Epoxy Derivatives of Aromatic Polycyclic Hydrocarbons

THE SYNTHESIS OF DIBENZ[*a,c*]ANTHRACENE 10,11-OXIDE AND ITS METABOLISM BY RAT LIVER PREPARATIONS

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(Received 4 May 1972)

The synthesis of dibenz[*a,c*]anthracene 10,11-oxide is described. The oxide was unstable and was rapidly decomposed with cold mineral acid into a mixture of 10- and 11-hydroxydibenz[*a,c*]anthracene. The oxide was converted by rat liver microsomal preparations and homogenates into a product that is probably 10,11-dihydro-10,11-dihydroxydibenz[*a,c*]anthracene and which was identical with the metabolite formed when dibenz[*a,c*]anthracene was metabolized by rat liver homogenates. The oxide did not react either chemically or enzymically with GSH. 10,11-Dihydrodibenz[*a,c*]anthracene and 10,11-dihydrodibenz[*a,c*]anthracene 12,13-oxide were both metabolized by rat liver preparations into *trans*-10,11,12,13-tetrahydro-10,11-dihydroxydibenz[*a,c*]anthracene and the oxide was converted chemically into this dihydroxy compound, and it reacted chemically but not enzymically with GSH. In the alkylation of 4-(*p*-nitrobenzyl)pyridine, the 'K-region' epoxide, dibenz[*a,h*]anthracene 5,6-oxide, was more active than either dibenz[*a,c*]anthracene 10,11-oxide or 10,11-dihydrobenz[*a,c*]anthracene 12,13-oxide.

Of the aromatic polycyclic hydrocarbons commonly used in the study of chemical carcinogenesis, dibenz[*a,c*]anthracene (I) is the only one whose molecule does not possess a 'K-region', that is a bond of high electron density similar in nature to the 9,10-bond of phenanthrene. The hydrocarbon (I) is usually considered to be inactive as a carcinogen (Barry *et al.*, 1935; Heidelberger *et al.*, 1962), although Lijinsky *et al.* (1970) found the compound to be weakly active. Covalent binding of the ³H-labelled hydrocarbon (I) to the nucleic acids and proteins of mouse skin was observed when the animals were painted with solutions of the compound (Brookes & Lawley, 1964; Goshman & Heidelberger, 1967), and to the nucleic acids and proteins of rodent embryo cells that were incubated with the compound (Duncan *et al.*, 1969; Duncan & Brookes, 1972; Kuroki & Heidelberger, 1971).

There is now good evidence that aromatic hydrocarbons are metabolized by microsomal enzymes to arene oxides (Jerina *et al.*, 1970; Selkirk *et al.*, 1971; Grover *et al.*, 1971*b*), and that these are further metabolized to dihydrodiols by 'epoxide hydrases' located in the microsomal fractions (Jerina *et al.*, 1968, 1970; Brooks *et al.*, 1970; Pandov & Sims, 1970; Oesch & Daly, 1971) and to glutathione conjugates by a 'glutathione transferase' located in the soluble fractions of rat liver preparations (Boyland & Williams, 1965). Dibenz[*a,c*]anthracene (I) is itself metabolized by rat liver homogenates to a dihydrodiol

that was tentatively identified as 10,11-dihydro-10,11-dihydroxydibenz[*a,c*]anthracene (X; Scheme 1) (Sims, 1970), so that the intermediate formation of dibenz[*a,c*]anthracene 10,11-oxide (VIII) in the metabolism of the hydrocarbon (I) seems likely. It is also possible that the oxide (VIII) is involved in the binding to macromolecules observed when mouse skin and rodent embryo cells are treated with the hydrocarbon (I), since 'K-region' epoxy derivatives of aromatic hydrocarbons are known to react with nucleic acids and proteins both chemically (Grover & Sims, 1970) and when incubated with cells in culture (Grover *et al.*, 1971*a*; Kuroki *et al.*, 1972). 'K-region' oxides will also induce malignant transformations in rodent cells (Grover *et al.*, 1971*c*; Huberman *et al.*, 1972) and mutational changes in

(I)

mammalian cells (Huberman *et al.*, 1971), bacteriophages (Cookson *et al.*, 1971) and bacteria (Ames *et al.*, 1972). It was therefore decided to prepare dibenz[*a,c*]anthracene 10,11-oxide (VIII) and to examine some of its chemical and biochemical properties. Some properties of the related 10,11-dihydrodibenz[*a,c*]anthracene 12,13-oxide (VII) were examined.

Experimental

Methods

Melting points. These are uncorrected.

