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Epoxy Derivatives of Aromatic Polycyclic Hydrocarbons

THE PREPARATION AND METABOLISM OF EPOXIDES RELATED TO BENZO[*a*]PYRENE AND TO 7,8- AND 9,10-DIHYDROBENZO[*a*]PYRENE

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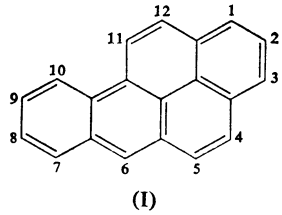
Products that appeared to be mainly benzo[*a*]pyrene 7,8-oxide and benzo[*a*]pyrene 9,10-oxide were synthesized and their chemical and biochemical properties were investigated. The oxides were unstable and readily rearranged to phenols. They were converted by rat liver homogenates and microsomal preparations into phenols and dihydrodiols, but glutathione conjugates were not formed in appreciable amounts. The dihydrodiols formed from benzo[*a*]pyrene 7,8- and 9,10-oxide by rat liver microsomal preparations were identical in their chromatographic and spectrographic properties with dihydrodiols formed when benzo[*a*]pyrene was metabolized by rat liver homogenates. 9,10-Dihydrobenzo[*a*]pyrene 7,8-oxide and 7,8-dihydrobenzo[*a*]pyrene 9,10-oxide were also synthesized. They were converted by rat liver homogenates and microsomal preparations into the related *cis*- and *trans*-dihydroxy compounds. Glutathione conjugates were formed from the oxides by rat liver homogenates. Both 7,8- and 9,10-dihydrobenzo[*a*]pyrene were metabolized by rat liver homogenates to mainly the *trans*-isomers of the related dihydroxy compounds. In experiments with boiled homogenates, the benzo[*a*]pyrene oxides were converted into phenols, whereas the dihydrobenzo[*a*]pyrene oxides yielded small amounts of the related dihydroxy compounds.

Recent work on the biological action of the 'K-region' epoxides of polycyclic aromatic hydrocarbons has shown that many of them will induce malignant transformation of rodent cells *in vitro* (Grover *et al.*, 1971*b*) and mutational changes in mammalian cells and bacteriophages (Huberman *et al.*, 1971; Cookson *et al.*, 1971). The parent hydrocarbons and the phenols and the dihydrodiols that are formed from the epoxides by metabolism are usually either inactive or much less active in these systems than the epoxides themselves. There is now abundant evidence that epoxides are the intermediates formed during the metabolism of aromatic hydrocarbons by hepatic microsomal enzymes (Jerina *et al.*, 1970; Selkirk *et al.*, 1971; Grover *et al.*, 1971*a*), and it is possible that they are also the intermediates responsible for the biological effects induced by the parent hydrocarbons.



(I)

Many polycyclic aromatic hydrocarbons, including benzo[*a*]pyrene (I), are metabolized by rat liver preparations at bonds other than those of the 'K-regions' (Sims, 1970), so that an examination of the properties of the 'non-K-region' epoxides derived from these hydrocarbons is of great interest. The synthesis of one 'non-K-region' epoxide, benz[*a*]anthracene 8,9-oxide, has been described (Sims, 1971) and the present paper describes the synthesis of compounds that are believed to be benzo[*a*]pyrene 7,8- and 9,10-oxide [(VI, Scheme 1) and (XV, Scheme 2)].

During the preparation of the arene oxides (VI and XV), 9,10-dihydrobenzo[*a*]pyrene 7,8-oxide (V) and 7,8-dihydrobenzo[*a*]pyrene 9,10-oxide (XIV) were also synthesized. Their metabolism has been examined and compared with that of the parent hydrocarbons, 9,10-dihydrobenzo[*a*]pyrene (II) and 7,8-dihydrobenzo[*a*]pyrene (XI).

Experimental

Methods

Melting points. These are uncorrected.

Thin-layer chromatography. This 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 Chromogram sheets (silica gel with fluorescent indicator) (Eastman–Kodak Co., Rochester, N.Y., U.S.A.). The coated glass plates were developed with

solvent (*a*) benzene, solvent (*b*) benzene – ethanol (19:1, v/v) or solvent (*c*) benzene – ethanol (9:1, v/v) and the Chromogram sheets with solvent (*d*) 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 NH_3. The properties of the compounds examined by t.l.c. are recorded in Table 1.

Paper chromatography. This was carried out on Whatman no. 1 chromatography paper. The chromatograms were developed downwards overnight with solvent (*e*) butan-1-ol–propan-1-ol–aq. 2M-NH_3 (2:1:1, by vol.). The dried chromatograms were examined in u.v. light and were then dipped in a solution of 0.2% ninhydrin in acetone and heated in an oven for 5min at 110°C.

U.v.-absorption spectra. These were usually measured in ethanol with a Unicam SP. 800 recording spectrophotometer. The spectra of compounds separated by chromatography were measured after elution of the appropriate spots or bands removed from thin-layer chromatograms with ethanol or elution of the appropriate bands cut from paper chromatograms with methanol containing 1% (v/v) of aq. NH_3 (sp.gr. 0.88). The u.v. spectra of some of the compounds in this work have been described by Sims (1968).

Rat liver preparations. Homogenates (Sims & Grover, 1968) and microsomal and soluble fractions (Booth *et al.*, 1960) were prepared from the livers of 35-day-old male rats of the Chester Beatty strain as previously described.

In experiments with the dihydrobenzo[*a*]pyrene oxides (V and XIV), the oxides (50mg) in acetone (5ml) were added to homogenates prepared from 40g of rat liver. The mixtures were incubated at 37°C for 30min and were then extracted with equal volumes of ethyl acetate. The extracts were dried over Na_2SO_4 and evaporated and the residues were examined as described below. The aqueous layers were filtered to remove protein, they were acidified to pH4 with acetic acid and were treated with charcoal (2g). The charcoal was filtered off and washed with water, and the adsorbed material was eluted with

Table 1. *Properties on thin-layer chromatograms of compounds related to benzo[a]pyrene*

Details are given in the text. —, R_F not measured.

Compound	R_F in solvent (*a*)	(*b*)	(*c*)	(*d*)	Fluorescence: Immediate	Fluorescence: After exposure to NH_3
7,8,9,10-Tetrahydro-7-oxobenzo[*a*]pyrene	0.43	0.93	—	0.52	Violet, turning yellow	Yellow
7,8,9,10-Tetrahydro-8-oxobenzo[*a*]pyrene (IX)	0.32	0.83	—	0.40	Violet	Violet
7,8,9,10-Tetrahydro-9-oxobenzo[*a*]pyrene (XVIII)	0.37	0.85	—	0.41	Violet	Violet
9,10-Dihydrobenzo[*a*]pyrene 7,8-oxide (V)	0.60	0.93	—	0.58	Blue–green	Blue–green
7,8-Dihydrobenzo[*a*]pyrene 9,10-oxide (XIV)	*	*	*	0.58	Blue–green	Blue–green
cis-7,8,9,10-Tetrahydro-7,8-dihydroxybenzo-[*a*]pyrene (Xa)	0.00	0.22	0.41	—	Blue–green	Blue–green
trans-7,8,9,10-Tetrahydro-7,8-dihydroxybenzo-[*a*]pyrene (Xb)	0.00	0.18	0.40	—	Blue–green	Blue–green
cis-7,8,9,10-Tetrahydro-9,10-dihydroxybenzo-[*a*]pyrene (XIXa)	0.00	0.25	0.40	—	Blue–green	Blue–green
trans-7,8,9,10-Tetrahydro-9,10-dihydroxy-benzo[*a*]pyrene (XIXb)	0.00	0.22	0.39	—	Blue–green	Blue–green
7-Hydroxybenzo[*a*]pyrene (VIII)	0.30	0.43	—	0.09	Blue	Orange
8-Hydroxybenzo[*a*]pyrene	0.20	0.31	—	0.09	Blue	Green
9-Hydroxybenzo[*a*]pyrene (XVII)	0.25	0.35	—	0.09	Blue	Bright green
Benzo[*a*]pyrene 7,8-oxide (VI)	*	*	—	0.48	Violet	Violet
Benzo[*a*]pyrene 9,10-oxide (XV)	*	*	—	0.48	Violet	Violet
Metabolite [probably 7,8-dihydro-7,8-di-hydroxybenzo[*a*]pyrene (VII)]	0.00	0.19	0.48	—	Violet	Violet
Metabolite [probably 9,10-dihydro-9,10-di-hydroxybenzo[*a*]pyrene (XVI)]	0.00	0.15	0.41	—	Violet	Violet

* Product decomposed completely during the loading and developing of the chromatogram.

methanol (250ml) containing 5% (v/v) aq. NH_3 (sp.gr. 0.88). The solvent was distilled off and the residues were examined by paper chromatography in solvent (*e*). The results are described below.

