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MICROSOMAL MIXED-FUNCTION OXIDASES AND EPOXIDE HYDRATASE CONVERT BENZO[a]PYRENE STEREOSPECIFICALLY TO OPTICALLY ACTIVE DIHYDROXYDIHYDROBENZO[a]PYRENES

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The microsomal mixed-function oxidases and epoxide hydratase catalyze the metabolism of a large variety of xenobiotics including drugs, pesticides and carcinogens. Any stereospecific action of these enzyme systems may be significant to the specificity of drug action as well as to carcinogen activation and detoxification. Earlier studies showed that mixtures of the (+) and the (-) enantiomers of dihydrodiols have been found in the urine of rats and rabbits fed with anthracene, phenanthrene and naphthalene (see reviews in Ref. 1). In the metabolism of naphthalene by rat liver microsomes of mice, rats, rabbits and guinea pigs, (-)trans-1,2-dihydroxy-1,2-dihydronaphthalene was formed in large excess of the (+) enantiomer (2), although another report (3) suggested that (+) enantiomer was formed with mouse liver microsomes.

Benzo[a]pyrene (BP) is a common polycyclic hydrocarbon carcinogen present as a pollutant in the environment and may be a cause of human cancer (4). BP is converted to trans-diols by the action of mixed-function oxidases which form epoxide intermediates (1,5,6) which are then converted to dihydrodiols by the action of epoxide hydratase (7-9). We recently demonstrated (10,11) that BP is metabolized via r-7,t-8-dihydroxy-7,8-dihydrobenzo[a]pyrene (trans-7,8-diol) predominantly to r-7,t-8-dihydroxy-t-9,10-oxy-7,8,9,10-tetrahydrobenzo[a]-pyrene (diol-epoxide I).* In mammalian cells, the latter compound is extraordinarily more mutagenic (10) than fifteen other BP metabolites tested including the K-region epoxide (6,7), three dihydrodiols (12,13) and four phenols (14). These studies and the metabolic formation of diol-epoxide I suggest that the latter may be the ultimate carcinogenic form of benzo[a]-pyrene. Analysis of the hydrolysis products of: (1) two synthetic isomeric diol-epoxides, (2) the diol epoxide formed from the metabolically made trans-7,8-diol and (3) those formed

*The nomenclature of compounds in this report is designated according to: Nomenclature of Organic Compounds (Eds. J. H. Fletcher, O. C. Dermer and R. B. Fox), No. 126, pp. 103-119. American Chemical Society, Washington, D.C. (1974).

from synthetic racemic trans-7,8-diol indicates that the trans-7,8-diol is converted predominantly to one enantiomeric form of diol-epoxide I (11). These results indicated that the metabolically formed trans-7,8-diol would be optically active.

In this report, we have isolated and measured directly the optical activity of three metabolically formed BP trans-dihydrodiols: trans-7,8-diol, the precursor of the highly active mutagenic diol-epoxide I, r-9,t-10-dihydroxy-9,10-dihydrobenzo[a]pyrene (trans-9,10-diol) and the K-region dihydrodiol, r-4,t-5-dihydroxy-4,5-dihydrobenzo[a]pyrene (trans-4,5-diol). Each of the three metabolically formed trans-diols exhibits optical activity, and their respective specific rotations are shown in Table 1.

Table 1. Specific rotation of three enzymatically formed trans-dihydroxydihydrobenzo[a]-pyrenes*

Metabolite	$[\alpha]^{25°}$ (c, 0.025, methanol) deg.	
	λ = 400 nm	λ = 500 nm
trans-4,5-diol	N.M.	-145 ± 70
trans-7,8-diol	-3284 ± 70	-767 ± 60
trans-9,10-diol	-1290 ± 60	-575 ± 60

*Optical rotations were measured on a Perkin-Elmer model 241-MC polarimeter with a cell of 0.2 dm light pathlength. The unit of c is g/100 ml. N.M. indicates not measurable. The trans-diols were prepared enzymatically from benzo[a]pyrene and isolated by high-pressure liquid chromatography (S. K. Yang, D. W. McCourt and H. V. Gelboin, manuscript in preparation). Synthetic racemic trans-diols measured under identical conditions were optically inactive.