Chromatography. T.l.c. was carried out either on glass plates (20cm×20cm) coated with layers of silica gel G (E. Merck A.-G., Darmstadt, Germany) of 0.25mm thickness or on Eastman 6060 Chromagram sheets (silica gel with fluorescent indicator) (Eastman-Kodak Co., Rochester, N.Y., U.S.A.). Chromatograms were developed in either solvent (a), benzene, or solvent (b), benzene-ethanol (9:1, v/v). In the chromatography of the oxides, Chromagram sheets were sometimes developed with solvent (c), cyclohexane-dioxan (9:1, v/v) (Selkirk *et al.*, 1971). The products were detected on the chromatograms by examining the wet plates in u.v. light, both before and after exposure to NH₃. The plates were then sprayed with a solution of Na₂Cr₂O₇ (5%, w/v) in 0.1M-H₂SO₄ and heated in an oven to 110°C for 10min. The properties of the compounds examined by t.l.c. are recorded in Table 1.

Paper chromatography was carried out on Whatman no. 1 chromatography paper. The chromatograms were developed overnight in butan-1-ol-propan-1-ol-aq. 2M-NH₃ (2:1:1, by vol.). The dried chromatograms were examined in u.v. light and then dipped in a solution of 0.2% ninhydrin in acetone and heated in an oven for 5min at 110°C.

Absorption spectra. The u.v.-absorption spectra of isolated compounds were measured in ethanol. The spectra of compounds separated by chromatography were measured after elution of the appropriate spots or bands removed from t.l.c. plates with ethanol or elution of the appropriate bands cut from paper chromatograms with methanol containing 1% (v/v) of aq. NH₃ soln. (sp.gr. 0.88). The spectra were measured on a Unicam SP.800 recording spectrophotometer.

Experiments with rat liver preparations. These were carried out as described previously (Waterfall & Sims, 1972) with microsomal and soluble fractions and homogenates of the livers of normal rats when the oxides (VII) and (VIII) were used as substrates, and with homogenates of the livers of rats that had been treated 48h previously with 3-methylcholanthrene when the hydrocarbons (I) and (IV) were used. Ethyl acetate- and water-soluble fractions were

obtained as described previously (Waterfall & Sims, 1972) and examined to give the results described below. Control experiments with homogenates that had been boiled for 5min were also carried out.

Alkylation of 4-(*p*-nitrobenzyl)pyridine by epoxides. This was done as previously described (Grover & Sims, 1970), with 10,11-dihydrodibenz[*a,c*]anthracene 12,13-oxide (VII) and dibenz[*a,c*]anthracene 10,11-oxide (VIII) as substrates. In a comparative experiment, the alkylation of 4-(*p*-nitrobenzyl)pyridine by the K-region epoxide, dibenz[*a,h*]anthracene 5,6-oxide, was also examined. The results are shown in Fig. 1.

Materials

Triphenylene was obtained from Ralph N. Emanuel Ltd., Wembley, Middx., U.K. Dibenz[*a,h*]anthracene 5,6-oxide was prepared as described by Boyland & Sims (1965).

Preparation of 10,11,12,13-tetrahydro-10-oxodibenz[*a,c*]anthracene (II). This was prepared from triphenylene by the method of Buu-Hoï *et al.* (1959). It formed needles from aq. acetone, m.p. 186°C (Found: C, 89.2; H, 5.2. Calc. for C₂₂H₁₆O: C, 89.2; H, 5.4%), λ_{max.} at 255 (inflexion), 260 (inflexion), 269, 304, 324 and 368nm (log ε 4.55, 4.62, 4.72, 4.03, 3.93 and 2.95 respectively). Buu-Hoï *et al.* (1959) did not record the m.p. of the ketone (II).

Preparation of 10-hydroxy-10,11,12,13-tetrahydrodibenz[*a,c*]anthracene (III). The ketone (II) (3.5g) in benzene-methanol (1:1, v/v; 50ml) was treated with an excess of NaBH₄, added in small portions during 1h. The solvent was removed under reduced pressure and the residue washed with water and recrystallized from benzene to yield the *hydroxy compound* (III) (3.1g) as needles, m.p. 155–156°C (Found: C, 88.6; H, 6.0; C₂₂H₁₈O requires C, 88.6; H, 6.1%), λ_{max.} at 246 (inflexion), 254, 263, 275 (inflexion), 289, 305 (inflexion), 316, 323, 330, 338 and 347nm (log ε 4.35, 4.65, 4.93, 4.32, 4.15, 3.56, 3.28, 3.27, 3.18, 2.88 and 2.76 respectively).

