8,9-DIHYDRO-8,9-DIHYDROXYBENZ(A)ANTHRACENE 10,11-OXIDE: A NEW TYPE OF POLYCYCLIC AROMATIC HYDROCARBON METABOLITE

J. BOOTH and P. SIMS

The Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, Fulham Road, London SW3 6JB, UK

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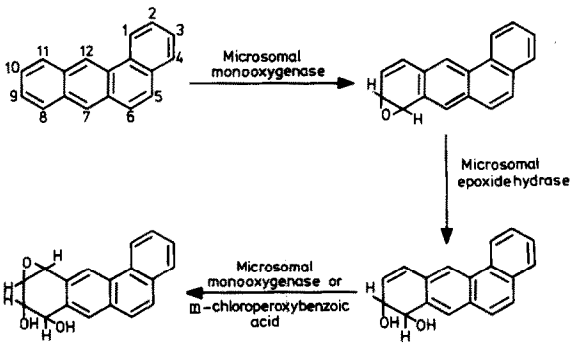
1. Introduction

One of the major routes of metabolism of aromatic hydrocarbons involves the formation of dihydrodiols [1]. These metabolites arise by the action of microsomal monooxygenases on the aromatic double bonds which forms epoxide intermediates [2,3] that are subsequently converted into dihydrodiols by microsomal 'epoxide hydrase' [4] or into glutathione conjugates by the soluble 'glutathione *S*-epoxide transferase' [5]. The dihydrodiols are themselves further metabolized by rat-liver preparations to intermediates that yield glutathione conjugates in the presence of the 'transferase [6]. This paper reports the synthesis of one such intermediate, 8,9-dihydro-8,9-dihydroxybenz(a) anthracene 10,11-oxide, and also presents evidence that this compound is formed from the benz(a)anthracene metabolite, 8,9-dihydro-8,9-dihydroxybenz(a)anthracene, by the microsomal fraction of rat-liver. The routes involved are summarized in scheme 1.



Scheme 1. Routes leading to the formation of 8,9-dihydro-8,9-dihydroxybenz(a)anthracene 10,11-oxide.

2. Materials and methods

2.1. *Materials*

^{3}H-Labelled 8,9-dihydro-8,9-dihydroxybenz(a) anthracene (sp. act. 150 mCi/mmole) and the unlabelled dihydrodiol were prepared enzymically from ^{3}H-labelled benz(a)anthracene and unlabelled benz(a) anthracene respectively [1].

In the preparation of 8,9-dihydro-8,9-dihydroxybenz (a)anthracene 10,11-oxide, the 8,9-dihydrodiol (4 mg) and *m*-chloroperoxybenzoic acid (6 mg), in chloroform (3 ml), were kept at 0°C for 48 hr. The solution was washed once with 2 M NaOH (5 ml), three times with water (5 ml) and dried (Na_2SO_4) and the solvent removed under reduced pressure. The residue was chromatographed in system (b) (table 1), the band containing the oxide removed and the product eluted with ethanol. The U.V. spectrum of the oxide, shown in fig. 1 (curve C), is similar to that of phenanthrene (fig. 1, curve D) and supports the proposed structure. The mass spectrum showed M^+, 278 ($C_{18}H_{14}O_3$ requires M, 278). The ^{3}H-labelled oxide was similarly prepared from the ^{3}H-labelled 8,9-dihydrodiol in 44% yield, as determined by radioactivity measurements.

2.2. *Liver preparations*

Microsomal and soluble cell fractions were prepared [7] from the livers of male Chester Beatty strain rats that had been pre-treated with 3-methylcholanthrene [1].