The epoxides (V and XIV) were also incubated at 37°C for 30min with rat liver microsomal fractions. The incubation mixtures were each extracted with ethyl acetate (50ml) and the extracts were examined by t.l.c. to give the results described below.

In the experiments with the arene oxides (VI and XV), the oxides (5mg) in acetone (0.5ml) were incubated at 37°C for 30min with homogenates or microsomal fractions prepared from 4g of rat liver. The ethyl acetate-soluble fractions were examined as described above, together with the aqueous fractions from the incubations with the homogenates. The oxides (5mg) were also incubated at 37°C for 1h with soluble fractions prepared from 20g of rat liver and containing GSH (5mg). Ethyl acetate-soluble and aqueous fractions were examined as described above.

The metabolism of the hydrocarbons (I, II and XI) was studied by using homogenates prepared from the livers of rats that had been treated 48h previously with 3-methylcholanthrene (5mg) in arachis oil (0.5ml), administered by intraperitoneal injection. Each hydrocarbon (5mg) in acetone (5ml) was incubated at 37°C for 30min with homogenate prepared from 40g of rat liver containing cofactors in the amounts previously described (Sims & Grover, 1968). Ethyl acetate-soluble and aqueous fractions were examined as described above.

Control experiments were carried out with rat liver homogenates that had been boiled for 5min.

Materials

7,8-Dihydrobenzo[*a*]pyrene (XI), 9,10-dihydrobenzo[*a*]pyrene (II), *cis*-7,8,9,10-tetrahydro-7,8-dihydroxybenzo[*a*]pyrene (Xa), *cis*-7,8,9,10-tetrahydro-9,10-dihydroxybenzo[*a*]pyrene (XIXa), 7,8,-9,10-tetrahydro-7-oxobenzo[*a*]pyrene, 7,8,9,10-tetrahydro-8-oxobenzo[*a*]pyrene (IX), 7,8,9,10-tetrahydro-9-oxobenzo[*a*]pyrene (XVIII), 7-hydroxybenzo[*a*]pyrene (VIII), 8-hydroxybenzo[*a*]pyrene and 9-hydroxybenzo[*a*]pyrene (XVII) were prepared as described by Sims (1968).

The light petroleum used in this work had b.p. 80–100°C. Apparatus used in the preparation and manipulation of the arene oxides was rinsed with aq. 2M-NH_3 and dried at 110°C before use.

Preparation of 7,8-*dibromo*-7,8,9,10-*tetrahydrobenzo*[*a*]*pyrene* (*III*). 9,10-Dihydrobenzo[*a*]pyrene (II) (10g) in carbon tetrachloride (200ml) was treated at room temperature with bromine (2ml) in carbon tetrachloride (10ml), added with stirring during 5min. The solvent was removed and the yellow oil crystallized from light petroleum to yield the *dibromide* (III) in pale-yellow needles (12.4g), m.p. 153–154°C (Found: C, 58.0; H, 3.5; Br, 38.8; $C_{20}H_{14}Br_2$ requires C, 58.0; H, 3.4; Br, 38.6%), $\lambda_{max.}$ at 262.5, 285, 315, 329 and 345nm ($\log\epsilon$ 4.55, 4.39, 4.04, 4.45 and 4.65 respectively).

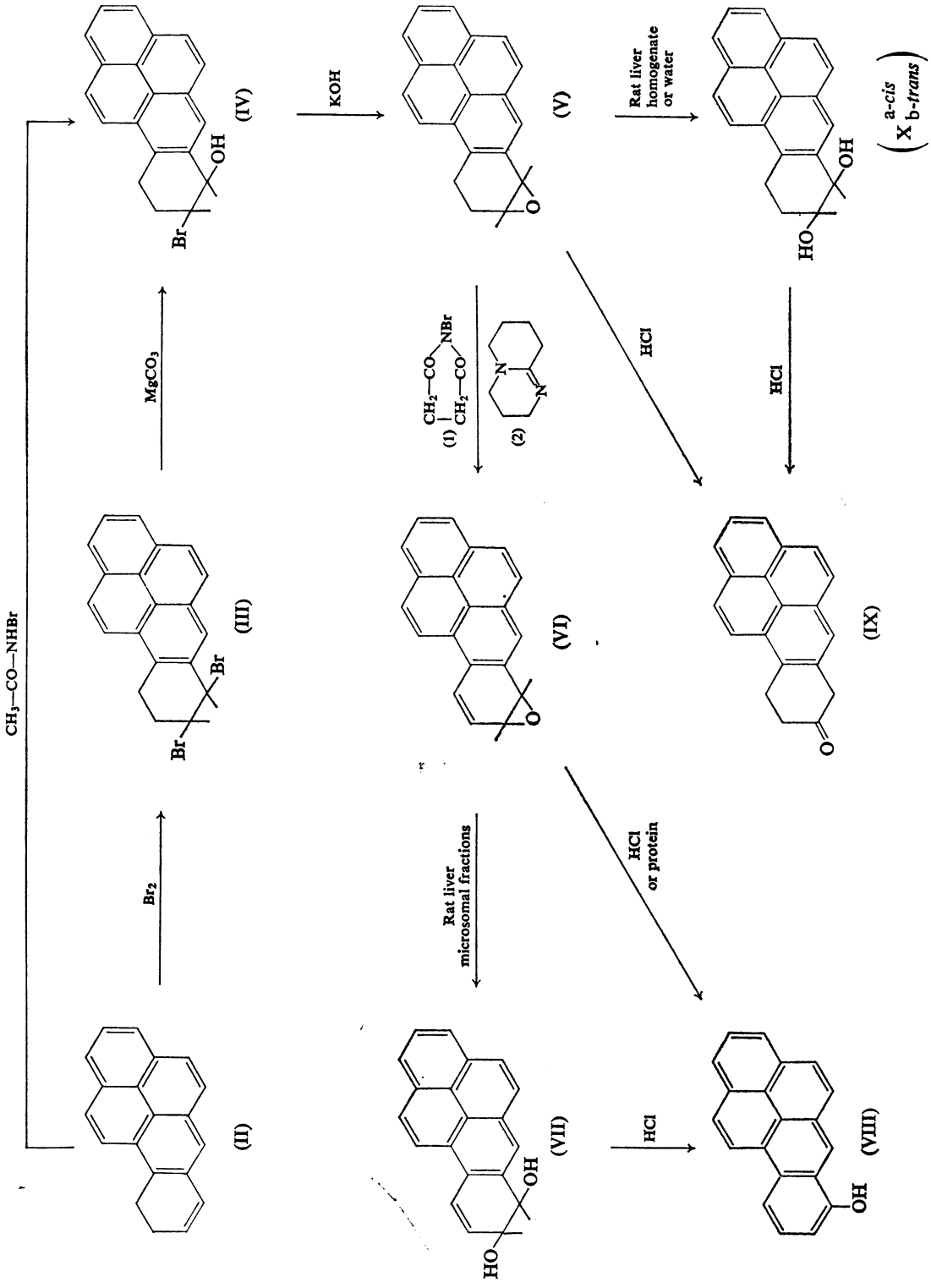
Preparation of 7,8,9,10-*tetrahydrobenzo*[*a*]*pyrene*-7,8-*bromohydrin* (*IV*). (*a*) The dibromo compound (III) (6g) in aq. 50% (v/v) acetone (500ml) was heated under reflux with an excess of $MgCO_3$ for 30min. The mixture was filtered and the filtrate was evaporated under reduced pressure to remove acetone. The solid that separated was recrystallized from benzene–light petroleum to yield the *bromohydrin* (IV) in needles, m.p. 158–159°C (Found: C, 68.4; H, 4.5; Br, 22.0; $C_{20}H_{15}BrO$ requires C, 68.4; H, 4.3; Br, 22.8%), $\lambda_{max.}$ at 237.5, 247, 251.5, 267.5, 279, 314.5, 329 and 345nm ($\log\epsilon$ 4.53, 4.84, 3.85, 4.35, 4.66, 4.04, 4.49 and 4.65 respectively).

