Epoxy Derivatives of Aromatic Polycyclic Hydrocarbons

THE PREPARATION OF BENZ[a]ANTHRACENE 8,9-OXIDE AND 10,11-DIHYDROBENZ[a]ANTHRACENE 8,9-OXIDE AND THEIR METABOLISM BY RAT LIVER PREPARATIONS

By P. SIMS

*Chester Beatty Research Institute, Institute of Cancer Research:
Royal Cancer Hospital, London SW3 6JB, U.K.*

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The syntheses of 10,11-dihydrobenz[a]anthracene 8,9-oxide, benz[a]anthracene 8,9-oxide and 9-hydroxybenz[a]anthracene are described, together with those of a number of related compounds. The epoxides react both chemically and enzymically with water to yield the corresponding dihydrodiols and with reduced glutathione to form glutathione conjugates, and they react chemically with *N*-acetylcysteine to yield the corresponding mercapturic acids. 8,9-Dihydro-8,9-dihydroxybenz[a]anthracene, formed enzymically from benz[a]anthracene 8,9-oxide, was identical with a dihydrodiol formed when benz[a]anthracene was metabolized by rat liver homogenates. Similarly 10,11-dihydrobenz[a]anthracene 8,9-oxide yielded a dihydrodiol identical with the product formed when 10,11-dihydrobenz[a]anthracene was metabolized.

It is now becoming widely accepted that the formation of an epoxide is the primary step in the hydroxylation of compounds with aromatic bonds by mammalian microsomal enzyme systems. With the aromatic hydrocarbons, a limited number of these epoxides have been available for study and, apart from those of benzene (Vogel, Böll & Günther, 1965) and naphthalene (Vogel & Klärner, 1968), are all related to phenanthrene 9,10-oxide. In these compounds the oxirane rings are formed by the addition of oxygen at the so-called 'K-regions' (Newman & Blum, 1964; Boyland & Sims, 1965*a,b*; Sims, 1966), which are the bonds in the molecules with the highest electron densities. Since with many aromatic hydrocarbons metabolism occurs mainly on bonds other than those of the 'K-regions' (Sims, 1970), it seemed desirable to attempt the synthesis of a series of 'non-K-region' epoxides and to examine their properties. The present paper describes the synthesis and metabolism in rat liver preparations of the first arene oxide in this series, benz[a]anthracene 8,9-oxide. The metabolism of 10,11-dihydrobenz[a]anthracene and its related epoxide, 10,11-dihydrobenz[a]anthracene 8,9-oxide, was also examined.

EXPERIMENTAL

Methods

Melting points. These are uncorrected.

Thin-layer chromatography. This was carried out on silica gel G as described by Sims (1966). The chromato-

grams were developed with (a) benzene, (b) benzene-ethanol (19:1, v/v) or (c) benzene-ethanol (9:1, v/v). They were examined in u.v. light while still wet, both before and after exposure to NH_3 , and were then sprayed either with a solution of 2,6-dichloroquinonechloroimide (0.5% in ethanol) followed by aq. Na_2CO_3 (10%, w/v) or with a solution of $\text{Na}_2\text{Cr}_2\text{O}_7$ (5%, w/v) in 0.1M- H_2SO_4 . With the latter reagent the colours were fully developed by heating the chromatograms in an oven at 110°C for 10 min. The properties of compounds detected on 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 for 18 h in 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 then either dipped in a solution of 0.2% ninhydrin in acetone or sprayed with the $\text{K}_2\text{Cr}_2\text{O}_7$ - AgNO_3 reagent of Knight & Young (1958). The properties of compounds detected on paper chromatograms are recorded in Table 2.

Spectra. I.r. spectra were measured as mulls in Nujol on a Perkin-Elmer 257 grating i.r. spectrometer and u.v.-absorption spectra on a Unicam SP. 800 recording spectrophotometer. The spectra of compounds separated by chromatography were measured after elution with ethanol of the appropriate spots or bands removed from thin-layer chromatograms or elution with methanol containing 1% (v/v) of aq. NH_3 (sp. gr. 0.88) of the appropriate bands cut from paper chromatograms.

Materials

Benz[a]anthracene. This was obtained from Koch-Light Laboratories Ltd. (Colnbrook, Bucks., U.K.). It was purified by passing a solution in light petroleum (b.p. 80–100°C) through a bed of alumina (Spence type H)

Table 1. Properties of benz[a]anthracene derivatives on t.l.c.

Benz[a]anthracene derivative	R_F in solvent			Fluorescence		Colour	
				Immediate	After exposure to NH_3	With $\text{Na}_2\text{Cr}_2\text{O}_7\text{-H}_2\text{SO}_4$	With dichloroquinone-chloroimide- Na_2CO_3
	(a)	(b)	(c)				
8-Hydroxy-8,9,10,11-tetrahydro (IV)	0.11	0.36	0.55	None	None	Brown	None
9-Hydroxy-8,9,10,11-tetrahydro (XIII)	0.05	0.30	0.48	None	None	Red	None
8-Oxo-8,9,10,11-tetrahydro (III)	0.27	0.62	0.89	Blue	Blue	Brown	None
9-Oxo-8,9,10,11-tetrahydro (IX)	0.24	0.57	0.85	None	Blue	Red	Brown
<i>cis</i> -8,9-Dihydroxy-8,9,10,11-tetrahydro (X)	0.00	0.17	0.35	None	None	Red	None
<i>trans</i> -8,9-Dihydroxy-8,9,10,11-tetrahydro	0.00	0.16	0.34	None	None	Red	None
8,9-Epoxy-8,9,10,11-tetrahydro (VIII)	0.49	0.85	0.98	None	None	Red	None
8-Hydroxy (XI)	0.35	0.52	0.73	Violet	Orange	Brown	Violet
9-Hydroxy (XIV)	0.23	0.46	0.68	Violet	Green	Brown	Green
8,9-Dihydro-8,9-dihydroxy (XVI)	0.00	0.29	0.42	Dark violet	Dark violet	Red	None
8,9-Epoxy-8,9-dihydro (XV)*	—	—	—	—	—	—	—