2.3. *Incubation of ^{3}H-labelled 8,9-dihydro-8,9-dihydroxybenz(a)anthracene with microsomal fractions*

The reaction mixture (60 ml) contained microsomal

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Table 1
Thin-layer chromatography of 8,9-dihydro-8,9-dihydroxybenz(a)anthracene 10,11-oxide and related compounds

Compound	R_F × 100 in chromatographic system		
	(a)	(b)	(c)
8,9-Dihydro-8,9-dihydroxybenz(a)anthracene	44	62	67
8,9-Dihydro-8,9-dihydroxybenz(a)anthracene 10,11-oxide (synthetic)	37	53	58
8,9-Dihydro-8,9-dihydroxybenz(a)anthracene 10,11-oxide (microsomal metabolite)	37	54	57
Metabolite, probably 8,9,10,11-tetrahydro-8,9,10,11-tetrahydroxybenz(a)anthracene	14	10	12

Chromatographic system (a), 0.25 mm layers of silica gel G developed with benzene:ethanol (87:13 v/v); (b), Eastman 6060 Chromogram sheets developed with cyclohexane:dioxan (1:1 v/v); (c) Eastman 6060 Chromogram sheets developed with ethyl acetate:n-hexane:acetic acid (15:4:1 by vol).

fraction (≡ 10 g liver), NADP (18 mg), glucose 6-phosphate (90 mg), glucose 6-phosphate dehydrogenase (42 units), cyclohexene oxide (60 μmoles in 0.6 ml ethanol) and ^{3}H-labelled 8,9-dihydro-8,9-dihydroxybenz(a)anthracene (3 μmoles in 1.2 ml ethanol) in 0.1 M pyrophosphate buffer (pH 8.0). The mixture was shaken at 37°C for 1 hr and extracted with diethyl ether (100 ml). The dried (Na_2SO_4) extract was concentrated and examined by t.l.c. [system (b), table 1]. The major metabolite, which was visible as a dark absorbing area in U.V. light and was radioactive, was chromatographically identical with 8,9-dihydro-8,9-dihydroxybenz(a)anthracene 10,11-oxide. The metabolite, eluted from the appropriate areas of chromatograms with ethanol, was incubated with 0.1 M phosphate buffer, pH 7.4 (9 ml), soluble fraction of rat-liver (1 ml) and GSH (3 mg) at 37°C for 1 hr. The mixture was extracted with ethyl acetate (15 ml) and the GSH conjugate in the aqueous phase examined by column chromatography [8].

3. Results and discussion

Evidence that 8,9-dihydro-8,9-dihydroxybenz(a)anthracene 10,11-oxide is a microsomal metabolite of the 8,9-dihydrodiol is presented in table 1. When ether extracts, obtained from incubations of rat-liver microsomal fraction with the tritiated 8,9-dihydrodiol were examined by t.l.c., two radioactive products were

Table 2
Factors affecting the metabolism of 8,9-dihydro-8,9-dihydroxybenz(a)anthracene by the microsomal fraction of rat-liver

Modification to complete reaction mixture	Radioactivity (cpm × 10^{-3}) associated with metabolites	
	10,11-oxide	8,9,10,11-Tetrahydrotetrol
Microsomal fraction heated* before incubation	0.5	0
NADPH omitted	0.2	0
Cyclohexene oxide omitted	18.6	14.3
None	18.0	4.5

* Heated at 100° for 5 min.
Incubation conditions and the composition of the complete reaction mixture are described in the text. The mixtures (5 ml) were extracted with ether (10 ml) and the dried extract evaporated to dryness. The residues were dissolved in ethanol (60 μl) and portions (10 μl) of the solutions applied to Chromogram sheets (system (b), table 1). Appropriate areas of the chromatogram were cut out and the radioactivity present determined by liquid scintillation counting.