The data in Table 2 suggest that the metabolically formed trans-7,8-diol is an optically pure enantiomer. The racemic trans-7,8-diols are metabolically converted to two diol-epoxides which yield upon hydrolysis four stereoisomeric tetrols. These have been fully characterized by mass and ultraviolet absorption spectra and by chromatographic mobilities. The metabolically formed trans-7,8-diol, however, yields predominantly diol-epoxide I upon further metabolism by the mixed-function oxidases which upon hydrolysis yields two stereoisomeric tetrols and one triol. The two additional tetrols and one triol observed in the metabolism of the racemic trans-7,8-diol are present in small quantities. Thus, the metabolically formed trans-7,8-diol is essentially an optically pure enantiomer with the specific rotation indicated in Table 1. The optical purity of the metabolically formed (-)trans-7,8-diol is further indicated by comparison with the high pressure liquid chromatographically resolved and optically pure (-)trans-7,8-diol which has a specific rotation of $[\alpha]^{25°}_{400}$ -3730 ± 160 deg. (c, 0.019, methanol) (S. K. Yang, H. V. Gelboin, J. D. Weber, D. L. Fischer, V. Sankaran and J. F. Engel, manuscript in preparation). The optical purity of the trans-4,5-

diol and the trans-9,10-diol has not been established. Their formation may be stereospecifically similar to the formation of trans-7,8-diol or they may be formed stereoselectively by the action of mixed-function oxidases and epoxide hydratase.

Table 2. The properties of tetrols and triols derived metabolically from trans-7,8-diol and from synthetic diol-epoxides

Metabolite*	t_r on HPLC, min+	Molecular ion (M+), m/e‡	Amount of metabolite (nmoles)§ From metab. formed (-)trans-7,8-diol‖	From racemic trans-7,8-diol¶
(7,10/8,9)-tetrol (I)	26.7	320	1.51	1.25
(7,9/8,10)-tetrol (II)	27.8	320	0.03-0.15	0.14
(7/8,9,10)-tetrol (I)	30.6	320	0.38	0.32
(7/8,9)-triol (I)	34.0	304	0.23	0.28
(7,9,10/8)-tetrol (II)	36.1	320	0.03-0.05	0.33
(7,9/8)-triol (II)	44.8	304	0.01-0.04	0.30

*Tetrol and triol are abbreviations for tetrahydroxytetrahydrobenzo[a]pyrene and trihydroxypentahydrobenzo[a]pyrene respectively. Ultraviolet absorption spectra indicate that all tetrols and triols contain saturated carbons at the 7, 8, 9 and 10 positions of benzo[a]pyrene. The numbers in parentheses preceding tetrol or triol indicate the relative stereoconfigurations of the hydroxyl groups designated according to Nomenclature of Organic Compounds (See footnote on first page of this communication). The roman numeral in parentheses following tetrol and triol indicates that it is identical to the products derived from the synthetic diol-epoxide I or II in its chromatographic mobilities on HPLC, ultraviolet absorption and mass spectra. The detailed structural characterization of the tetrols (hydrolysis products) and triol (reduction products by NADPH of diol-epoxides I and II) will be published elsewhere (S. K. Yang, P. P. Roller and H. V. Gelboin, manuscript in preparation). Other metabolites structurally unrelated to diol-epoxides are also formed and have not been identified (11). The synthesis and stereochemical characterization of two isometric diol-epoxides have been reported (15-17).

+The metabolites of trans-7,8-diol were analyzed on a Spectra-Physics model 3500 high-pressure liquid chromatograph fitted with a 6.2 mm I.D. x 250 mm Dupont "Zorbax" octadecyltrimethoxysilane (ODS) column (10,11). The column was eluted with a linear gradient of 75% methanol in water to 100% methanol for 100 min at ambient temperature. The solvent flow rate was 0.5 ml/min. The absorption of the eluent was monitored at 248 nm and fractions were collected and the radioactivity was determined by liquid scintillation counting. The retention times were the elution time of the chromatographic peaks.