(*b*) 9,10-Dihydrobenzo[*a*]pyrene (II) (4.5g), in aq. 50% (v/v) tetrahydrofuran (250ml), was stirred for 4h with *N*-bromoacetamide (2.45g) and sodium acetate (2.5g). The solution was evaporated to 50ml under reduced pressure and the residue was diluted with water. The solid that separated was recrystallized from benzene–light petroleum to yield the bromohydrin (IV) (3.8g) in needles, m.p. and mixed m.p. 158–159°C.

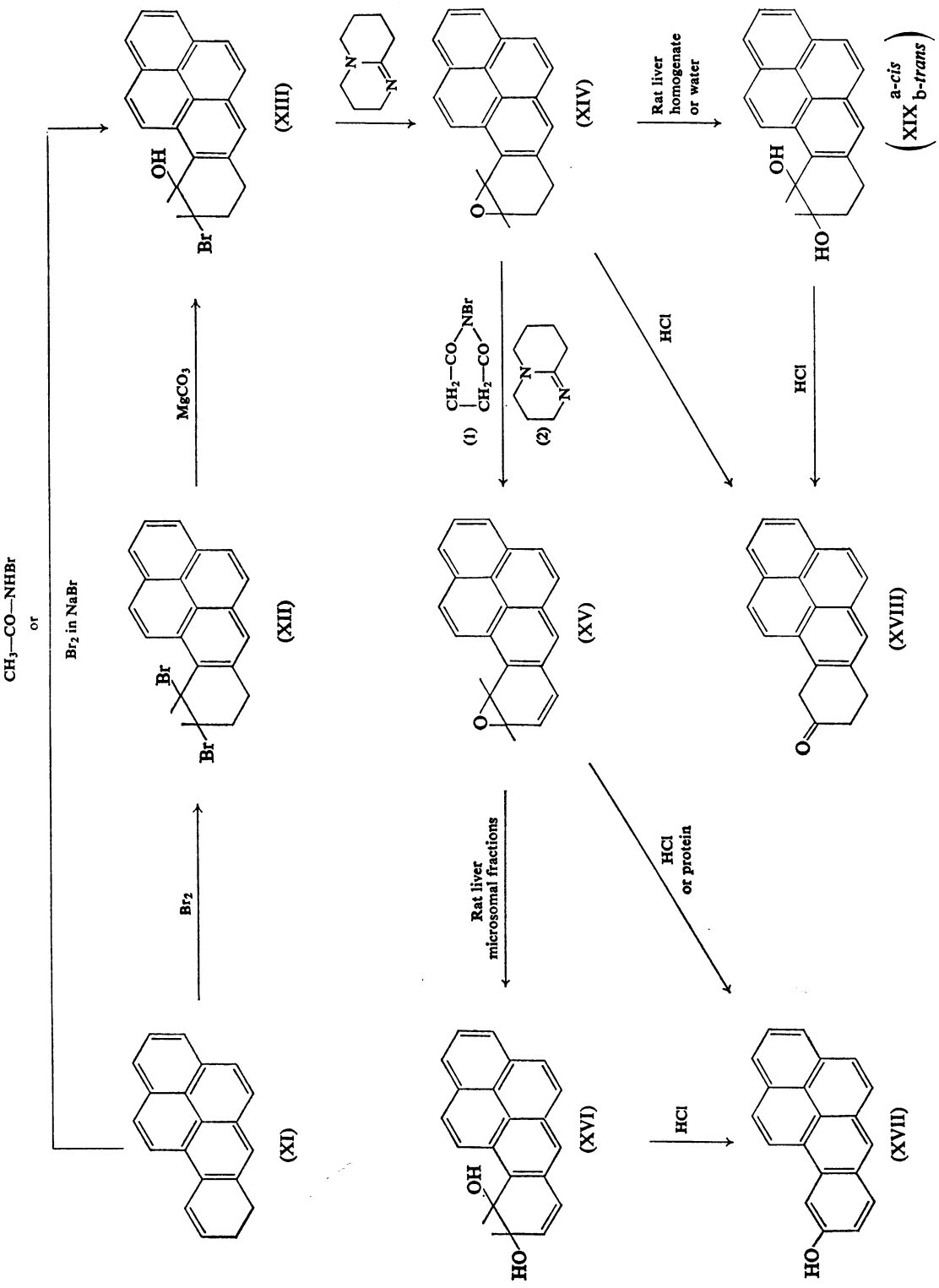
Preparation of 9,10-*dihydrobenzo*[*a*]*pyrene* 7,8-*oxide* (7,8-*epoxy*-7,8,9,10-*tetrahydrobenzo*[*a*]*pyrene*) (*V*). A solution of the bromohydrin (IV) (3g) in ethanol (600ml) was cooled and stirred while a solution of KOH (800mg) in methanol (8ml) was added during 30min. The product that separated was collected and recrystallized from light petroleum to yield the *oxide* (V) in plates, m.p. 157–160°C (decomp.) (softening at 143–145°C) (Found: C, 88.6; H, 5.2; $C_{20}H_{12}O$ requires C, 88.9; H, 5.2%), $\lambda_{max.}$ at 243, 252.5, 269, 280.5, 312.5, 327, 342.5 and 381nm ($\log\epsilon$ 4.59, 4.93, 4.39, 4.63, 4.03, 4.44, 4.64 and 3.45 respectively).

The oxide (V) was decomposed by heating it on a steam bath for 10min with acetic acid containing a few drops of conc. HCl to yield a product with the chromatographic properties and the u.v.-absorption spectrum of 7,8,9,10-tetrahydro-8-oxobenzo[*a*]-pyrene (IX).

Preparation of benzo[*a*]*pyrene* 7,8-*oxide* (7,8-*epoxy*-7,8-*dihydrobenzo*[*a*]*pyrene*) (*VI*). In a typical preparation, 9,10-dihydrobenzo[*a*]pyrene 7,8-oxide (V) (400mg) in carbon tetrachloride (50ml) was heated under reflux for 2.5h with *N*-bromosuccinimide (2g) and $\alpha\alpha'$-azoisobutyrodinitrile (50mg). The mixture turned yellow, then green and finally brown, and a precipitate of succinimide was formed. The mixture was cooled and filtered and the filtrate was evaporated to about 5ml under reduced pressure. A solid separated as a red crystalline powder (185mg) that appeared to consist mainly of a bromo-epoxide (presumably 10-bromo-9,10-dihydrobenzo[*a*]pyrene



Scheme 1. *Reactions involved in the synthesis and metabolism of benzo[a]pyrene 7,8-oxide (VI) and some related compounds*
For details see the text.



Scheme 2. *Reactions involved in the synthesis and metabolism of benzo[a]pyrene 9,10-oxide (XV) and some related compounds*

For details see the text.

7,8-oxide). Further crops of the compound were obtained from the mother-liquor, but they proved to be less pure than the first crop. The u.v. spectrum of the product was similar to that of the epoxide (V) and showed $\lambda_{max.}$ at 243.5 (inflexion), 252, 269, 281, 300 (inflexion), 313, 328, 344, 360 (inflexion) and 381 nm. When the crystals were examined by t.l.c. in solvent (*d*), one major product at R_F 0.45 was detected together with a second product at R_F 0.40, which appeared as pale-yellow and red spots respectively when the chromatograms were examined in daylight. Attempts to recrystallize the bromo-epoxide were unsuccessful: it usually decomposed to a green gum that was shown by t.l.c. to contain 8-hydrobenzo[*a*]pyrene. The product similarly decomposed when kept at room temperature for more than a few hours. Attempts to chromatograph the product on columns of silica gel, alumina or Florisil yielded only coloured decomposition products. In attempts to prepare the corresponding chloro-epoxide from the epoxide (V) and *N*-chlorosuccinimide, only starting materials were recovered from the reaction mixtures.