Preparation of 10,11-dihydrodibenz[*a,c*]anthracene (IV). The hydroxy compound (III) (3g) was heated on a steam bath for 15min with acetic acid (50ml) containing a few drops of conc. HCl. The solution was diluted with water and the product dissolved in light petroleum (b.p. 80–100°C) and the solution passed through a bed of alumina (Spence type H). The solvent was evaporated under reduced pressure and the residue recrystallized from ethanol to yield the *hydrocarbon* (IV) (2.4g) as silky needles, m.p. 168–169°C (Found: C, 94.4; H, 5.8; C₂₂H₁₆ requires C, 94.2; H, 5.8%), λ_{max.} at 230 (inflexion), 238, 257 (inflexion), 265, 274.5, 293, 305, 319, 342 and 360nm (log ε 4.08, 4.25, 4.58, 4.81, 4.98, 4.18, 4.18, 3.37 and 2.67 respectively).

Table 1. *Properties of dibenz[*a,c*]anthracene derivatives on t.l.c. plates*

Details are given in the text. —, R_F not measured; d, compound decomposed completely on the chromatograms. The fluorescences recorded are those of the compounds chromatographed on silica gel-coated glass plates; the compounds were detected on Chromagram sheets by the formation in u.v. light of dark or fluorescent spots against an orange fluorescent background. The colours were obtained after chromatography on silica gel-coated glass plates.

Compound	Fluorescence		R_F on silica gel on glass plates		R_F on Chromagram sheets					Colour with the $\text{Na}_2\text{Cr}_2\text{O}_7\text{-H}_2\text{SO}_4$ reagent
	Immediate	After exposure to NH_3	Solvent (a)	Solvent (b)	Solvent (a)	Solvent (b)	Solvent (c)			
Dibenz[<i>a,c</i>]anthracene (I)	Bright violet	Bright violet	0.96	—	—	—	—	—	None	
10,11-Dihydrodibenz[<i>a,c</i>]anthracene (IV)	Violet	Violet	0.96	—	—	—	—	—	Red-brown	
10,11,12,13-Tetrahydro-10-oxodibenz[<i>a,c</i>]anthracene (II)	Blue	Bright blue	0.27	0.90	0.50	—	—	—	None	
10,11,12-13-Tetrahydro-11-oxodibenz[<i>a,c</i>]anthracene (XII)	None	None	0.23	0.83	0.46	—	—	—	Red-brown	
<i>trans</i> -10,11,12,13-Tetrahydro-10,11-dihydroxydibenz[<i>a,c</i>]anthracene (XIII)	None	None	0.00	0.13	0.00	0.39	—	—	Red-brown	
10,11-Dihydrodibenz[<i>a,c</i>]anthracene 12,13-oxide (VII)	None	None	0.52	0.93	0.72	—	—	0.56	Red-brown	
10-Hydroxydibenz[<i>a,c</i>]anthracene (IX)	Violet	Yellow	0.33	0.67	0.13	—	—	0.09	Grey	
11-Hydroxydibenz[<i>a,c</i>]anthracene (XI)	Violet	Green	0.23	0.57	0.06	—	—	0.40	Brown	
Dibenz[<i>a,c</i>]anthracene 10,11-oxide (VIII)	—	—	d	d	d	0.93*	0.40*	—	—	
10,11-Dihydro-10,11-dihydroxydibenz[<i>a,c</i>]anthracene (X)	Dark violet	Dark violet	0.00	0.18	0.00	0.47	—	—	Brown	

* Some decomposition of the oxide (VIII) occurred during the loading and developing of the chromatograms.

Fig. 1. Alkylation of 4-(*p*-nitrobenzyl)pyridine by epoxides

This was measured as the increase in colour formation with time of incubation at 37°C resulting from the reaction of 0.2 μmol of dibenz[*a,h*]anthracene 5,6-oxide (●), 10,11-dihydrodibenz[*a,c*]anthracene 12,13-oxide (VII) (▲) and dibenz[*a,c*]anthracene 10,11-oxide (VIII) (■) with 4-(*p*-nitrobenzyl)pyridine at pH 7.4.