* The epoxide (XV) could not be chromatographed in any of the above solvents: decomposition to the phenols (X) and (XIV) occurred during both the spotting and the running of the chromatogram.

and recrystallizing the recovered hydrocarbon from ethanol.

Benz[a]anthracene 5,6-oxide. This was prepared as previously described (Newman & Blum, 1964; Boyland & Sims, 1965b).

β -(3-Phenanthryl)propionic acid (I) (Scheme 1). This acid was prepared as described by Haworth & Mavin (1933): it was purified by the method of Bachmann & Bradbury (1937) and it then separated from benzene in slender prisms, m.p. 155–156°C. Haworth & Mavin (1933) record m.p. 157–158°C for this compound.

γ -(3-Phenanthryl)butyric acid (II). A mixture of the keto acid (I) (35g), KOH (35g) and hydrazine hydrate (50ml) in diethylene glycol (500ml) was heated under reflux for 4h. The mixture was diluted with water and acidified with HCl, and the product was recrystallized from benzene to yield the butyric acid (II) (32g) in prisms, m.p. 138–139°C. Haworth & Mavin (1933) report m.p. 138–139°C for the substituted butyric acid obtained by reducing the keto acid (I) with Zn–HCl.

8-Oxo-8,9,10,11-tetrahydrobenz[a]anthracene (III). The butyric acid (II) was converted into its acid chloride with SOCl_2 and cyclized with SnCl_4 as described by Bachmann (1939) to yield the ketone (III), a portion of which was recrystallized from ethanol in prisms, m.p. 179–180°C, λ_{max} 210, 222, 230 (inf.), 263 (inf.), 271, 294, 308.5, 359 and 375nm ($\log \epsilon$ 4.12, 4.24, 4.00, 4.57, 4.71, 4.17, 3.97, 2.98 and 2.90 respectively). The i.r. spectrum showed strong carbonyl absorption at 5.95 μm . Haworth & Mavin (1933) report m.p. 179–180°C for this ketone.

8-Hydroxy-8,9,10,11-tetrahydrobenz[a]anthracene (IV). The crude ketone (III) (15g) was suspended in methanol (300ml) and treated with an excess of NaBH_4 . When the ketone had dissolved, the solution was evaporated to about 100ml under reduced pressure and the residue diluted with water. The hydroxy compound (IV) (14.5g) separated and was recrystallized from benzene in needles, m.p. 122–123°C, λ_{max} at 210, 217, 225, 250 (inf.), 256, 270 (inf.), 279, 286, 298, 317, 327, 334 and 350nm ($\log \epsilon$ 4.25, 4.52, 4.42, 4.72, 4.80, 4.32, 4.22, 4.08, 4.07, 2.76, 2.59, 2.74, 2.58 and 2.72 respectively). Bachmann (1939) reports m.p. 124–125°C for the product obtained when the ketone (III) was reduced with aluminium isopropoxide.

10,11-Dihydrobenz[a]anthracene (V). The hydroxy compound (IV) (12g) in acetic acid (100ml) was heated on the steam bath for 15min with a few drops of conc. HCl. The solution was diluted with water, and the product that separated was purified by dissolving it in light petroleum (b.p. 80–100°C) and passing the solution through a bed of alumina. The purified product was recrystallized from ethanol to yield 10,11-dihydrobenz[a]anthracene (V) (9.5g) in flat needles, m.p. 112–113°C, λ_{max} at 212, 221, 227, 260 (inf.), 265, 272, 295, 308, 330 (inf.), 345, 358 and 364nm ($\log \epsilon$ 4.27, 4.08, 4.08, 4.56, 4.78, 4.87, 4.27, 4.16, 3.69, 2.64, 2.34 and 2.52 respectively). Bachmann (1939) reports m.p. 112–113.5°C for the compound (V) prepared by the dehydrochlorination of the 7-chlorotetrahydro compound.

8,9-Dibromo-8,9,10,11-tetrahydrobenz[a]anthracene (VI). The dihydro compound (V) (9g) in carbon tetrachloride (150ml) was treated at room temperature with a solution of bromine (2ml) in carbon tetrachloride (10ml). The solvent was immediately removed under reduced pressure

to yield a yellow oil that rapidly crystallized. The dibromo compound (VI) (15g) was used without further purification, but a portion recrystallized from light petroleum (b.p. 80–100°C) formed pale-yellow fluffy needles, darkening at 122°C and melting at 133°C (with decomposition) (Found: Br, 40.5. $C_{18}H_{14}Br_2$ requires Br, 41.0%), λ_{max} . at 219, 225, 250 (infl.), 258, 268 (infl.), 277 (infl.), 294, 326 (infl.), 339 and 361 nm ($\log \epsilon$ 4.44, 4.42, 4.57, 4.70, 4.62, 4.43, 4.14, 2.71, 2.71 and 2.54 respectively).