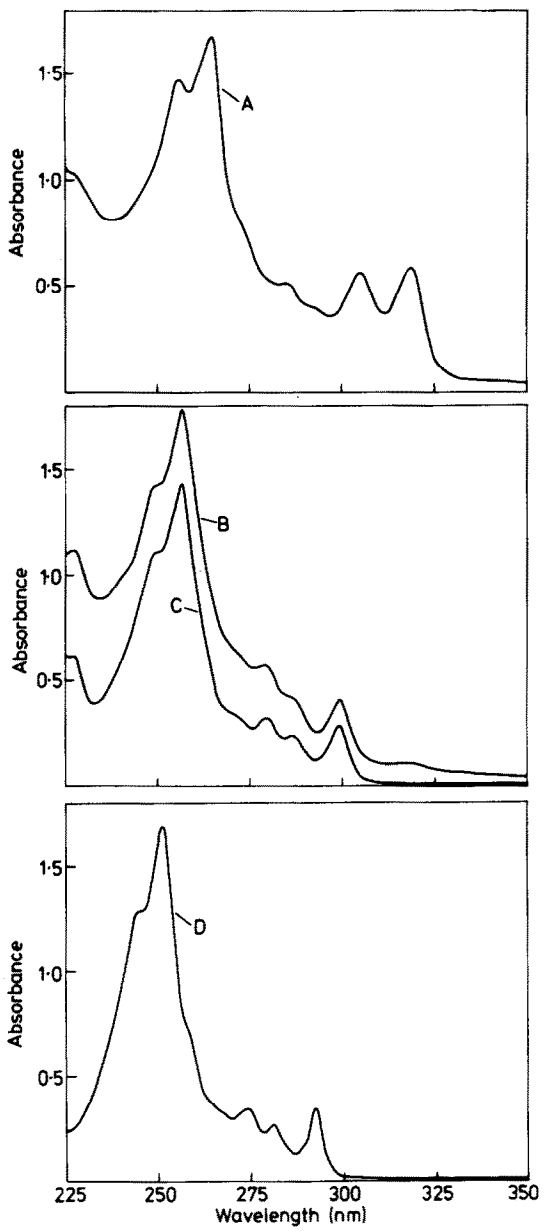

Fig. 1. Ultra-violet absorption spectra measured in ethanol, of (A) 8,9-dihydro-8,9-dihydroxybenz(a)anthracene; (B) 8,9-dihydro-8,9-dihydroxybenz(a)anthracene 10,11-oxide obtained from microsomal oxidations; (C) 8,9-dihydro-8,9-dihydroxybenz(a)anthracene 10,11-oxide obtained by synthesis; (D) phenanthrene. The spectra of A, B and C were measured on samples purified by t.l.c. as described in the text.

located. The major, faster moving product exhibited the chromatographic properties of the synthetically-prepared 10,11-oxide in three chromatographic systems. The more polar product is probably the 8,9, 10,11-tetrahydrotetrol derivative formed by the further metabolism of the 10,11-oxide, since epoxides are hydrated to dihydrodihydroxy derivatives by microsomal 'epoxide hydrase' [4]. This is supported by the finding that more of the polar metabolite is present in incubation mixtures that do not contain the 'epoxide hydrase' inhibitor, cyclohexene oxide [4] (table 2). Table 2 also demonstrates that microsomal oxidation of the isolated 10,11-bond of the 8,9-dihydrodiol is an enzymic reaction that requires NADPH.

Confirmation of the structure of the microsomal metabolite of the 8,9-dihydrodiol is provided by curves B and C in fig. 1, which shows that the metabolite and the synthetically prepared 8,9-dihydro-8,9-dihydroxybenz(a)anthracene 10,11-oxide exhibit similar U.V. absorption spectra that differ from that of the parent 8,9-dihydrodiol (curve A).

Furthermore, the microsomal metabolite reacted with GSH in the presence of the soluble cell fraction to form a GSH conjugate. The elution profile obtained with this conjugate, using a column chromatography system designed to separate GSH conjugates [8], was identical to that obtained with a similar conjugate prepared enzymically from synthetic 8,9-dihydro-8,9-dihydroxybenz(a)anthracene 10,11-oxide and glutathione (G. R. Keysell, J. Booth and P. Sims, unpublished observations) and the U.V. absorption spectra of the two conjugates were identical.

The results show, therefore, that 8,9-dihydro-8,9-dihydroxybenz(a)anthracene, which is a metabolite of benz(a)anthracene in rats, rabbits and mice [9], in rat liver and lung preparations [10], and in hamster embryo cells [11] and human lymphocytes [12], is itself further metabolized to an epoxide. The reactions of this diol-epoxide with DNA are described in the following paper [13].

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