Preparation of 10,11-dibromo-10,11,12,13-tetrahydrodibenz[*a,c*]anthracene (V). The dihydro derivative (IV) (2g) in CCl₄ (20ml) was treated at room temperature with bromine (0.4ml) in CCl₄ (10ml), added during 5 min. The solvent was removed to yield a pale-yellow gum that rapidly crystallized. The product was recrystallized from light petroleum (b.p. 80–100°C) to yield the *dibromo compound* (V) (2.1g) as needles, m.p. 124–126°C (decomp.) (Found: Br, 35.9; C₂₂H₁₆Br₂ requires Br, 36.3%), λ_{max.} at 254, 262, 277 (inflexion), 288.5 and 308nm (log ε 4.51, 4.73, 3.90, 3.80 and 3.00 respectively).

Preparation of 10,11,12,13-tetrahydrodibenz[*a,c*]anthracene 10,11-bromohydrin (VI). The dibromo compound (V) (1.8g) in aq. 50% (v/v) acetone (250ml) was heated under reflux for 30min with an excess of MgCO₃. The mixture was filtered and the filtrate evaporated under reduced pressure to remove acetone. The solid that separated was recrystallized from benzene to yield the *bromohydrin* (VI) (0.95g) as needles, m.p. 191–193°C (decomp.) (Found: Br, 20.9; C₂₂H₁₇BrO requires Br, 21.2%), λ_{max.} at 245 (inflexion), 254, 262.5, 276 (inflexion), 288, 305 (inflexion), 316, 323, 330, 338 and 347nm (log ε 4.38, 4.46, 5.17, 4.24, 3.66, 3.40, 3.12, 3.20, 3.08, 2.75 and 2.64 respectively).

Preparation of 10,11-dihydrodibenz[*a,c*]anthracene 12,13-oxide (10,11-epoxy-10,11,12,13-tetrahydrodibenz[*a,c*]anthracene) (VII). The bromohydrin (VI) (800mg) in tetrahydrofuran (10ml) was kept overnight with 1,5-diazabicyclo[4,3,0]non-5-ene (1ml). The mixture was diluted with water (100ml) and the solution extracted with diethyl ether (50ml). The ethereal solution was washed three times with portions (50ml) of water and dried over Na₂SO₄. The diethyl ether was removed and the residue recrystallized, first from aq. acetone (to remove a minor by-product that was detected by t.l.c. as a blue fluorescent compound in u.v. light, which had R_F 0.46 in solvent *a*), and then from benzene to yield the *oxide* (VII) (255mg) in plates, m.p. 142–145°C (Found: C, 88.7; H, 5.2; C₂₂H₁₆O requires C, 89.2; H, 5.4%), λ_{max.} at 246 (inflexion), 254, 263, 276 (inflexion), 289, 307 (inflexion), 318, 331, 338 and 347nm (log ε 4.40, 4.72, 4.96, 4.13, 4.05, 3.24, 2.24, 2.18, 2.13 and 2.01 respectively).

Preparation of dibenz[*a,c*]anthracene 10,11-oxide (10,11-epoxy-10,11-dihydrodibenz[*a,c*]anthracene) (VIII). The oxide (VII) (160mg) in CCl₄ (10ml) was heated under reflux with *N*-bromosuccinimide (500mg) and αα'-azoisobutyrodinitrile (10mg) for 1h. The mixture slowly became brown and succinimide separated. The mixture was cooled and filtered and the filtrate evaporated under reduced pressure until crystals separated. The product (130mg) formed a light-brown powder, and it is presumed to be 10-bromo-10,11-dihydrodibenz[*a,c*]anthracene 12,13-oxide: its u.v.-absorption spectrum had certain similarities with that of the oxide (VII) in that it showed λ_{max.} at 262nm and inflexions at 254 and 288nm, but the fine structure of the spectrum of the oxide (VII) was absent in that of the bromo-epoxide. The product could not be further characterized because attempts to recrystallize it from any one of a number of organic solvents led to its decomposition.

The crude bromo-epoxide (100mg), in tetrahydrofuran (2ml), was kept overnight with 1,5-diazabicyclo[4,3,0]non-5-ene (0.1ml). The u.v.-absorption spectrum of the material in solution progressively changed from that typical of a 10,11,12,13-tetrahydrodibenz[*a,c*]anthracene to that typical of a 10,11-dihydrodibenz[*a,c*]anthracene (see Fig. 2). The mixture, diluted with ether (50ml), was washed with water (3 × 50ml) and the ethereal solution dried (with Na₂SO₄) and evaporated under reduced pressure. The residue was dissolved at room temperature in the minimum amount of acetone and precipitated by the dropwise addition of water to yield the *oxide* (VIII) (60mg) as a pale-yellow powder, m.p. 75–78°C (decomp.) (Found: C, 89.2; H, 5.0; C₂₂H₁₄O requires C, 89.8; H, 4.8%), λ_{max.} at 246, 260 (inflexion), 268, 278, 299 (inflexion), 308 (inflexion), 324 and 362nm (log ε 4.11, 4.20, 4.29, 4.42, 4.03, 3.82, 3.75 and 2.81 respectively).