8,9,10,11-Tetrahydrobenz[a]anthracene-8,9-bromohydrin (VII). The crude dibromo compound (VI) (12g) in aq. 50% (v/v) acetone was heated under reflux for 30 min with an excess of $MgCO_3$. The mixture was filtered and the filtrate evaporated under reduced pressure to remove the acetone. The solid that separated was recrystallized from benzene–light petroleum (b.p. 80–100°C) to yield the bromohydrin (VII) (8g) in needles, m.p. 151–152°C (Found: C, 66.2; H, 4.4; Br, 24.2. $C_{18}H_{15}BrO$ requires C, 66.1; H, 4.6; Br, 24.4%), λ_{max} . at 213, 222, 225, 250 (infl.), 262, 270 (infl.), 279, 286, 298, 314, 328, 331, 344 and 351 nm ($\log \epsilon$ 4.54, 4.57, 4.45, 4.70, 4.80, 4.25, 4.21, 4.07, 3.04, 2.86, 2.96, 2.36 and 2.96 respectively).

10,11-Benz[a]anthracene 8,9-oxide (VIII). A solution of the bromohydrin (VII) (8g) in ethanol (200 ml) was cooled to 0°C and stirred while a solution of KOH (1.5g) in methanol (25 ml) was added in portions during 15 min. The solid that separated was recrystallized from light petroleum (b.p. 80–100°C) to yield 10,11-dihydrobenz[a]anthracene 8,9-oxide (8,9-epoxy-8,9,10,11-tetrahydrobenz[a]anthracene) (VIII) (4.3g) in plates, m.p. 158–160°C (Found: C, 87.6; H, 5.9. $C_{18}H_{14}O$ requires C, 87.8; H, 5.7%), λ_{max} . at 217, 225, 250 (infl.), 257, 272, 278.5, 285, 296, 313, 320, 328, 335.5, 343 and 361 nm ($\log \epsilon$ 4.43, 4.32, 4.70, 4.84, 4.11, 4.16, 4.06, 3.95, 2.73, 2.63, 2.54, 2.68, 2.51 and 2.63 respectively). The i.r. spectrum showed a band at 11.25 μ m that may be attributed to the oxirane structure (cf. Bellamy, 1956).

The epoxide (VIII) (100mg) in acetic acid (1 ml) containing a few drops of conc. HCl was heated on the steam bath for 10 min. The cooled solution was diluted with water, and the product that separated was recrystallized from ethanol to yield the ketone (IX) (65mg) in plates, m.p. and mixed m.p. 147–148°C.

11-Bromo-10,11-dihydrobenz[a]anthracene 8,9-oxide (XII). The oxide (VIII) (2g), *N*-bromosuccinimide (1.46g) and $\alpha\alpha'$ -azoisobutyronitrile (50mg) in carbon tetrachloride (100 ml) were heated under reflux until the reaction commenced, as indicated by a darkening in colour of the reaction mixture and the separation of succinimide. The time needed to initiate the reaction varied between experiments and the initiation appeared to be facilitated if the reaction was carried out in direct sunlight. Once the reaction had started it was allowed to continue for 30 min, and the solution was cooled and succinimide filtered off. The filtrate was evaporated under reduced pressure and the residual gum extracted with dry ether (100 ml) at room temperature. The solid (850mg) that separated on keeping the ethereal solution overnight at 0°C is presumed to be 11-bromo-10,11-dihydrobenz[a]anthracene 8,9-oxide (XII), by analogy with the product obtained when 1,2-epoxy-1,2,3,4-tetrahydronaphthalene is treated under similar conditions with *N*-bromosuccinimide (Vogel & Klärner, 1968). The bromo oxide (XII)

was recrystallized from ether in pale-pink plates, m.p. 80–82°C (decomp.) (Found: C, 66.2; H, 4.0. $C_{18}H_{13}BrO$ requires C, 66.5; H, 4.0%), λ_{max} . at 216, 224 (infl.), 250 (infl.), 256.5, 270 (infl.), 279, 284 (infl.), 2.97, 306 (infl.), 317 (infl.), 334 and 351 nm ($\log \epsilon$ 4.42, 4.32, 4.38, 4.79, 4.26, 4.18, 4.11, 3.00, 3.10, 3.10 and 2.88 respectively). Attempts to recrystallize the epoxide from higher-boiling solvents such as cyclohexane resulted in its decomposition to a dark-green gum. Similar gums were formed when the solid was kept at room temperature for some days. Examination of these gums by t.l.c. showed the presence of 9-hydroxybenz[a]anthracene (XIV) together with a number of unidentified substances.

Benz[a]anthracene 8,9-oxide (XV). A solution of the crude bromo epoxide (XII) (500mg) in tetrahydrofuran (5 ml) was kept overnight with 1,5-diazobicyclo[4.3.0]non-5-ene (Ralph N. Emanuel Ltd., Wembley, Middx., U.K.) (0.5 ml). The mixture was diluted with ether (50 ml) and the ethereal solution was washed three times with water and dried (Na_2SO_4). The ether was removed under reduced pressure and the residue recrystallized from aq. acetone to yield benz[a]anthracene 8,9-oxide (8,9-epoxy-8,9-dihydrobenz[a]anthracene) (XV) (380mg) in clusters of tiny needles, decomposing at 112–115°C (Found: C, 88.3; H, 5.0. $C_{18}H_{12}O$ requires C, 88.5; H, 5.0%), λ_{max} . at 221, 226, 235, 260 (infl.), 268, 275 (infl.), 285 (infl.), 295, 308, 322, 334 (infl.), 347, 356 and 366 nm ($\log \epsilon$ 4.28, 4.12, 4.17, 4.66, 4.69, 4.74, 4.39, 4.12, 4.06, 4.26, 4.30, 3.03, 3.09, 2.79 and 3.03 respectively). The i.r. spectrum showed a band at 11.30 μ m, attributable to the oxirane structure. Newman & Blum (1964) found a band at a similar wavelength in the spectrum of the isomeric benz[a]anthracene 5,6-oxide.