The crude bromo-epoxide (150mg), in tetrahydrofuran (5ml), was kept overnight with 1,5-diazabicyclo[4,3,0]non-5-ene (0.5ml). The u.v.-absorption spectrum of the material in solution progressively changed from that typical of a tetrahydrobenzo[*a*]pyrene to that typical of a 7,8-dihydrobenzo[*a*]pyrene. The solution was diluted with diethyl ether (50ml) and the mixture was washed successively with water (25ml), aq. 2M-KOH (25ml) and with water (2×25ml). The ethereal solution was dried over Na_2SO_4 and the diethyl ether was evaporated under reduced pressure in the presence of 1 drop of the diazabicyclononene reagent. The residue was washed with a little dry ice-cold diethyl ether to yield a product (80mg) that appeared to be mainly benzo[*a*]pyrene 7,8-oxide (VI), which formed a yellow powder that turned black at 139–142°C (but did not melt below 300°C). Attempts to recrystallize the crude oxide from any one of a number of organic solvents were unsuccessful: spontaneous decomposition occurred to yield a product that was shown by t.l.c. to consist mainly of 7-hydroxybenzo[*a*]pyrene (VIII). Attempts to purify the oxide by preparative t.l.c. were also unsuccessful. Because of this an acceptable elemental analysis could not be obtained. In attempts to measure the mass spectrum of the epoxide (VI), the spectrum recorded was that of the phenol (VIII).

When examined by t.l.c. in solvent (*d*), the epoxide (VI), as usually prepared, was found to contain one minor impurity, which formed a small absorbing spot in u.v. light with R_F 0.58. Some 7-hydroxybenzo[*a*]pyrene (VIII) was also detected in relatively large amounts on these chromatograms, but it appeared to have been formed during the loading of the chromatograms, as the characteristic u.v.-absorption peaks of the phenol were not present in the u.v.-absorption spectrum of the epoxide.

In one preparation of the epoxide (VI), the bromo-epoxide (48mg) was obtained from the epoxide (V) (200mg) as a yellow solid that was successfully recrystallized from methylene chloride as yellow crystals that darkened at 78–79°C and melted at 153–154°C. The dehydrobromination of this product was done as described above except that the reaction was done under N_2 and it yielded what was believed to be pure epoxide (VI) (22mg), which turned black at 180–186°C. The u.v. spectrum of this product is shown in Fig. 1, together with that of 7,8-dihydrobenzo[*a*]pyrene (XI).

The epoxide (VI) was rapidly decomposed by conc. HCl to yield mainly a phenol that had the chromatographic properties and the u.v.-absorption spectrum of 7-hydroxybenzo[*a*]pyrene (VIII). No 8-hydroxybenzo[*a*]pyrene was detected.

Preparation of 9,10-*dibromo*-7,8,9,10-*tetrahydrobenzo[a]pyrene* (*XII*). 7,8-Dihydrobenzo[*a*]pyrene (XI) (2g) was brominated in carbon tetrachloride in the manner described above. The product appeared to be unstable, as HBr was evolved during the working-up procedures. Fractional crystallization of the product from light petroleum yielded two products. The more soluble product (850mg) appeared to be 9,10-*dibromo*-7,8,9,10-*tetrahydrobenzo[a]pyrene* (XII), which separated from light petroleum in golden-yellow needles, m.p. 159–161°C (Found: Br, 38.2; $C_{20}H_{14}Br_2$ requires Br, 38.6%). Its u.v.-absorption spectrum resembled that of 7,8,9,10-tetrahydrobenzo[*a*]pyrene and showed $\lambda_{max.}$ at 227.5 (inflexion), 237, 246.5, 252, 267, 278.5, 313, 327, 343 and 359nm.

The less-soluble product appeared to arise from the dibromo-compound (XII) by loss of HBr and was probably 9- or 10-*bromo*-7,8-*dihydrobenzo[a]pyrene*. It separated from light petroleum in pale-yellow plates, m.p. 176°C (Found: C, 72.2; H, 4.1; Br, 23.6; $C_{20}H_{13}Br$ requires C, 72.1; H, 3.9; Br, 24.0%), $\lambda_{max.}$ at 228, 250, 258, 272.5, 283, 294.5, 322, 336, 352, 372 and 393nm. The spectrum thus resembles that of 7,8-dihydrobenzo[*a*]pyrene (XI).

Preparation of 7,8,9,10-*tetrahydrobenzo[a]pyrene*-9,10-*bromohydrin* (*XIII*). (*a*) The dibromo compound (XII) (500mg), in aq. 50% (v/v) acetone (10ml), was treated with $MgCO_3$ as described above to yield the *bromohydrin* (XIII) (250mg), which separated from light petroleum in needles, m.p. 133°C (Found: C, 68.4; H, 4.3; Br, 23.1; $C_{20}H_{15}BrO$ requires C, 68.4; H, 4.3; Br, 22.8%), $\lambda_{max.}$ at 237, 247, 257, 267.5, 278.5, 300, 313, 327, 343 and 376nm ($\log \epsilon$ 4.64, 4.88, 4.25, 4.46, 4.69, 3.86, 4.19, 4.49, 4.63 and 3.47 respectively).

(*b*) 7,8-Dihydrobenzo[*a*]pyrene (XI) (4.1g) in aq. 50% (v/v) tetrahydrofuran (200ml) was treated with *N*-bromoacetamide (2.23g) and sodium acetate

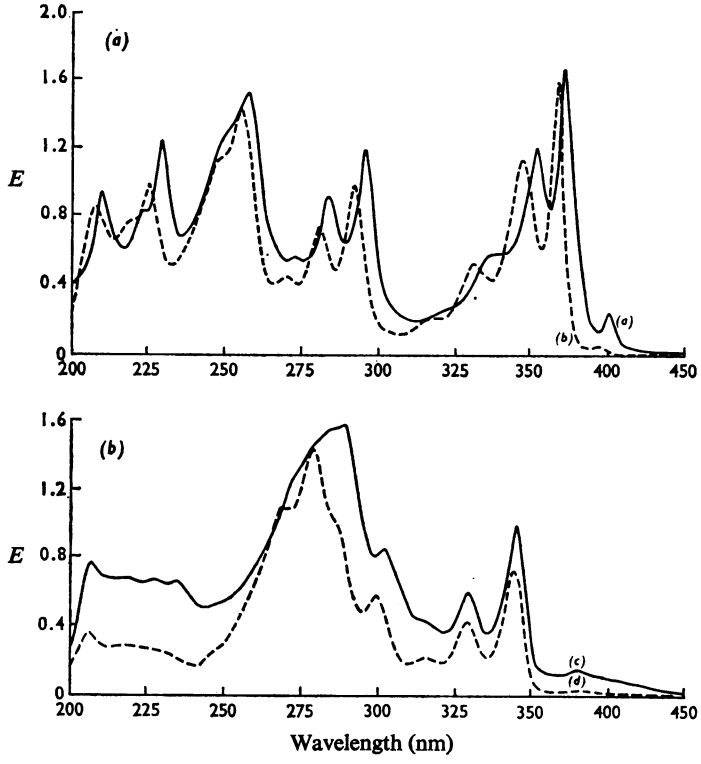


Fig. 1. *U.v.-absorption spectra of* (*a*) *benzo*[*a*]*pyrene* 7,8-*oxide* (*VI*), (*b*) 7,8-*dihydrobenzo*[*a*]*pyrene* (*XI*), (*c*) *benzo*[*a*]*pyrene* 9,10-*oxide* (*XV*) *and* (*d*) 9,10-*dihydrobenzo*[*a*]*pyrene* (*II*)

The spectra of 7,8-dihydro-7,8-dihydroxybenzo[*a*]pyrene (VII) and 9,10-dihydro-9,10-dihydroxybenzo[*a*]pyrene (XVI) were similar to those of the hydrocarbons (XI) and (II) respectively.

(2.5g) as described above to yield the bromohydrin (XIII) (3.1g), crystallizing from light petroleum in needles, m.p. and mixed m.p. 133°C. The filtrate was evaporated to a small volume and the material that separated was fractionally crystallized from ethanol to yield two unidentified bromine-containing products that formed thick prisms, m.p. 141°C, and needles, m.p. 139–140°C, respectively. The u.v.-absorption spectra of the two products were similar to each other and to that of the bromohydrin (XIII) and showed $\lambda_{max.}$ at 237, 247, 257, 267.5, 278.5, 300, 313, 327, 343 and 376nm. When examined by t.l.c., the products each had R_F 0.78 in solvent (*d*): the bromohydrin (XIII) had R_F 0.51 in this solvent. The products were not examined further.

(*c*) 7,8-Dihydrobenzo[*a*]pyrene (XI) (1g) in aq. 50% (v/v) tetrahydrofuran (100ml) was stirred for 4h at room temperature with a solution of bromine (0.2ml) in aq. 10% (v/v) NaBr (10ml). The solution was evaporated to a small volume under reduced pressure and the product that separated was recrystallized from light petroleum to yield the bromohydrin (XIII) (750mg), in needles, m.p. and mixed m.p. 133°C. The product (150mg) of m.p. 141°C described above was recovered from the mother-liquors.