Scheme 1. Synthesis and reactions of dibenz[*a,c*]anthracene 10,11-oxide (VIII)

For details see the text.

Attempts to recrystallize the oxide (VIII) from solvents such as benzene, cyclohexane or light petroleum (b.p. 40–60°C), or to chromatograph it on silica gel, alumina or Florisil columns, led to its decomposition.

The epoxide (VIII) was decomposed immediately when treated with conc. HCl and it yielded products with the chromatographic characteristics and u.v.-absorption spectra of 10- and 11-hydroxydibenz[*a,c*]anthracene (IX and XI). A comparison of the absorption spectra of the two products showed that the phenol (IX) was present in much greater amount than the phenol (XI). The solid epoxide (VIII) also yielded the phenols (IX) and (XI) when it was kept at 60°C for 1 h.

*Preparation of 10,11,12,13-tetrahydro-11-oxodibenz[*a,c*]anthracene (XII).* The oxide (VII) (100 mg) in acetic acid (2 ml) was heated on a steam bath with a few drops of conc. HCl for 15 min. The solution was cooled and diluted with water and the product that separated recrystallized three times from aq. acetone. The ketone (XII) (25 mg) was obtained as plates, m.p. 197–199°C (Found: C, 89.2; H, 5.5; C₂₂H₁₆O requires C, 89.2; H, 5.4%), $\lambda_{\max.}$ at 245 (inflexion), 253, 262, 273 (inflexion), 288, 308 (inflexion), 323 and 329 nm (log ϵ 4.23, 4.51, 4.72, 4.13, 3.98, 3.38, 3.11 and 2.96 respectively). Examination by t.l.c. of the mother liquor from the recrystallization showed the presence of a small amount of a compound with the chromatographic properties of the ketone (II).

*Preparation of trans-10,11,12,13-tetrahydro-10,11-dihydroxydibenz[*a,c*]anthracene (XIII).* The oxide (VII) (50 mg), in acetone (10 ml) and water (1 ml), was heated under reflux for 24 h. The acetone was distilled off and the product that separated recrystallized from aq. ethanol to yield the dihydroxy compound (XIII) (25 mg) in plates, m.p. 170–172°C (decomp.) (Found: C, 84.2; H, 5.6; C₂₂H₁₈O₂ requires C, 84.1; H, 5.8%), $\lambda_{\max.}$ at 245 (inflexion), 253, 262, 275 (inflexion), 288, 305 (inflexion), 316, 323, 330, 338 and 347 nm (log ϵ 4.37, 3.68, 4.91, 4.34, 4.17, 3.57, 3.29, 3.29, 3.20, 2.90 and 2.77 respectively). The compound failed to discharge the blue colour of the potassium triacetylosmate reagent of Criegee *et al.* (1942): *trans*-dihydroxy compounds do not react with this reagent. When the dihydroxy compound was heated on a steam bath with conc. HCl for 15 min and the products were examined by t.l.c., a compound with the chromatographic properties and u.v.-absorption spectrum of the ketone (XII) was detected.

*Preparation of 10-hydroxydibenz[*a,c*]anthracene (IX).* The ketone (II) (100 mg) and sulphur (25 mg) were heated together at 220°C for 30 min, and the product was then separated from the reaction mixture by sublimation at 270°C (bath)/15 mm Hg (2000 N/m²). The 10-hydroxydibenz[*a,c*]anthracene (15 mg) was recrystallized from benzene–cyclohexane in pale-yellow needles, m.p. 220–222°C (Found: C, 89.5; H, 5.0; C₂₂H₁₄O requires C, 89.8; H, 4.8%),

$\lambda_{\max.}$ at 244, 251, 262, 272, 283, 295, 336 (inflexion), 360 and 380 nm (log ϵ 3.86, 4.34, 4.22, 4.26, 4.45, 4.56, 3.36, 3.20 and 3.18 respectively).

*Preparation of 11-hydroxydibenz[*a,c*]anthracene (XI).* The ketone (XII) (25 mg) was heated with sulphur (10 mg) and the product (2 mg) isolated as described above. Because of the small amount obtained it was not possible to characterize the product fully, but its chromatographic properties and u.v.-absorption spectrum suggested that it was mainly 11-hydroxydibenz[*a,c*]anthracene (XII), $\lambda_{\max.}$ at 210, 217 (inflexion), 231, 250, 265, 276, 286.5, 320, 332, 348, 362 and 386 nm.