The epoxide (10mg) in aq. 50% (v/v) acetone (5 ml) was heated under reflux for 8 h. The acetone was distilled off and the aqueous mixture extracted with ether (10 ml). The gummy residue obtained on evaporation of the ether was chromatographed on a t.l.c. plate in solvent (c). A number of products were detected when the chromatogram was examined in u.v. light, including compounds with the chromatographic properties and u.v.-absorption spectra of 8- and 9-hydroxybenz[a]anthracene (XI and XIV). A third product, present in small amounts, was identical in its chromatographic and spectroscopic properties with the 8,9-dihydrodiol (XVI) (Scheme 2) described below.

cis-8,9-Dihydroxy-8,9,10,11-tetrahydrobenz[a]anthracene (X). 10,11-Dihydrobenz[a]anthracene (V) (2g) in benzene (20 ml) was treated with osmium tetroxide (2g) at room temperature. A rapid reaction set in, and after 2 h the solid osmium complex was filtered off and dissolved in methylene chloride (100 ml). This solution was shaken with an excess of a solution of mannitol (10%, w/v) and KOH (1%, w/v) in water. After about 2 h the methylene chloride layer had become almost colourless. It was separated and dried (Na_2SO_4), and the methylene chloride was removed under reduced pressure. The residue was crystallized from benzene to yield cis-8,9-dihydroxy-8,9,10,11-tetrahydrobenz[a]anthracene (X) (1.8g) in needles, m.p. 190–191°C (Found: C, 82.1; H, 5.9. $C_{18}H_{16}O_2$ requires C, 81.8; H, 6.1%), λ_{max} . at 216, 224, 250 (infl.), 255.5, 270 (infl.), 278, 285.5, 298, 317, 326, 334, 342 and 350 nm ($\log \epsilon$ 4.51, 4.36, 4.73, 4.82, 4.27, 4.25, 4.10, 4.10, 2.85, 2.83, 2.82, 2.64 and 2.81 respectively). The diacetate

Scheme 2. Metabolic and chemical reactions of benz[*a*]anthracene 8,9-oxide (XV). 10,11-Dihydrobenz[*a*]anthracene 8,9-oxide (VIII) undergoes similar reactions.

formed prisms from ethanol, m.p. 152°C (Found: C, 75.9; H, 5.8. $C_{22}H_{20}O_4$ requires C, 75.8; H, 5.8%). The diol (X) rapidly changed the colour of the potassium triacetylosmate reagent of Criegee, Marchand & Wannowius (1942) from blue to grey: this is a reaction of *cis*-diols, but not of *trans*-diols.

9-Oxo-8,9,10,11-tetrahydrobenz[*a*]anthracene (IX). A solution of the diol (X) (2g) in acetic acid (20ml) was heated on the steam bath for 15 min with a few drops of conc. HCl. The solution was diluted with water, and the product that separated was recrystallized from ethanol in pale-pink plates (1.5g), m.p. 147–148°C. When a portion of the product was chromatographed in benzene on silica gel, the pure *ketone* (IX) was obtained, which formed colourless plates from ethanol, m.p. 147–148°C (Found: C, 87.6; H, 5.9. $C_{18}H_{14}O$ requires C, 87.8; H, 5.7%), $\lambda_{\max.}$ at 216, 222 (infl.), 248 (infl.), 254.5, 271, 278, 283, 291, 317, 324.5, 332, 340 and 348 nm ($\log \epsilon$ 4.43, 4.29, 4.67, 4.79, 4.25, 4.20, 4.11, 4.09, 2.86, 2.72, 2.72, 2.22 and 2.55 respectively). The i.r. spectrum showed strong carbonyl absorption at 5.86 μ m.

9-Hydroxy-8,9,10,11-tetrahydrobenz[*a*]anthracene (XIII). The crude *ketone* (IX) (500mg) was reduced in methanol with $NaBH_4$. The product was recovered by evaporating off most of the methanol under reduced pressure and diluting the residue with water. It was recrystallized from benzene to yield the *hydroxy compound* (XIII) (480mg), m.p. 126°C (Found: C, 86.9; H, 6.3. $C_{18}H_{16}O$ requires C, 87.1; H, 6.5%), $\lambda_{\max.}$ at 217, 225, 249 (infl.), 225, 273.5, 280, 286, 298, 318, 326, 334, 342 and

349 nm ($\log \epsilon$ 4.51, 4.37, 4.73, 4.80, 4.26, 4.10, 4.09, 2.82, 2.60, 2.78, 2.60 and 2.77 respectively).

trans-8,9-Dihydroxy-8,9,10,11-tetrahydrobenz[*a*]anthracene. 10,11-Dihydrobenz[*a*]anthracene 8,9-oxide (VIII) (1g) was heated under reflux with aq. 50% (v/v) acetone (50ml) for 24 h. The solution was diluted with water, and the product that separated was recrystallized from benzene to yield the *trans-diol* (800mg) in plates, m.p. 190–191°C (Found: C, 82.3; H, 6.2. $C_{18}H_{16}O$ requires C, 81.8; H, 6.1%), $\lambda_{\max.}$ at 217, 224.5, 250 (infl.), 278, 285.5, 298, 317, 326, 334, 342 and 350 nm ($\log \epsilon$ 4.49, 4.38, 4.73, 4.83, 4.28, 4.25, 4.11, 4.11, 2.83, 2.81, 2.28, 2.59 and 2.80 respectively). A mixture with the *cis*-diol (X) had m.p. 155–165°C. The *trans*-diol failed to discharge the blue colour of the potassium triacetylosmate reagent.

