grown in culture in the presence of benz(a)anthracene [2].

Benzo(a)pyrene is converted into the 4,5-, 7,8- and 9,10-dihydrodiols [3, 4] but only the 7,8-isomer is further metabolized by hamster liver microsomal fraction to an active intermediate that reacts with DNA [5].

Furthermore, when the DNA from hamster embryo cells that had been treated with $^3$H-labelled benzo(a)pyrene was hydrolysed to nucleosides and the nucleosides separated by column chromatography, only the 7,8-dihydrodiol formed a derivative that was coincident with a nucleoside derived from the hydrocarbon [6]. This paper reports that the major route of microsomal metabolism of benzo(a)-pyrene 7,8-dihydrodiol is different from that of the 9,10-isomer.

$^3$H-labelled 7,8- and 9,10-dihydrodiols of benzo(a)pyrene (sp. act. 352 mCi/m-mole) were prepared enzymically [7] and microsomal and soluble fractions were prepared [1] from the livers of male rats that had been pretreated with 3-methylcholanthrene [7]. Incubation mixtures (30 ml), in 0·1 M pyrophosphate buffer (pH 8·0) contained microsomal fraction (≡ 5 g liver), $^3$H-labelled 7,8- or 9,10-dihydrodiol (150 μg added in 0·6 ml acetone) and an NADPH generating system that consisted of $NADP^+$ (9 mg), glucose 6-phosphate (45 mg) and glucose 6-phosphate dehydrogenase (36 units). After incubation for 1 hr, the mixtures were extracted twice with ethyl acetate (40 ml). The extracts were dried with $Na_2SO_4$, concentrated and part of the solutions chromatographed on thin layers of Silica gel G developed with chloroform:methanol (4:1, v/v). The chromatograms were divided into 23 fractions and the radioactivity associated with each fraction determined by liquid scintillation counting. The profiles of radioactivity are shown in Fig. 1.

Compounds A in Fig. 1(a) and 1(b) were unreacted substrates since they had u.v. absorption spectra and chromatographic properties that were identical with those of the 7,8-dihydrodiol and the 9·10-dihydrodiol respectively.

Compound B (Fig. 1(a)), which is the major metabolite of the 7,8-dihydrodiol, is more polar ($R_f$ 0·37) than the parent compound ($R_f$ 0·72) and is not formed in the absence of NADPH or $O_2$. The metabolite was probably the 7,8,9,10-tetrahydrotetrol of benzo(a)pyrene since the u.v. absorption spectrum was very similar to that of 7,8,9,10-tetrahydrobenzo(a)pyrene (Fig. 2(a) and (b)). This was supported by measurement of the mass spectrum which showed $M^+$, 320 ($C_{20}H_{16}O_4$ requires M, 320). Metabolism of a bond of the 7,8-dihydrodiol, other than the adjacent 9,10-bond, would not give products showing a 'pyrene type' spectrum. The results therefore suggest that the dihydrodiol is converted by the microsomal mono-oxygenases into a diol–expoxide by metabolism of the olefinic

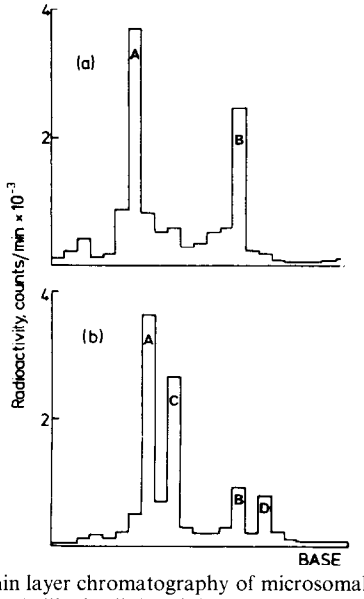


Fig. 1. Thin layer chromatography of microsomal metabolites of (a) 7,8-dihydrodiol and (b) 9,10-dihydrodiol. Details of thin-layer chromatography are described in the text.

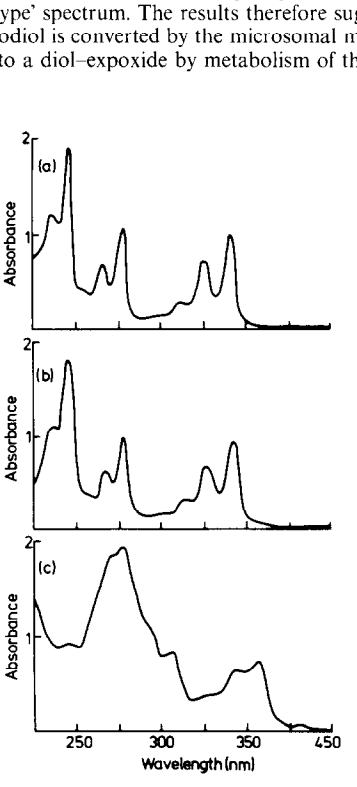


Fig. 2. Ultra-violet absorption spectra measured in ethanol of (a) 7,8,9,10-tetrahydrotetrol derived from either the 7,8- or 9,10-dihydrodiol of benzo(a)pyrene; (b) 7,8,9,10-tetrahydrobenzo(a)pyrene; (c) metabolite C (Fig. 1(b)) derived from the 9,10-dihydrodiol.