Preparation of 7,8-*dihydrobenzo*[*a*]*pyrene* 9,10-*oxide*(9,10-*epoxy*-7,8,9,10-*tetrahydrobenzo*[*a*]*pyrene*) (*XIV*). The 9,10-bromohydrin (XIII) in tetrahydrofuran (10ml) was kept overnight with 1,5-diazabicyclo[4,3,0]non-5-ene (3ml). The solid that separated was recrystallized from benzene–light petroleum to yield the *oxide* (XIV) in plates, m.p. 149°C (darkening at 140°C) (Found: C, 88.8; H, 5.3; $C_{20}H_{12}O$ requires C, 88.9; H, 5.2%), $\lambda_{max.}$ at 238, 247.5, 258, 268.5, 280, 302, 314, 328, 344.5 and 378nm ($\log \epsilon$ 4.63, 4.86, 4.25, 4.46, 4.68, 3.80, 4.17, 4.48, 4.60 and 3.45 respectively). The epoxide (XIV) decomposed into unidentified products when it was chromatographed in solvents (*a*), (*b*) or (*c*), but it could be chromatographed with some decomposition in solvent (*d*).

When a portion of the epoxide (XIV) was heated with conc. HCl in acetic acid, a product with the

chromatographic properties and the u.v.-absorption spectrum of 7,8,9,10-tetrahydro-9-oxobenzo[*a*]-pyrene (XVIII) was obtained.

Preparation of benzo[a]pyrene 9,10-oxide (9,10-epoxy-9,10-dihydrobenzo[a]pyrene) (XV). In a typical preparation, 7,8-dihydrobenzo[*a*]pyrene 9,10-oxide (XIV) (350mg) in carbon tetrachloride (50ml) was heated under reflux for 2.5h with $\alpha\alpha'$-azoisobutyrodinitrile (50mg) and *N*-bromosuccinimide (1.8g). The solution gradually turned brown and succinimide separated. The mixture was cooled and filtered and the filtrate was evaporated to about 5ml under reduced pressure. A purple gum separated that soon solidified to a purple–brown powder (140mg), which is presumed to be mainly 7-bromo-7,8-dihydrobenzo[*a*]pyrene 9,10-oxide. A second crop (75mg) of the bromo-epoxide was obtained as brown plates when the mother liquor was kept at 0°C for some hours, which proved to be purer than the first. The u.v.-absorption spectrum of the bromo-epoxide showed $\lambda_{max.}$ at 248, 258, 268, 279, 300 (inflexion), 314.5, 328, 349 and 387 (inflexion) nm. The spectrum was similar to that of the oxide (XIV) except that there was a loss of fine structure in the 250–275nm region. Attempts to recrystallize the bromo-epoxide from any one of a number of solvents were unsuccessful; decomposition usually occurred to yield green gums that were shown by t.l.c. to contain 9-hydroxybenzo[*a*]pyrene (XVII). The bromo-epoxide also decomposed when chromatographed on silica gel, alumina or Florisil columns.

The crude bromo-epoxide (160mg) in tetrahydrofuran (5ml) was kept overnight with 1,5-diazabicyclo[4,3,0]non-5-ene (0.5ml). The u.v.-absorption spectrum of the material in solution changed progressively from that typical of a 7,8,9,10-tetrahydro-[*a*]pyrene to that typical of a 9,10-dihydrobenzo[*a*]-pyrene. The solution was diluted with diethyl ether (50ml) and washed with water (50ml), with aq. 2M-KOH (50ml) and with water again (2×50ml). The ethereal solution was dried over Na_2SO_4 and was evaporated to dryness in the presence of 1 drop of the diazabicyclononene reagent. The residue was washed with a little dry ice-cold diethyl ether to yield a product (85mg) that appeared to be mainly benzo-[*a*]pyrene 9,10-oxide (XV); it formed a yellow powder that softened and turned black at 135–139°C. The u.v.-absorption spectrum was similar to that of 9,10-dihydrobenzo[*a*]pyrene (II) (see Fig. 1). Attempts to recrystallize the epoxide (XV) were unsuccessful; spontaneous rearrangement to 9-hydroxybenzo[*a*]pyrene (XVII) readily occurred. An acceptable elemental analysis for the oxide (XVI) could not therefore be obtained. When the crude oxide was examined by t.l.c. in solvent (*d*), two minor impurities were normally detected as small u.v.-absorbing spots at R_F 0.58 and 0.64. Some 9-hydroxybenzo[*a*]pyrene (XVII) was always detected on these chromatograms, but it was probably formed from the epoxide (XV) during the loading of the chromatogram, as the characteristic u.v.-absorption peaks of the phenol were absent from the u.v.-absorption curve of the oxide.

The oxide (XV) was decomposed immediately when treated with conc. HCl to yield a product with the chromatographic properties and u.v.-absorption characteristics of 9-hydroxybenzo[*a*]pyrene (XVII).

Preparation of trans-7,8,9,10-tetrahydro-7,8-dihydroxybenzo[a]pyrene (Xb). 9,10-Dihydrobenzo-[*a*]pyrene 7,8-oxide (V) (100mg) was heated under reflux for 24h with acetone (25ml) containing 5% (v/v) of water. The acetone was distilled off under reduced pressure and the solid that separated was dissolved in the minimum amount of boiling benzene. The solid that crystallized out on cooling was recrystallized from benzene to yield *trans*-7,8,9,10-*tetrahydro*-7,8-*dihydroxybenzo*[*a*]*pyrene* (Xb) (22mg) in plates, m.p. 234–235°C (decomp.) (Found: C, 83.4; H, 5.4; $C_{20}H_{16}O_2$ requires C, 83.3; H, 5.6%). $\lambda_{max.}$ at 237, 247, 257, 267, 278, 301, 314, 328, 344, 356, 370 and 377nm (log ϵ 4.68, 4.90, 4.27, 4.49, 4.69, 3.87, 4.19, 4.50, 4.65, 3.61, 3.73 and 3.48 respectively). Examination of the mother-liquors by t.l.c. showed the presence of a compound with the chromatographic properties and u.v.-absorption spectrum of the *cis*-diol (Xa). The *trans*-diol (Xb) failed to discharge the colour of the potassium triacetylosmate reagent of Criegee *et al.* (1942), whereas, under similar conditions, the *cis*-isomer (Xa) changed the colour from blue to grey.

A small portion of the original mixtures of isomers (Xa and Xb) was chromatographed in solvent (*c*). The bands containing the isomers were removed separately from the chromatogram and the adsorbed material was eluted from the silica gel with ethanol (5ml). A comparison of the u.v.-absorption spectra of the solution showed that the ratio of the *cis*- to the *trans*-isomer in the original mixture was 1:8.5.

When the *trans*-diol (Xb) was heated to 100°C with conc. HCl in acetic acid, a product with the chromatographic properties of the ketone (IX) was formed.

Attempted preparation of trans-7,8,9,10-tetrahydro-9,10-dihydroxybenzo[a]pyrene (XIXb). 7,8-Dihydrobenzo[*a*]pyrene 9,10-oxide (XIV) (50mg) in aq. 10% (v/v) acetone (50ml) was stirred at room temperature for 5 days. The acetone was distilled off and the product that separated was applied to the base-line of a preparative thin-layer chromatogram that was developed in solvent (*c*). Two dihydroxy compounds were detected when the chromatogram was examined in u.v. light. The bands containing these compounds were removed and the adsorbed material was eluted from the silica gel with diethyl ether. The material in the faster-moving band was identified as *cis*-7,8,9,10-tetrahydro-9,10-dihydroxybenzo[*a*]pyrene (XIXa), m.p. and mixed m.p. 189–190°C. The diol changed

the colour of the potassium triacetylosmate reagent from blue to grey. The material in the slower-moving band was apparently *trans*-7,8,9,10-tetrahydro-9,10-dihydroxybenzo[*a*]pyrene (XIX); it failed to discharge the colour of the potassium triacetylosmate reagent. The u.v.-absorption spectrum of the *trans*-dihydroxy compound was similar to that of the *cis*-isomer and showed $\lambda_{max.}$ at 236.5, 246, 256, 278, 300, 312.5, 326, 342 and 276nm. When the *trans*-compound was heated with conc. HCl in acetic acid, a product was obtained that was identified by t.l.c. as 7,8,9,10-tetrahydro-9-oxobenzo[*a*]pyrene (XVIII). The ratio of the *cis*- to the *trans*-isomer in the original reaction mixture was found to be 5:1 when measured as described above.