The *trans*-diol was decomposed with HCl in acetic acid in a similar manner to the *cis*-diol to yield the 9-*ketone* (IX), identified by its chromatographic properties and its u.v.-absorption spectrum.

9-Hydroxybenz[*a*]anthracene (XIV). The *ketone* (IX) (250mg) and sulphur (150mg) were heated at 220°C for 30 min, and the product was then separated from the reaction mixture by sublimation at 270°C (bath)/15 mmHg. **9-Hydroxybenz[*a*]anthracene (XIV)** (65mg) was recrystallized from benzene in pale-yellow needles, m.p. 212–215°C (darkening at 200°C) (Found: C, 88.1; H, 5.0. $C_{15}H_{12}O$ requires C, 88.5; H, 4.9%), $\lambda_{\max.}$ at 211, 224, 233, 249 (infl.), 254, 277, 287, 306, 330, 355 (infl.), 370 (infl.) and 392 nm ($\log \epsilon$ 4.27, 4.31, 4.41, 4.46, 4.62, 4.82, 4.32, 3.61, 3.51, 3.31 and 3.08 respectively). The *methyl ether*,

prepared by treating the phenol (XIV) in methanol with a solution of diazomethane in ether, separated from light petroleum (b.p. 80–100°C) in plates, m.p. 185°C (Found: C, 88.5; H, 5.4. $C_{19}H_{14}O$ requires C, 88.3; H, 5.4%), $\lambda_{\max}$. at 210, 224, 231, 247 (infl.), 252, 275, 285, 290 (infl.), 301, 325, 332, 340, 348 (infl.), 355 (infl.) and 386 nm ($\log \epsilon$ 4.38, 4.44, 4.45, 4.52, 4.58, 4.78, 4.99, 4.80, 4.50, 3.83, 3.81, 3.80, 3.67, 3.54 and 3.14 respectively).

8-Hydroxybenz[a]anthracene (XI). This was prepared by heating the ketone (III) with sulphur as described above. It formed pale-yellow needles from benzene, m.p. 212–215°C (decomp.), $\lambda_{\max}$. at 221.5, 226, 272, 284, 295, 340 (infl.), 360 and 386 nm ($\log \epsilon$ 4.39, 4.44, 4.65, 4.59, 4.54, 3.51, 3.59 and 3.35 respectively). It yielded a methyl ether in needles from light petroleum (b.p. 80–100°C), m.p. 185°C. Cook & Schoental (1952) report m.p. 217–218°C for the phenol and m.p. 186–187°C for the methyl ether.

Reactions of the epoxides (VIII) and (XV) with *N*-acetylcysteine and with GSH

The epoxide (VIII or XV) (10 mg), $NaHCO_3$ (20 mg) and either *N*-acetylcysteine or GSH (10 mg) in aq. 50% (v/v) acetone (20 ml) were heated under reflux for 6 h. The acetone was distilled off under reduced pressure and the aqueous mixtures were extracted with equal volumes of ether. The residues remaining when the ether was evaporated were examined by t.l.c. The aqueous layers were acidified to pH 4 with acetic acid, and activated charcoal (British Drug Houses Ltd., Poole, Dorset, U.K.) (2 g) was added. The charcoal was filtered off and washed with water, and the absorbed material was eluted with methanol (500 ml) containing 5% (v/v) of aq. NH_3 (sp.gr. 0.88). The solutions were evaporated and the residues examined on paper chromatograms.

The ether extracts from the reactions of the epoxide (XV) with the thiols contained compounds indistinguishable chromatographically and spectroscopically from 8- and 9-hydroxybenz[a]anthracene (XI) and (XIV). The chromatographic properties of the *N*-acetylcysteine and GSH conjugates present in the aqueous layers are shown in Table 2. Both conjugates had identical u.v.-absorption spectra that closely resembled that of the dihydrodiol (XVI), $\lambda_{\max}$. at 210, 222, 227, 258, 266, 274 (infl.), 284, 293, 306, 321, 345, 352 and 362 nm. Both conjugates were decomposed rapidly by cold 2M-HCl to yield mixtures of products: 8- and 9-hydroxybenz[a]anthracene were identified by t.l.c. as before and products that were probably the arylmercapturic acid and GSH conjugates [$\lambda_{\max}$. at 211, 232, 243, 268 (infl.), 279, 280, 350 and 388 nm] were detected when the mixtures from the respective conjugates were examined on paper chromatograms. *NN*-Diacylcysteine and GSSG were also detected in the products of the acid decomposition of the respective conjugates by using the methods described by Boyland, Ramsay & Sims (1961): *N*-acetylcysteine and GSH were not detected.

Hydrogenolysis of the GSH conjugate with Raney nickel by the method previously described (Boyland & Sims, 1962) showed that 9-hydroxybenz[a]anthracene (XIV) was the main phenolic product but that small amounts of the isomeric 8-hydroxy compound (XI) were also formed. Thus the *N*-acetylcysteine and GSH conjugates probably have the structures (XVII) and (XVIII) respectively shown in Scheme 2, but the presence of small amounts of the isomeric 9-substituted compound is possible.