Reactions of the oxides (VII) and (VIII) with GSH

The oxides (VII) and (VIII) (5 mg) were each stirred with a solution of NaHCO₃ (10 mg) and GSH (5 mg) in aq. 50% (v/v) acetone (10 ml) for 24 h. The acetone was distilled off under reduced pressure and the aqueous residues were extracted with ethyl acetate (10 ml). The ethyl acetate-soluble material was examined by t.l.c. and the aqueous layers, after evaporation to dryness and dissolving in methanol, were examined on paper chromatograms.

With the oxide (VII), the ethyl acetate-soluble fractions contained products with the chromatographic properties and u.v.-absorption spectra of the ketone (XII) and the dihydroxy compound (XIII). The aqueous layer contained a product of R_F 0.02 that gave a purple colour with ninhydrin. The u.v.-absorption spectrum was similar to that of the epoxide (VII) and showed $\lambda_{\max.}$ at 208, 248 (inflexion), 256, 264, 276, 290, 307, 319, 332, 338 and 347 nm. Thus the product is probably *S*-(10,11,12,13-tetrahydro-11-hydroxydibenz[*a,c*]anthracen-10-yl)glutathione.

The ethyl acetate-soluble fraction obtained from the reaction of the oxide (VIII) with GSH contained products with the chromatographic properties and u.v.-absorption spectra of 10- and 11-hydroxydibenz[*a,c*]anthracene (IX and XI). No evidence was obtained for the presence of GSH conjugates in the aqueous layer.

Results

*Metabolism of dibenz[*a,c*]anthracene 10,11-oxide (VIII)*

The oxide (VIII) (2 mg) was incubated as indicated above with the microsomal fraction prepared from rat liver (4 g). Examination of the ethyl acetate-soluble fraction by t.l.c. showed the presence of three products, two of which were identified by their chromatographic properties and u.v.-absorption spectra as 10- and 11-hydroxydibenz[*a,c*]anthracene (IX and XI). From a comparison of the absorption curves, it was evident that the 10-hydroxy compound (IX) was the major product.

Fig. 2. U.v.-absorption spectra of (a) 10,11-dihydrodibenz[a,c]anthracene 12,13-oxide (VII), (b) dibenz[a,c]anthracene 10,11-oxide (VIII) and (c) 10,11-dihydro-10,11-dihydroxydibenz[a,c]anthracene (X)

For details see the text.

The third product was a dihydrodiol, the u.v.-absorption spectrum (Fig. 2) of which was almost identical with that of 10,11-dihydrodibenz[a,c]anthracene (IV) and showed $\lambda_{\max}$ at 230 (inflexion), 240, 257 (inflexion), 265, 274.5, 293, 306, 320, 342 and 355 nm. When heated with conc. HCl the metabolite yielded products that were identified by t.l.c. as the phenols (IX and XI). Measurements of their u.v.-absorption spectra showed that the phenol (IX) was the major product. The metabolite therefore appears to be 10,11-dihydro-10,11-dihydroxydibenz[a,c]anthracene (X).

The ethyl acetate-soluble products obtained when the oxide (VIII) (5mg) was incubated with homo-

genate prepared from 40g of rat liver also contained three products that were identified by t.l.c. as the phenols (IX) and (XI) and the dihydrodiol (X). Examination by the methods indicated above of the aqueous fraction from this incubation failed to reveal the presence of GSH conjugates.

In control experiments with boiled homogenates, the phenols (IX) and (XI), but not the dihydrodiol (X), were formed.

In an experiment in which the oxide (VIII) (2mg) and GSH (5mg) were incubated in the presence of rat liver soluble fraction obtained as indicated above, no evidence for the formation of GSH conjugates could be obtained. This fraction contains the enzyme 'glutathione-S-epoxide transferase' described by Boyland & Williams (1965).

Metabolism of dibenz[a,c]anthracene (I)

The hydrocarbon (I) (5mg) was incubated with homogenate prepared from the livers (40g) of rats that had been previously treated with 3-methylcholanthrene (Waterfall & Sims, 1972). The ethyl acetate-soluble fraction contained, apart from the hydrocarbon (I), only one product: this had the chromatographic properties and the u.v.-absorption spectrum of the dihydrodiol (X). When heated with conc. HCl the metabolite yielded two products, which were identified by t.l.c. as the phenols (IX) and (XI).