Reaction of the epoxides with GSH

Each of the epoxides (10mg) was stirred with a solution of $NaHCO_3$ (20mg) and GSH (10mg) in aq. 50% (v/v) acetone (20ml) for 24h. The acetone was distilled off under reduced pressure and the aqueous residues were extracted with ethyl acetate (10ml). The ethyl acetate-soluble material was examined by t.l.c. The aqueous layers were evaporated to dryness and the residues were examined on paper chromatograms.

Reaction of 9,10-*dihydrobenzo[a]pyrene* 7,8-*oxide* (*V*). The ethyl acetate fraction contained material with the chromatographic properties of the *cis*- and *trans*-dihydroxy compounds (Xa and Xb). The aqueous layer contained a product that appeared to be *S*-(7,8,9,10-tetrahydro-8-hydroxybenzo[*a*]pyren-7-yl)-glutathione. It had R_F 0.17 in solvent (*e*) and gave a purple colour with ninhydrin. Its u.v.-absorption spectrum showed $\lambda_{max.}$ at 249, 269, 280, 301.5, 314.5, 328, 344, 358 and 379nm.

Reaction of 7,8-*dihydrobenzo[a]pyrene* 9,10-*oxide* (*XIV*). Products with the chromatographic properties of the dihydroxy compounds (XIXa and XIXb) were detected in the ethyl acetate extract. The aqueous fraction contained a product that appeared to be *S*-(7,8,9,10-tetrahydro-9-hydroxybenzo[*a*]pyren-10-yl)glutathione. It had R_F 0.12 in solvent (*e*) and gave a purple colour with ninhydrin. Its u.v. spectrum showed $\lambda_{max.}$ at 239 (inflexion), 247, 258 (inflexion), 269, 279.5, 303, 316.5, 330, 346.5, 358 (inflexion), 368 and 377nm.

Reaction of benzo[a]pyrene 7,8-*oxide* (*VI*). The main product in the ethyl acetate fraction was identified by t.l.c. as 7-hydroxybenzo[*a*]pyrene (VIII). There was no evidence for the presence of glutathione conjugates in the aqueous layer.

Reaction of benzo[a]pyrene 9,10-*oxide* (*XV*). The main product in the ethyl acetate fraction was identified by t.l.c. as 9-hydroxybenzo[*a*]pyrene (XVII). A small amount of a product was present in the aqueous layer that gave a purple colour with ninhydrin and whose u.v.-absorption spectrum showed $\lambda_{max.}$ at 275 (inflexion), 282, 287 (inflexion), 298 (inflexion), 328 and 343nm. This is the type of spectrum expected of a glutathione conjugate with a 9,10-dihydrobenzo[*a*]pyrene ring structure.

Alkylations of 4-(*p-nitrobenzyl*)*pyridine by epoxides*

These were carried out at 37°C as described by Grover & Sims (1970). Fig. 2 shows the results obtained with 9,10-dihydrobenzo[*a*]pyrene 7,8-oxide (V) and 7,8-dihydrobenzo[*a*]pyrene 9,10-oxide (XIV). The arene oxides (VI) and (XV) failed to react with the reagents. This was probably because they rapidly underwent rearrangement to the phenols (VIII) and (XVII) respectively, as (after the reaction mixtures had been incubated for 5min) the presence of these phenols could be demonstrated by spectrofluorimetry.

Results

Metabolism of benzo[a]pyrene 7,8-*oxide* (*VI*)

The epoxide was incubated with rat liver microsomal fractions as described above. Two major products were detected in the ethyl acetate-soluble

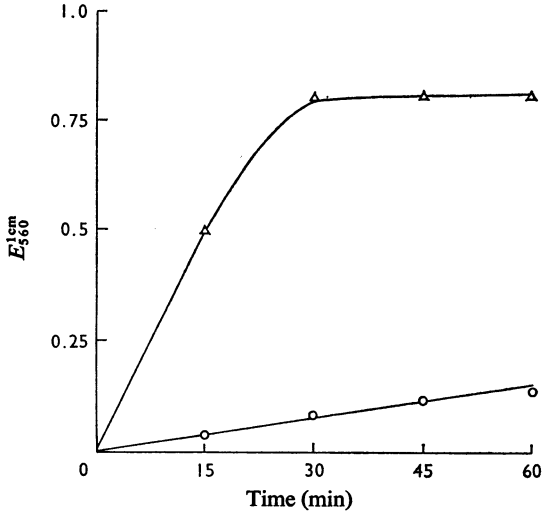


Fig. 2. *Alkylation of* 4-(*p-nitrobenzyl*)*pyridine by epoxides*

Increase in colour formation with time of incubation at 37°C resulting from the reaction of 0.2μmol of 9,10-dihydrobenzo[*a*]pyrene 7,8-oxide (V) (○) and 7,8-dihydrobenzo[*a*]pyrene 9,10-oxide (XIV) (△) with 4-(*p*-nitrobenzyl)pyridine at pH7.4 was measured.

fraction when the chromatogram was examined in u.v. light. The first was identified as 7-hydroxybenzo[*a*]pyrene (VIII) by its chromatographic properties and its u.v.-absorption spectrum.

The second product had the chromatographic properties of a dihydrodiol, the u.v.-absorption spectrum of which was almost identical with that of 7,8-dihydrobenzo[*a*]pyrene (XI) (see Fig. 1) and showed $\lambda_{max.}$ at 226, 250 (inflexion), 256, 272, 281.5, 293, 318 (inflexion), 331.5, 347.5, 367 and 394nm. On heating with conc. HCl the metabolite yielded a product with the chromatographic properties and u.v.-absorption spectrum of 7-hydroxybenzo[*a*]pyrene (VIII). The metabolite therefore appears to be 7,8-dihydro-7,8-dihydroxybenzo[*a*]pyrene (VII).

Examination of the products of the incubation of the epoxide with GSH in the presence of rat liver soluble fraction showed that the ethyl acetate extract contained small amounts of products with the chromatographic properties of 7-hydroxybenzo[*a*]pyrene (VIII) and the dihydrodiol (VII). The presence of glutathione conjugates in the aqueous fraction could not be demonstrated.

Examination by t.l.c. of the products of the incubation of the epoxide (VI) with rat liver homogenates also showed that the phenol (VIII) and small amounts of the dihydrodiol (VII) were formed; no glutathione conjugates were detected.

In experiments with boiled homogenates, the phenol (VIII), but not the dihydrodiol (VII), was detected.

Metabolism of benzo[a]pyrene 9,10-*oxide* (*XV*)

With rat liver microsomal fractions, the epoxide (XV) yielded a phenol with the chromatographic properties and u.v.-absorption spectrum of 9-hydroxybenzo[*a*]pyrene (XVII), together with a dihydrodiol that, on treatment with conc. HCl, yielded a product that was identified by t.l.c. as 9-hydroxybenzo[*a*]pyrene (XVII). The u.v.-absorption spectrum of the metabolite was similar to that of 9,10-dihydrobenzo[*a*]pyrene (II) (see Fig. 1) and showed $\lambda_{max.}$ at 230, 270 (inflexion), 278, 284 (inflexion), 298.5, 314.5, 328, 344, 350 (inflexion), 379 and 400nm. The metabolite is therefore presumed to be 9,10-dihydro-9,10-dihydroxybenzo[*a*]pyrene (XVI).

When the epoxide (XV) was incubated with rat liver supernatant, the ethyl acetate extract of the reaction mixture was shown by t.l.c. to contain small amounts of the phenol (XVII) and the dihydrodiol (XVI). Glutathione conjugates were not detected in the aqueous layer.

Incubation of the epoxide (XV) with rat liver homogenate also yielded products with the chromatographic properties of the phenol (XVII) and the dihydrodiol (XVI). Trace amounts of a glutathione conjugate were detected in the aqueous fraction; the u.v.-absorption spectrum of this product was similar to the conjugate obtained from the chemical reaction of the epoxide (XV) with GSH. In control experiments with boiled homogenates, the phenol (XVII), but not the dihydrodiol (XVI), was formed.