The ethereal extracts obtained after the reaction of the epoxide (VIII) with *N*-acetylcysteine and with GSH both contained compounds with the chromatographic properties and u.v.-absorption spectrum of 8,9-dihydroxy-8,9,10,11-tetrahydrobenz[a]anthracene. The residues

Table 2. Properties of the *N*-acetylcysteine and GSH conjugates of benz[a]anthracene on paper chromatograms

Details are given in the text.

Probable structure of conjugate	R_F	Fluorescence	Colour with ninhydrin	Reaction with $Na_2Cr_2O_7$ -AgNO ₃ reagent
<i>N</i> -Acetyl- <i>S</i> -(9-hydroxy-8,9,10,11-tetrahydrobenz[a]anthracen-8-yl)cysteine	0.52	None	None	++
<i>S</i> -(9-Hydroxy-8,9,10,11-tetrahydrobenz[a]anthracen-8-yl)glutathione	0.12	None	Purple	++
<i>N</i> -Acetyl- <i>S</i> -(9-hydroxy-8,9-dihydrobenz[a]anthracen-8-yl)cysteine (XVII)	0.37	Dark violet	None	++
<i>N</i> -Acetyl- <i>S</i> -(benz[a]anthracen-8-yl)cysteine (benz[a]anthracene 8-mercapturic acid)	0.51	Violet	None	+
<i>S</i> -(9-Hydroxy-8,9-dihydrobenz[a]anthracen-8-yl)-glutathione (XVIII)	0.10	Dark violet	Purple	++
<i>S</i> -(Benz[a]anthracen-8-yl)-glutathione	0.18	Violet	Purple	+

from the aqueous layers contained compounds with the expected chromatographic properties (Table 2) of the *N*-acetylcysteine and GSH conjugates. The u.v.-absorption spectra of the conjugates closely resembled those of the related dihydroxy derivative (X) and both showed $\lambda_{\max}$. at 220, 250 (infl.), 259, 272 (infl.), 281, 287 (infl.), 299, 321, 329, 336, 344 and 352 nm. When the conjugates, obtained by eluting the appropriate bands from paper chromatograms with methanol, were heated with conc. HCl on the steam bath for 15 min, they both yielded a product that was identical in its chromatographic properties and u.v.-absorption spectrum with the 9-ketone (IX), indicating that the conjugates were the 9-hydroxy-8,9,10,11-tetrahydrobenz[a]anthracen-8-yl derivatives.

Experiments with rat liver preparations

Homogenates and microsomal fractions were prepared from the livers of 35-day-old male rats of the Chester Beatty strain as described previously (Sims, 1970). In the experiments with epoxides, incubations were carried out with homogenates prepared from 40 g of liver or with microsomal fractions prepared from 5 g of the livers of normal rats: no cofactors were added. In the experiments with hydrocarbons, incubations were carried out with homogenates prepared from the livers of rats that had each been treated 48 h previously with 3-methylcholanthrene (5 mg) in arachis oil (5 ml) administered by intraperitoneal injection. In these incubations the cofactors in the amounts previously described (Sims & Grover, 1968) were added. All the incubations were carried out for 30 min at 37°C and the incubation mixtures were each extracted with equal volumes of ethyl acetate. The extracts were dried (Na_2SO_4) and examined as described below. In some experiments the aqueous layers were filtered to remove protein, and water-soluble metabolites were recovered from the filtrates with charcoal as described above. The recovered metabolites were examined on paper chromatograms as indicated below.

Control experiments in which homogenates that had boiled for 5 min were also carried out.

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

These were carried out at 37°C, as previously described (Grover & Sims, 1970), with benz[a]anthracene 5,6-oxide, benz[a]anthracene 8,9-oxide (XV) and 10,11-dihydrobenz[a]anthracene 8,9-oxide (VIII) as substrates. The results are shown in Fig. 1.

RESULTS

Metabolism of benz[a]anthracene 8,9-oxide (XV) by a rat liver homogenate. The epoxide (XV) (35 mg) in acetone (5 ml) was incubated with rat liver homogenate as described above. The ethyl acetate extract was evaporated and the residue applied to the base line of a t.l.c. plate that was developed for 15 cm with solvent (c). Three main products were detected when the chromatogram was examined in u.v. light. Two were identified by their u.v.-absorption spectra as 8- and 9-hydroxybenz[a]anthracene (XI and XIV). The third product

Fig. 1. Increase in colour formation with time of incubation at 37°C resulting from the reaction of 0.2 μmol of benz[a]anthracene 5,6-oxide (○), 10,11-dihydrobenz[a]anthracene 8,9-oxide (VIII) (△) and benz[a]anthracene 8,9-oxide (XV) (□) with 4-(*p*-nitrobenzyl)pyridine at pH 7.4.

formed a thin band with a dark-violet fluorescence: the silica gel in this region of the chromatogram was removed and the absorbed material eluted with ether. Evaporation of the ether yielded a product that was recrystallized from benzene to yield what is presumed to be the *trans* form of 8,9-dihydro-8,9-dihydroxybenz[a]anthracene (XVI) (9 mg) in needles, m.p. 196°C (Found: C, 82.0; H, 5.4. $\text{C}_{18}\text{H}_{14}\text{O}$ requires C, 82.4; H, 5.4%), $\lambda_{\max}$. at 210, 221, 228, 256, 264.5, 272 (infl.), 285, 291 (infl.), 304.5, 318, 344, 351 and 362 nm (log ϵ 4.08, 4.04, 4.04, 4.54, 4.62, 4.26, 4.05, 3.94, 4.15, 4.20, 2.46, 2.40 and 2.34 respectively).