GSH conjugates could not be detected in the aqueous layer. The dihydrodiol (X) was not detected when the hydrocarbon (I) was incubated with boiled homogenate.

Metabolism of 10,11-dihydrodibenz[a,c]anthracene 12,13-oxide (VII)

The oxide (VII) (2mg) was incubated with the microsomal fraction prepared from rat liver (4g). The only product detected when the ethyl acetate fraction was examined by t.l.c. had the chromatographic properties and u.v.-absorption spectrum of *trans*-10,11,12,13-tetrahydro-10,11-dihydroxydibenz[a,c]anthracene (XIII). When heated with acid the metabolite yielded a product that was identified by t.l.c. as the ketone (XII). The product failed to discharge the blue colour of the potassium triacetylosmate reagent.

The dihydroxy compound (XIII) was the only metabolite detected when the epoxide (VII) (5mg) was incubated with homogenate prepared from rat liver (40g). The identity of the metabolite was confirmed by its u.v.-absorption spectrum and by its decomposition by hot conc. HCl into the ketone (XII). The metabolite was not formed in a control experiment with boiled homogenate.

GSH conjugates were not detected, either in the aqueous fraction from the metabolism of the epoxide

(VII) by the homogenate or as a product of the incubation of the epoxide (VII) with GSH in the presence of rat liver soluble fraction.

Metabolism of 10,11-dihydrodibenz[a,c]anthracene (IV)

The hydrocarbon (IV) (5mg) was incubated with homogenate prepared from the livers (40g) of rats that had previously been treated with 3-methylcholanthrene, and the ethyl acetate-soluble fraction was examined by t.l.c. The only product detected was identical in its chromatographic properties and u.v.-absorption spectrum with the dihydroxy compound (XIII). When heated with conc. HCl the metabolite yielded a product that was identified by t.l.c. as the ketone (XII). The metabolite failed to discharge the blue colour of the potassium triacetylosmate reagent.

Discussion

The evidence for the structure of dibenz[a,c]anthracene 10,11-oxide (VIII) rests mainly on its method of synthesis and on its u.v.-absorption spectrum (see Fig. 2), which is similar to those of the dihydrodibenz[a,c]anthracene (IV) and of the dihydrodiol (X), except that the maxima are shifted to longer wavelengths in the spectrum of the epoxide. This shift to longer wavelengths has been observed in comparisons between the spectra of other arene oxides and their related dihydrodiols (Waterfall & Sims, 1972).

The chemical and biochemical properties of the oxide (VIII) are similar to those previously reported for the other 'non-K-region' oxides, benz[a]anthracene 8,9-oxide (Sims, 1971) and benzo[a]pyrene 7,8- and 9,10-oxide (Waterfall & Sims, 1972). All are unstable and readily rearrange to phenols, either rapidly with conc. HCl or, in biochemical systems, by a slower non-enzymic reaction. Other 'non-K-region' oxides such as those of benzene and naphthalene (Jerina *et al.*, 1968, 1970) and 'K-region' oxides such as benz[a]anthracene and dibenz[a,h]anthracene 5,6-oxide (Boyland & Sims, 1965) also undergo this non-enzymic rearrangement. In both types of rearrangement, however, the direction in which the oxiran ring opens appears to depend on the molecular structure of each individual oxide. Thus benz[a]anthracene 8,9-oxide yields mixtures of 8- and 9-hydroxybenz[a]anthracene (Sims, 1971), where the ring-opening has given rise to compounds with hydroxyl groups on the newly formed aromatic rings in positions both α and β to the original aromatic ring system. With benzo[a]pyrene 7,8- and 9,10-oxide, however, the oxiran ring opens in one direction only to give 7- and 9-hydroxybenzo[a]pyrene respectively (Waterfall & Sims, 1972). In the rearrangements of the 7,8-oxide, the products have hydroxyl groups in the α -positions

to the original aromatic ring system, whereas in those of the 9,10-oxide the products have hydroxyl groups in the β -positions. In the present work, the rearrangements of the oxide (VIII) yield mixtures of phenols (IX) and (XI) in which 10-hydroxydibenz[a,c]anthracene (IX), where the hydroxy group is in the α -position on the newly formed aromatic ring, is the major product. In all these cases the related dihydrodiols are decomposed by acid to give phenols that are the same as those formed by the rearrangement of the parent oxides. Thus, in the present work, both the oxide (VIII) and the dihydrodiol (X) yield mixtures of 10- and 11-hydroxydibenz[a,c]anthracene (IX and XI), in which the phenol (IX) predominates.