Metabolism of benzo[a]pyrene (*I*)

The ethyl acetate-soluble products from the metabolism of the hydrocarbon (I) by rat liver homogenates contained a phenol, which was identified by t.l.c. as 3-hydroxybenzo[*a*]pyrene. Two dihydrodiols were also detected and they were separated by preparative t.l.c. in solvent (*c*). The u.v. spectrum of the faster-running metabolite was of the 7,8-dihydrobenzo[*a*]pyrene type and had $\lambda_{max.}$ at 226, 250 (inflexion), 256, 276, 281.5, 293, 318 (inflexion), 331.5, 347.5, 367 and 394nm. The metabolite was indistinguishable on chromatograms developed in solvents (*b*) and (*c*) from the dihydrodiol obtained in the metabolism of the 7,8-oxide (VI) and, when heated with conc. HCl, it yielded a product with the chromatographic properties and u.v.-absorption spectrum of 7-hydroxybenzo[*a*]pyrene (VIII). It therefore appears that the metabolite is 7,8-dihydro-7,8-dihydroxybenzo[*a*]pyrene (VII).

The u.v. spectrum of the slower-running dihydrodiol was of the 9,10-dihydrobenzo[*a*]pyrene type and it had $\lambda_{max.}$ at 230, 270 (inflexion), 278, 284 (inflexion), 298.5, 314.5, 328, 344, 350 (inflexion), 379 and 400nm. When examined by t.l.c. in solvents (*b*) and (*c*) it was indistinguishable from the dihydrodiol formed in the metabolism of the 9,10-oxide (XV). When heated with acid, the metabolite yielded a phenol with the chromatographic properties and u.v.-absorption spectrum of 9-hydroxybenzo[*a*]pyrene (XVII). The metabolite therefore appears to be 9,10-dihydro-9,10-dihydroxybenzo[*a*]pyrene (XVI).

An examination of the aqueous fraction by paper chromatography failed to reveal the presence of glutathione conjugates.

Metabolism of 9,10-*dihydrobenzo[a]pyrene* 7,8-*oxide* (*V*)

When the epoxide (V) was incubated with rat liver homogenate, two products were detected by t.l.c. in the ethyl acetate fraction and were separated by preparative t.l.c. Both appeared to be dihydroxy compounds that, when heated to 100°C with conc. HCl, yielded products with the chromatographic properties and u.v.-absorption spectra of 7,8,9,10-tetrahydro-8-oxobenzo[*a*]pyrene (IX). The u.v.-absorption spectra of both metabolites were similar and showed $\lambda_{max.}$ at 237, 246.5, 256.5, 267, 278, 301, 314, 327.5, 344, 356, 370 and 277nm. The faster-

moving metabolite was identical in its chromatographic properties with *cis*-7,8,9,10-tetrahydro-7,8-dihydroxybenzo[*a*]pyrene (Xa) and the slower-moving metabolite with those of *trans*-7,8,9,10-tetrahydro-7,8-dihydroxybenzo[*a*]pyrene (Xb). The stereochemistry of the metabolites was confirmed by their action on the potassium triacetylosmate reagent; the metabolite believed to be the *cis*-dihydroxy compound changed the colour of the reagent from blue to grey, whereas that believed to be the *trans*-compound was without effect. The ratio of the *cis*- to the *trans*-isomer present in the incubation mixture was 1:7.9.

The aqueous fraction was shown by paper chromatography to contain a glutathione conjugate that formed a fluorescent band in u.v. light at R_F 0.17 in solvent (*c*) and gave a purple colour with the ninhydrin reagent. It yielded a compound with the chromatographic properties of 7,8,9,10-tetrahydro-8-oxobenzo[*a*]pyrene (IX) when heated with conc. HCl. The u.v.-absorption spectrum of the conjugate showed $\lambda_{max.}$ at 250, 269, 280.5, 302, 314.5, 344.5, 358 and 379nm. These properties suggest that the conjugate was *S*-(7,8,9,10-tetrahydro-8-hydroxybenzo-[*a*]pyrene-7-yl)glutathione. The chromatographic properties and the u.v. spectrum of the conjugate were identical with those of the product obtained when the epoxide (V) and GSH were allowed to react chemically. The dihydroxy compounds (Xa and Xb) were formed when homogenates that had been previously boiled were used but spectroscopic measurements showed that the non-enzymic reaction was small as compared with the enzymic reaction. The glutathione conjugate was not detected in reactions with boiled homogenates.

The ethyl acetate-soluble products obtained when the oxide (V) was incubated with rat liver microsomal fractions showed the presence of compounds with the chromatographic properties of *cis*- and *trans*-7,8,9,10 - tetrahydro - 7,8 - dihydroxybenzo[*a*]pyrene (Xa and Xb). The ratio of the *cis*- to the *trans*-isomer was 1:8.5.

Metabolism of 9,10-*dihydrobenzo*[*a*]*pyrene* (*II*)

The ethyl acetate-soluble fraction obtained from the metabolism of 9,10-dihydrobenzo[*a*]pyrene (II) by rat liver homogenate contained a product that had the chromatographic properties and u.v.-absorption spectra characteristic of *trans*-7,8,9,10-tetrahydro-7,8-dihydroxybenzo[*a*]pyrene (Xb). A small amount of the product with the chromatographic properties of the *cis*-dihydroxy compound (Xa) was also detected. A product with the chromatographic properties of *S*-(7,8,9,10-tetrahydro-8-hydroxybenzo-[*a*]pyren-7-yl)glutathione was present in the aqueous phase. None of these products was detected when boiled homogenates were used.

Metabolism of 7,8-*dihydrobenzo*[*a*]*pyrene* 9,10-*oxide* (*XIV*)

When the oxide (XIV) was incubated with rat liver homogenate, the ethyl acetate-soluble fraction contained two dihydroxy compounds, which were separated by preparative t.l.c. Both metabolites yielded products with the chromatographic properties and u.v.-absorption spectrum of 7,8,9,10-tetrahydro-9-oxobenzo[*a*]pyrene (XVIII) when heated with conc. HCl. The u.v. spectra of the two products were similar and showed $\lambda_{max.}$ at 236, 246, 256, 267, 300, 312.5, 326, 342 and 376nm. The faster-moving product was identical in its chromatographic properties with *cis*-7,8,9,10-tetrahydro-9,10-dihydrobenzo[*a*]pyrene (XIXa) and the slower-moving product had chromatographic properties identical with the product that is probably *trans*-7,8,9,10-tetrahydro-9,10-dihydroxybenzo[*a*]pyrene (XIXb). This product failed to discharge the colour of the potassium triacetylosmate reagent, whereas the faster-moving product changed the colour of the reagent from blue to grey. The ratio of the *cis*- to the *trans*-isomer in the incubation was 1:1.

The aqueous phase contained a product that showed a violet fluorescence when paper chromatograms were examined in u.v. light. It gave a purple colour with ninhydrin and had R_F 0.12 on paper chromatograms developed with solvent (*e*). It yielded a product with the chromatographic properties of 7,8,9,10-tetrahydro-9-oxobenzo[*a*]pyrene (XVIII) when heated with acid. Its u.v.-absorption spectrum showed $\lambda_{max.}$ at 239 (inflexion), 247, 258 (inflexion), 269, 279.5, 304, 317, 330, 346.5, 358 (inflexion) and 378 nm. This evidence suggests that the conjugate was *S* - (7,8,9,10 - tetrahydro - 9 - hydroxybenzo[*a*]pyren-10-yl)glutathione. Its chromatographic properties and absorption spectrum were similar to those of the conjugate obtained when the epoxide (XIV) and GSH were allowed to react chemically.

The dihydroxy compounds (XIXa and XIXb) were formed when incubations were carried out with homogenates that had previously been boiled, but spectroscopic measurements showed that the amounts formed were small compared with those formed by fresh homogenates. The glutathione conjugate was not detected in the experiments with boiled homogenates.

The ethyl acetate-soluble products obtained from the incubation of the oxide (XIV) with rat liver microsomal fractions contained two products with the chromatographic properties of *cis*- and *trans*-7,8,9,10 - tetrahydro - 9,10 - dihydroxybenzo[*a*]pyrene (XIXa and XIXb). The ratio of the *cis*- to the *trans*-isomer in the reaction mixture was 1:1.

Metabolism of 7,8-*dihydrobenzo*[*a*]*pyrene* (*XI*)

Examination of the ethyl acetate-soluble products of the metabolism of 7,8-dihydrobenzo[*a*]pyrene (XI)

by rat liver homogenate showed the presence of two dihydroxy compounds with the chromatographic properties and u.v.-absorption spectra of *cis*- and *trans*-7,8,9,10-tetrahydro-9,10-dihydroxybenzo[*a*]-pyrene (XIXa and XIXb). The *trans*-isomer appeared to be the major product. Glutathione conjugates could not be detected in the aqueous phase. The dihydroxy compounds (XIXa and XIXb) were not detected in experiments with boiled homogenates.