When the dihydrodiol (XVI) was heated on the steam bath for 15 min with 5 *M*-hydrochloric acid, a mixture was obtained that was shown to contain products with the chromatographic properties and u.v.-absorption characteristics of 8- and 9-hydroxybenz[a]anthracene (XI and XIV).

An examination on paper chromatograms of the material obtained from the aqueous layer showed the presence of a product with the chromatographic and spectroscopic properties of 8-*S*-(9-hydroxy-8,9-dihydrobenz[a]anthracen-8-yl)glutathione (XVIII). The metabolite was decomposed by cold 2 *M*-hydrochloric acid in the same manner as the synthetic conjugate described above to yield 8- and 9-hydroxybenz[a]anthracene (XI and XIV) and the conjugate believed to be *S*-(benz[a]anthracen-8-yl)glutathione.

In a control experiment with boiled homogenate neither the dihydrodiol (XVI) nor the GSH conjugate (XVIII) was detected. 8- and 9-Hydroxybenz[a]anthracene (XI and XIV) were identified

when the ethyl acetate extract was examined by t.l.c.

Metabolism of benz[a]anthracene 8,9-oxide (XV) by rat liver microsomal fraction. The ethyl acetate extract was examined by t.l.c. and shown to contain products with the chromatographic characteristics and u.v.-absorption spectra of the phenols (XI and XIV) and the dihydrodiol (XVI).

Metabolism of benz[a]anthracene by a rat liver homogenate. The metabolism of this compound has already been investigated in detail (Boyland & Sims, 1964, 1965b; Sims, 1970) and one of the products tentatively identified as 8,9-dihydro-8,9-dihydroxybenz[a]anthracene (XVI). In the present work the ethyl acetate fraction obtained from the incubation of benz[a]anthracene (5 mg) with rat liver homogenates was evaporated and the residue applied to the base line of a t.l.c. plate that was then developed in solvent (c). The band of silica gel containing the metabolite believed to be the dihydrodiol (XVI) was removed and the metabolite eluted from the silica gel with ether. A direct comparison of this metabolite with that obtained from the epoxide (XV) as described above showed (1) that the two products were indistinguishable on t.l.c. with either solvent (b) or (c), (2) that the u.v.-absorption spectra of the two compounds were identical and (3) that both compounds, when treated with hot 5M-hydrochloric acid, yielded products with the chromatographic characteristics and u.v.-absorption spectra of 8- and 9-hydroxybenz[a]anthracene (XI and XIV). It is therefore concluded that the hydrocarbon and the epoxide are both metabolized to the dihydrodiol (XVI).

Metabolism of 10,11-dihydrobenz[a]anthracene 8,9-oxide (VIII) by a rat liver homogenate. The epoxide (VIII) (50 mg) in acetone (10 ml) was incubated with rat liver homogenate as described above. Portions of the ethyl acetate extract were examined by t.l.c. with solvents (b) and (c), when a compound with the properties of *trans*-8,9-dihydroxy-8,9,10,11-tetrahydrobenz[a]anthracene was the only product detected. The extract was evaporated to dryness under reduced pressure and the residue recrystallized from benzene to yield *trans*-8,9-dihydroxy-8,9,10,11-tetrahydrobenz[a]anthracene (15 mg) in plates, m.p. and mixed m.p. 190–191°C. The metabolite failed to discharge the colour of the potassium triacetylosmate reagent.

The aqueous layer contained a product with the properties on paper chromatograms and the u.v.-absorption spectrum of *S*-(9-hydroxy-8,9,10,11-tetrahydrobenz[a]anthracen-8-yl)glutathione. When the metabolite was heated with 5M-hydrochloric acid it yielded a product with the chromatographic properties and u.v.-absorption spectrum of 9-oxo-8,9,10,11-tetrahydrobenz[a]anthracene.

In a control experiment with boiled homogenate

unchanged epoxide (VIII) was recovered but the dihydroxy compound and the GSH conjugate were not detected.

Metabolism of 10,11-dihydrobenz[a]anthracene 8,9-oxide (VIII) by rat liver microsomal fraction. The ethyl acetate extract from this incubation was shown by t.l.c. to contain a product with the chromatographic properties and u.v.-absorption characteristics of *trans*-8,9-dihydroxy-8,9,10,11-tetrahydrobenz[a]anthracene.

Metabolism of 10,11-dihydrobenz[a]anthracene (V) by a rat liver homogenate. This incubation was carried out with a solution of the hydrocarbon (V) (5 mg) in acetone (5 ml). Examination of the ethyl acetate extract by t.l.c. showed that, apart from the hydrocarbon (V), only one product was present, which was identified by its chromatographic properties and u.v.-absorption spectrum as 8,9-dihydroxy-8,9,10,11-tetrahydrobenz[a]anthracene. It is presumed to have the *trans* configuration since it did not discharge the blue colour of the potassium triacetylosmate reagent.

A small amount of a product was detected in the aqueous layer that had the chromatographic and u.v.-absorption characteristics of *S*-(9-hydroxy-8,9,10,11-tetrahydrobenz[a]anthracen-8-yl)glutathione.