Since the dihydrodiol (X) is not formed when the oxide (VIII) is incubated with boiled rat liver homogenate, it is probable that in the incubations with fresh rat liver homogenates or with microsomal preparations the oxide (VIII) is hydrated enzymically by the microsomal 'epoxide hydrases' described by Jerina *et al.* (1968), Brooks *et al.* (1970) and Oesch & Daly (1971). The 'non-K-region' oxides, benz[a]anthracene 8,9-oxide (Sims, 1971) and benzo[a]pyrene 7,8- and 9,10-oxide (Waterfall & Sims, 1972) and the 'K-region' oxides, benz[a]anthracene 5,6-oxide (Grover *et al.*, 1971a) and dibenz[a,h]anthracene 5,6-oxide (Pandov & Sims, 1970), are also hydrated by these enzymes.

The oxide (VIII) did not react with GSH, either chemically or enzymically. This is unexpected since epoxides such as benz[a]anthracene 5,6-oxide, dibenz[a,h]anthracene 5,6-oxide and benz[a]anthracene 8,9-oxide react both chemically and enzymically with GSH to form acid-labile conjugates (Boyland & Sims, 1965; Sims, 1971).

In the metabolism of dibenz[a,c]anthracene (I), the dihydrodiol formed was shown by direct comparisons on t.l.c. plates to be identical with the dihydrodiol (X) formed in the metabolism of the oxide (VIII). The u.v.-absorption spectrum of the metabolite of the hydrocarbon (I) was identical with that of the metabolite of the oxide (VIII) (see Fig. 2) and both metabolites yielded the phenols (IX) and (XI) on treatment with acid. Neither of these phenols nor any metabolite other than the dihydrodiol (X) was detected in the experiment with the hydrocarbon (I), thus confirming the observation (Sims, 1970) that the dihydrodiol (X) was the only metabolite of dibenz[a,c]anthracene (I) formed by rat liver homogenates. If it is assumed that the formation of the phenols (IX) and (XI) in the metabolism of the oxide (VIII) is a non-enzymic reaction dependent on the experimental conditions used, then the metabolic formation of the dihydrodiol (X) both from the hydrocarbon (I) and the oxide (VIII) is in accord with the present concept that arene oxides are formed as intermediates in the metabolism of aromatic polycyclic hydrocarbons (Selkirk *et al.*, 1971; Grover *et al.*, 1971b).

10,11-Dihydrodibenz[*a,c*]anthracene (IV) and its related oxide (VII) were both metabolized by rat liver homogenates into the *trans*-dihydroxy compound (XIII). This suggests that the oxide (VII) is an intermediate in the formation of the dihydroxy compound (XIII) from the hydrocarbon (IV). Leibman & Ortiz (1970) have shown that epoxides are intermediates in the conversion of compounds such as styrene, cyclohexane and indene, which also contain olefinic double bonds, into glycols. The absence of GSH conjugates among the products of the incubations of the oxide (VII) was unexpected, since 10,11-dihydrobenz[*a*]anthracene 8,9-oxide (Sims, 1971) and 7,8-dihydrobenzo[*a*]pyrene 9,10-oxide and 9,10-dihydrobenzo[*a*]pyrene 7,8-oxide (Waterfall & Sims, 1972) yield GSH conjugates under similar experimental conditions.

If it can be assumed that, in the alkylation of 4-(*p*-nitrobenzyl)pyridine by epoxides, the intensity of the colour formed is independent of the nature of the alkylating groups, then the 'K-region' oxide dibenz[*a,h*]anthracene 5,6-oxide is a better alkylating agent in this reaction than either of the oxides (VII and VIII) derived from dibenz[*a,c*]anthracene (I). Comparative experiments on the alkylation of biological macromolecules by dibenz[*a,h*]anthracene 5,6-oxide and dibenz[*a,c*]anthracene 10,11-oxide (VIII) cannot be carried out until a ³H-labelled sample of the latter compound is available for study. The oxide (VIII) has not yet been tested for its biological activities, but the 'non-K-region' oxide benz[*a*]anthracene 8,9-oxide is less active than the 'K-region' epoxide benz[*a*]anthracene 5,6-oxide in producing malignant transformations in hamster embryo cells (Marquardt *et al.*, 1972; Huberman *et al.*, 1972) and mutations in bacteria (Ames *et al.*, 1972).

I thank Miss Janet Watson for technical assistance. This investigation was supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council and the Cancer Research Campaign.

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