Discussion

Benzo[*a*]pyrene 7,8- and 9,10-oxide (VI and XV) proved to be highly labile compounds that readily rearranged in both chemical and biological systems to the fully aromatic phenolic compounds (VIII and XVII) respectively. The gain in resonance energy when the non-aromatic state in the oxides is transformed into the aromatic state in the phenols presumably facilitates this rearrangement. Because of this instability, great difficulty was experienced in obtaining the pure epoxides. The evidence for their structure therefore rests mainly on their chemical conversion into phenols, their enzymic conversion into dihydrodiols and their u.v.-absorption characteristics. Fig. 1 shows that the absorption spectra of the epoxides are similar to, but not identical with, those of the corresponding dihydrobenzo[*a*]pyrenes and dihydrodiols; some of the absorption peaks were shifted to longer wavelengths in the spectra of the epoxides. Similar shifts have been observed in comparison between the spectra of 'K-region' epoxides and those of their related dihydrodiols (Boyland & Sims, 1965*b*; Sims, 1966) and between the spectrum of the 'non-K-region' epoxide benz[*a*]anthracene 8,9-oxide and that of its related dihydrodiol (Sims, 1971).

Jerina *et al.* (1968) found that the conversion of benzene oxide into phenol is catalysed by protein. The experiments with liver preparations reported here suggest that the benzo[*a*]pyrene oxides (VI and XV) are more labile than benzene oxide, as phenols were the major products; reasonable yields of the dihydrodiols (VII and XVI) were obtained only when liver microsomal fractions were used. Previous work has shown that 'K-region' epoxides undergo similar rearrangements to phenols in the presence of liver preparations (Boyland & Sims, 1965*a*,*b*; Sims, 1966) and in these experiments the conversion of the epoxides into dihydrodiols and glutathione conjugates in both chemical and enzymic reactions was readily demonstrated. The rearrangement of the epoxides (VI and XV) to the phenols (VIII and XVII) appears to be non-enzymic, since it occurred when boiled homogenates were used. The difficulties experienced in detecting chemical and enzymic conjugations of GSH with the benzo[*a*]pyrene oxides (VI and XV) were probably caused by the rapid rearrangement of the oxides to the corresponding phenols during the reactions. The enzymic hydrations of the benzo[*a*]pyrene oxides (VI and XV) are presumably catalysed by the 'epoxide hydrases' described by Jerina *et al.* (1968), Brooks *et al.* (1970) and Oesch & Daly (1971). The 'K-region' epoxides of phenanthrene and dibenz[*a*,*h*]anthracene are also hydrated by these enzymes (Pandov & Sims, 1970).

A direct comparison between the properties of the two dihydrodiols (VII and XVI) formed enzymically from benzo[*a*]pyrene 7,8- and 9,10-oxide (VI and XV) with those of two dihydrodiols formed from benzo[*a*]pyrene (I) by rat liver homogenates, showed that the two pairs of compounds are identical. In earlier work (Sims, 1967, 1970), the metabolite of benzo[*a*]pyrene, now shown to be 7,8-dihydro-7,8-dihydroxybenzo[*a*]pyrene (VII), was wrongly identified as 1,2-dihydro-1,2-dihydroxybenzo[*a*]pyrene. The published spectrum (Sims, 1970) is, however, clearly that of the dihydrodiol (VII). The phenols (VIII and XVII) are not detected as metabolites of benzo[*a*]pyrene (I) by rat liver homogenates. If the epoxides (VI and XV) are the intermediates formed in the metabolism of the hydrocarbon (I), then, under the experimental conditions used, their enzymic conversion into the dihydrodiols (VII and XVI) must be faster than their non-enzymic conversion into the phenols (VIII and XVII). It is now evident, therefore, that benzo[*a*]pyrene (I) is metabolized by rat liver homogenates at the 3-position to yield 3-hydroxybenzo[*a*]pyrene, at the 7,8-bond to yield 7,8-dihydro-7,8-dihydroxybenzo[*a*]pyrene (VII) and at the 9,10-bond to yield 9,10-dihydro-9,10-dihydroxybenzo[*a*]-pyrene (XVI). The dihydrodiols are presumed to have the *trans*-configuration by analogy with the configuration of dihydrodiols formed in the metabolism of other hydrocarbons and their related epoxides. Dihydrodiols formed at the 'K-regions' (the 4,5- and 11,12-bonds) of benzo[*a*]pyrene (I) have not been detected in experiments with rat liver homogenates. In the metabolism of the hydrocarbon (I) by rat liver microsomal fractions, however, evidence has been obtained (P. L. Grover, A. Hewer & P. Sims, unpublished work) that metabolism at the 4,5-bond does occur.

The enzymic formation of both the *cis*- and the *trans*-isomers of the dihydroxy compounds (X and XIX) from the dihydrobenzo[*a*]pyrenes (II and XI) and from their related epoxides (V and XIV) was unexpected, as 1,2-dihydronaphthalene and its oxide (Boyland & Sims, 1960) and 10,11-dihydrobenz[*a*]-anthracene and its oxide (Sims, 1971) yield only the *trans*-isomers of the corresponding dihydroxy compounds on metabolism. However, both indene (Brook&Youngs, 1956) and acenaphthylene (Hopkins *et al.*, 1962) are metabolized in rats to mixtures of *cis*- and *trans*-dihydroxy compounds. Indene epoxide is similarly metabolized by rats into a mixture of the

cis- and *trans*-dihydroxy compounds (Lewis, 1966) and, like the oxides (V and XIV), it is also converted chemically into a mixture of isomers. With rat liver microsomal fractions, however, indene and indene epoxide are both converted into the *trans*-dihydroxy compound only (Leibman & Ortiz, 1968). In the present work, the dihydrobenzo[*a*]pyrenes (II and XI) yielded with rat liver homogenates mainly the *trans*-isomer and only small amounts of the *cis*-isomer. The epoxides (V and XIV), however, yielded mixtures of isomers with both rat liver microsomal fractions and homogenates; the reactions appeared to be enzymic since only small amounts of the dihydroxy compounds were formed when boiled homogenates were used. Appreciable amounts of the *cis*-isomers were present in both of these mixtures, but the effect was most marked with the oxide (XIV), where, in the enzymic reactions, equal amounts of the isomers were formed and where, in the chemical reaction between the oxide (XIV) and water, the *cis*-dihydroxy compound was the major product. Leibman & Ortiz (1970) have shown that epoxides are intermediates in the metabolic conversion of compounds with olefinic double bonds into dihydroxy compounds. If, as seems likely, the epoxides (V and XIV) are intermediates in the enzymic formation of the dihydroxy compounds (X and XIX) from the dihydrobenzo[*a*]pyrenes (II and XI), then the observed differences between the proportions of the stereoisomers found in the metabolism of the hydrocarbons and of their epoxides are difficult to explain.

When tested by subcutaneous injection into mice, 7,8-dihydrobenzo[*a*]pyrene (XI) proved to be a powerful carcinogen, whereas 9,10-dihydrobenzo[*a*]-pyrene (II) was virtually inactive (P. Sims, unpublished work). The present work has shown that the hydrocarbons are both metabolized by rat liver homogenates at their olefinic double bonds, the oxides (V) and (XIV) presumably being formed as intermediates. It is not known if the oxide (XIV) is involved in the carcinogenic action of its parent hydrocarbon (XI), but in the alkylation of 4-(*p*-nitrobenzyl)pyridine this oxide is more active than the oxide (V) derived from the non-carcinogenic hydrocarbon (Fig. 2). Extension of this work to the investigation of the alkylation of cellular macromolecules must await the synthesis of the ^{3}H-labelled oxides.

A number of 'K-region' epoxides are active in producing malignant transformations in hamster embryo and C3H mouse ventral-prostate cells (Grover *et al.*, 1971*b*) and in inducing mutations in mammalian cells (Huberman *et al.*, 1971) and in bacteriophage (Cookson *et al.*, 1971). The epoxides described in the present work have not yet been tested in these systems, but the 'non-K-region' epoxide, benz[*a*]anthracene 8,9-oxide, proved to be less active than the corresponding 'K-region' epoxide, benz[*a*]anthracene 5,6-oxide.

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