DISCUSSION

It was suggested many years ago (Boyland, 1950) that epoxides are involved as intermediates in the metabolism of aromatic double bonds. Support for this hypothesis has come from studies on the metabolism of arene oxides such as phenanthrene 9,10-oxide (Boyland & Sims, 1965a), benz[a]anthracene and dibenz[a,h]anthracene 5,6-oxides (Boyland & Sims, 1965b), 3-methylcholanthrene 11,12-oxide (Sims, 1966), benzene oxide (Jerina, Daly, Witkop, Zaltzman-Nirenberg & Udenfriend, 1968) and naphthalene 1,2-oxide (Jerina, Daly, Witkop, Zaltzman-Nirenberg & Udenfriend, 1970), in which it was shown that the epoxides and their parent hydrocarbons yielded the same dihydrodiols and glutathione conjugates on metabolism. With phenanthrene, the same mercapturic acid was excreted when either phenanthrene or its 9,10-oxide was given to rats (Boyland & Sims, 1965a). Jerina *et al.* (1970) have also obtained evidence for the formation of naphthalene 1,2-oxide when naphthalene is incubated with hepatic microsomal fraction.

In all the experiments with the epoxides, phenols derived from the epoxides by their rearrangement were also obtained, although these are not necessarily formed when the parent hydrocarbons are metabolized (Sims, 1970). Jerina *et al.* (1968, 1970) have shown that the rearrangements of benzene

oxide and naphthalene oxide to phenols are catalysed by protein in a non-enzymic reaction.

In the present work, benz[*a*]anthracene 8,9-oxide (XV) was converted both by rat liver homogenates and microsomal fractions into 8- and 9-hydroxy-benz[*a*]anthracene (XI and XIV). Earlier work has shown that these phenols were excreted, as conjugates with sulphuric acid and glucuronic acid, when rats, rabbits and mice were dosed with benz[*a*]anthracene (Boyland & Sims, 1964), but they were not detected among the metabolic products formed when benz[*a*]anthracene was incubated with rat liver homogenates (Boyland & Sims, 1965b; Sims, 1970).

The dihydrodiol (XVI) formed from the epoxide (XV) by rat liver homogenates was, as far as could be determined, identical with that similarly formed from benz[*a*]anthracene: the configuration of the hydroxyl groups is presumably *trans* in both cases, but no direct evidence of this is available. The dihydrodiol (XVI) also appears to be the same as that detected, in conjugation with sulphuric acid and glucuronic acid, in the urine of rats, mice and rabbits dosed with benz[*a*]anthracene (Boyland & Sims, 1964). The dihydrodiol is presumably formed from the epoxide by the microsomal 'epoxide hydrases' described by Jerina *et al.* (1968), Brookes, Harrison & Lewis (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).

The mercapturic acid (XVII) obtained when the epoxide (XV) was allowed to react chemically with *N*-acetylcysteine had the same chemical and chromatographic properties as a mercapturic acid detected in the urine of animals treated with benz[*a*]anthracene. The epoxide (XV) reacted both chemically and enzymically with GSH to form the conjugate (XVIII). Presumably this reaction is catalysed by the enzyme 'glutathione *S*-epoxide-transferase' described by Boyland & Williams (1965). The reactions of other 'K-region' arene oxides with GSH are catalysed by this enzyme (Boyland, Sims & Williams, 1965).

Many arene oxides exist in equilibrium with their valence tautomers, the seven-membered cyclic oxepins. Thus benzene oxide is in equilibrium with oxepin itself (Günther, 1965). The chemical, physical and biochemical evidence, however, suggests that benz[*a*]anthracene 8,9-oxide (XV), like naphthalene 1,2-oxide (Vogel & Klärner, 1968), exists entirely in the oxirane form.

8,9,10,11-Tetrahydrobenz[*a*]anthracene 8,9-oxide (VIII) was more stable chemically than the arene oxide (XV); presumably the rearrangement to the phenols in the latter compound is facilitated by the gain in resonance energy when the non-aromatic state in the oxide is transformed into the

aromatic state in the phenols. In its other chemical and in its enzymic reactions, however, the oxide (VIII) behaved in a similar manner to the oxide (XV), yielding a dihydrodiol and a GSH conjugate that appeared to be identical with the corresponding metabolites formed from 10,11-dihydrobenz[*a*]anthracene (V). Leibman & Ortiz (1970) have shown that compounds such as styrene, cyclohexene and indene, which contain olefinic double bonds, are converted into epoxide intermediates by liver microsomal fractions. It is therefore likely that the epoxide (VIII) is formed as an intermediate in the metabolism of the hydrocarbon (V).

Benz[*a*]anthracene 5,6-oxide reacts both chemically (Grover & Sims, 1970) and in cells in culture (Grover, Forrester & Sims, 1971) with nucleic acids and proteins. Similar investigations with benz[*a*]anthracene 8,9-oxide (XV) await the preparation of the ³H-labelled compound, but in the alkylation of 4-(*p*-nitrobenzyl)pyridine the 5,6-oxide proved to be a better alkylating agent than either of the epoxides (VIII) or (XV). There are indications that arene oxides are the active intermediates concerned in the biological effects produced by aromatic hydrocarbons, and it has been found that benz[*a*]anthracene 5,6-oxide is more effective than benz[*a*]anthracene in producing malignant transformations in hamster embryo cells and in C3H mouse ventral-prostate cells (Grover *et al.* 1971). Dibenz[*a*]anthracene 5,6-oxide and 3-methylcholanthrene 11,12-oxide are also more active than are their parent hydrocarbons in these systems.

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