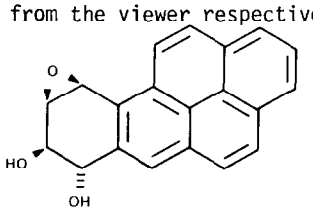
‡Mass spectra were performed on a JEOL model JMS-01SG-2 instrument at 70 eV with a solid probe.

§Amount of metabolites (nmoles/ml of incubation mixture) was determined by HPLC analysis. Either the metabolically formed or the synthetic racemic (^{3}H)trans-7,8-diol (420 nmoles) was added to a 10-ml incubation mixture containing liver microsomes equivalent to 2.4 mg protein from 3-methylcholanthrene-treated male Sprague-Dawley rats, 0.03 m-mole $MgCl_2$ and 0.5 m-mole Tris-HCl buffer at pH 7.5. The mixture was incubated at 37° for 10 min with gentle shaking. The metabolites were extracted and prepared for HPLC analysis as described (11,18).

‖Metabolically formed (^{3}H)trans-7,8-diol (sp. act. 377 mCi/m-mole) was prepared from (^{3}H)BP as described (11) (S. K. Yang, D. W. McCourt and H. V. Gelboin, manuscript in preparation).

¶Synthetic racemic (^{3}H)trans-7,8-diol (sp. act. 157 mCi/m-mole) was prepared under National Cancer Institute Contract NO1-CP-33387 by McCaustland et al. (19).

The sign of optical rotation had been related to the absolute stereochemistry for the r-1,t-2-dihydroxy-1,2-dihydronaphthalene (trans-1,2-dihydroxy-1,2-dihydronaphthalene) and r-1,t-2-dihydroxy-1,2-dihydroanthracene (trans-1,2-dihydroxy-1,2-dihydroanthracene) and the (-) enantiomer was deduced by its ORD and CD properties to have 1R,2R configuration (2,20,21). The ORD curves of the three enzymatically formed BP trans-diols are shown in Fig. 1. As indicated in Table 2, that the (-)trans-7,8-diol obtained enzymatically is an optically pure enantiomer, the stereochemistry of this (-)trans-7,8-diol may have the 7R,8R configuration by analogy to the deduced absolute configuration of (-)trans-1,2-dihydroxy-1,2-dihydronaphthalene and (-)trans-1,2-dihydroxy-1,2-dihydroanthracene (2,20,21). However the definitive proof for the absolute configuration should be determined by a direct method such as X-ray crystallography. If the enzymatically formed (-)trans-7,8-diol indeed has 7R,8R configuration, the structure of the predominant diol-epoxide formed metabolically would be as shown below in which the triangle and dotted line indicate that the substituent is toward and away from the viewer respectively.



Diol-epoxide I

The demonstration of stereospecificity in the formation of optically active metabolites by the mixed-function oxidases and epoxide hydratase may be of importance to understanding carcinogen as well as drug action since key receptors may be specific for only one form or enantiomer of a compound. These results also suggest the possibility that only one of the two enantiomers of drugs, carcinogens and other xenobiotics that contain asymmetric carbon atoms is formed and active biologically.

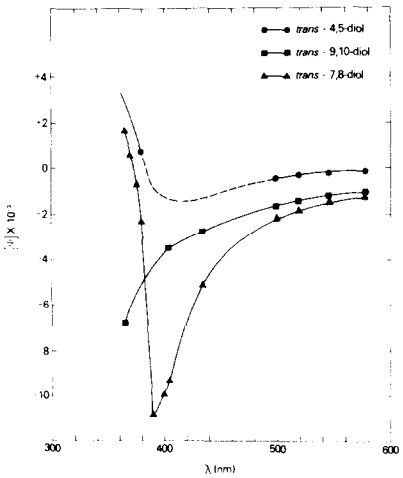


Fig. 1. ORD curves of three enzymatically formed benzo[a]pyrene trans-diols. The samples were obtained as indicated in the legend of Table 1. The molecular rotation [ϕ] is calculated by multiplying the specific rotation [α] by 2.86. The dotted line of trans-4,5-diol is the wavelength region where the optical rotations were not measurable due to opaqueness of the solution.

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