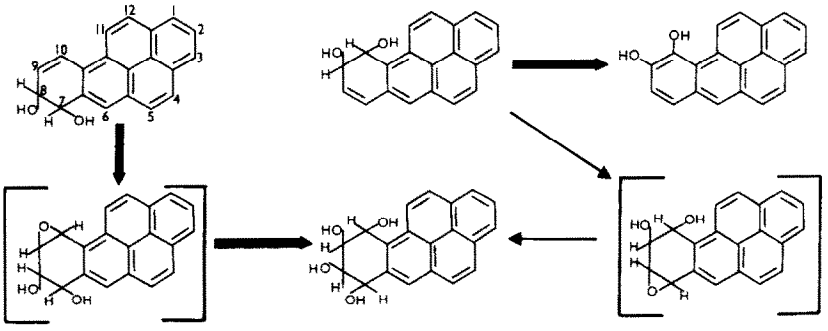

Fig. 3. Microsomal metabolism of benzo(a)pyrene 7,8- and 9,10-dihydrodiols. Thick arrows represent major and thin arrows minor routes. Compounds in brackets were not isolated.

9,10-bond and this epoxide is then hydrated to the corresponding tetrahydrotetrol by a microsomal 'epoxide hydrase' [8]. Furthermore, a chromatographically identical minor metabolite of the 9,10-dihydrodiol (B in Fig. 1(b)) had a u.v. absorption spectrum that was identical with that of the major metabolite of the 7,8-dihydrodiol. The same tetrahydrotetrol would only be obtained from both dihydrodiols if oxidation of the isolated adjacent bond of each isomer had occurred.

Compound C (Fig. 1(b)), the major metabolite ($R_f$ 0·60) of the 9,10-dihydrodiol ($R_f$ 0·69), is probably 9,10-dihydroxybenzo(a)pyrene since the mass spectrum shows a molecular ion $M^+$, 284 ($C_{20}H_{12}O_2$ requires 284) and the u.v. absorption spectrum (Fig. 2(c)) is that expected of a phenolic derivative. Furthermore, a 'diol-dehydrogenase' in mammalian liver has been reported [9] and dihydrodiols of aromatic hydrocarbons are converted into the corresponding *o*-dihydroxy derivatives by the soluble fraction of rabbit liver and $NADP^+$ [10]. When the 9,10-dihydrodiol of benzo(a)pyrene was incubated under these conditions, but using rat liver soluble fraction, the product was identical in its chromatographic and u.v. absorption properties to the microsomal metabolite described above, whereas the 7,8-dihydrodiol was not metabolized under similar conditions.

Compound C (Fig. 1(b)) is a minor metabolite of the 9,10-dihydrodiol of unknown structure that is more polar ($R_f$ 0·29) than any of the other metabolites and may arise through the further metabolism of 9,10-dihydroxybenzo(a)pyrene.

To study the formation of GSH conjugates, similar reaction mixtures but with the addition of soluble liver fraction (3 ml) and GSH (9 mg) were used. The amounts of GSH conjugates were determined by measuring the amount of radioactivity remaining in the aqueous phase after extraction of the mixtures with ethyl acetate [11]. Samples of the conjugates for measurements of u.v. absorption spectra were purified by chromatography of Sephadex G25 columns eluted with water [12]. The results, which showed that the presence of the microsomal fraction caused an 18-fold increase in the amount of radioactivity that remained in the aqueous phase in each case, demonstrated the formation of GSH conjugates [11]. Since epoxides are the only hydrocarbon metabolites known to be substrates for 'glutathione *S*-epoxide transferase' present in the soluble fraction, the synthesis of these conjugates probably takes place through the oxidation of the dihydrodiol to a diol–epoxide by the microsomal fraction and NADPH followed by conjugation of the diol–epoxide with GSH by the enzyme in the soluble fraction. The u.v. absorption spectra of both conjugates were similar to that of 7,8,9,10-tetrahydrobenzo(a)pyrene, confirming that microsomal oxidation occurs on the 9,10-bond of the 7,8-dihydrodiol and on the 7,8-bond of the 9,10-dihydrodiol. The principal absorption maxima were at 249, 268, 281, 332 and 348 nm and at 249, 268, 281, 328 and 344 nm for the conjugates derived from the 7,8- and 9,10-dihydrodiols respectively.

Although the structure of the major metabolite of the 9,10-dihydrodiol has not been conclusively established, it is clear that the two dihydrodiols studied are metabolized by different major pathways (Fig. 3). This difference may be related to the finding that only the 7,8-dihydrodiol is converted by cells into an intermediate that reacts with DNA [5, 6]; this isomer is apparently largely metabolized through a diol–epoxide intermediate whereas the main route of metabolism of the 9,10-isomer probably involves dehydrogenation.

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