Epoxy Derivatives of Aromatic Polycyclic Hydrocarbons

THE PREPARATION AND METABOLISM OF EPOXIDES RELATED TO 7,12-DIMETHYLBENZ[*a*]ANTHRACENE

By P. SIMS

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

(Received 20 September 1972)

The syntheses of 7,12-dimethylbenz[*a*]anthracene 5,6-oxide, 7-acetoxymethyl-12-methylbenz[*a*]anthracene 5,6-oxide and a product that appears to be mainly 7-hydroxymethyl-12-methylbenz[*a*]anthracene 5,6-oxide are described. The compounds readily rearranged to phenols in the presence of mineral acid, and 7,12-dimethylbenz[*a*]anthracene 5,6-oxide and its 7-hydroxymethyl derivative reacted slowly with water to yield *trans*-5,6-dihydro-5,6-dihydroxy-7,12-dimethylbenz[*a*]anthracene and *trans*-5,6-dihydro-5,6-dihydroxy-7-hydroxymethyl-12-methylbenz[*a*]anthracene respectively. Both epoxides were converted enzymically by rat liver microsomal fractions and homogenates into the related *trans*-dihydrodiols. The epoxides reacted chemically with GSH to form conjugates that were identical with the conjugates formed when the epoxides were incubated with rat liver homogenates. The GSH conjugates were more stable to acid than conjugates derived from other arene oxides. In the alkylation of 4-(*p*-nitrobenzyl)pyridine, 7,12-dimethylbenz[*a*]anthracene 5,6-oxide was more active than the 5,6-oxides of 7-methylbenz[*a*]anthracene and benz[*a*]anthracene.

It is now known that arene oxides are intermediates in the conversion of aromatic hydrocarbons into dihydrodiols and GSH conjugates by liver preparations (Jerina *et al.*, 1970; Selkirk *et al.*, 1971; Grover *et al.*, 1971*a*, 1972) and it is possible that the oxides are responsible for the biological effects induced by the parent hydrocarbons (Grover *et al.*, 1971*b*). The syntheses of 'K-region' epoxides of aromatic hydrocarbons were first described by Newman & Blum (1964), and since then the syntheses of other 'K-region' epoxides derived from both carcinogenic and inactive polycyclic hydrocarbons have been carried out (Boyland & Sims, 1965*c*; Sims, 1966). Of all the carcinogenic hydrocarbons, 7,12-dimethylbenz[*a*]anthracene (I, Scheme 1) is one of the most widely studied, but earlier attempts (Newman & Blum, 1964; P. Sims, unpublished work) to synthesize its 'K-region' epoxide, 7,12-dimethylbenz[*a*]anthracene 5,6-oxide (II), were unsuccessful. Since one of the pathways by which 7,12-dimethylbenz[*a*]anthracene (I) is metabolized by hepatic microsomal systems involves the hydroxylation of the methyl groups, to yield 7-hydroxymethyl-12-methylbenz[*a*]anthracene (IV) and 12-hydroxymethyl-7-methylbenz[*a*]anthracene (Boyland & Sims, 1965*a*), the possibility that the 'K-region' epoxides of the hydroxymethyl compounds are involved in the biological action of the parent hydrocarbon must also be considered.

The present paper describes the synthesis both of 7,12-dimethylbenz[*a*]anthracene 5,6-oxide (II) and of

a product that appears to be 7-hydroxymethyl-12-methylbenz[*a*]anthracene 5,6-oxide (VII). Some chemical and biochemical properties of the oxides have been investigated.

Experimental

Methods

Melting points. These are uncorrected.

Thin-layer chromatography. This was carried out either on glass plates (20 cm × 20 cm) coated with layers of silica gel G (E. Merck A.-G., Darmstadt, Germany) of 0.25 mm thickness or on Eastman 6060 Chromogram sheets (Eastman-Kodak Co., Rochester, N.Y., U.S.A.). The coated glass plates were developed with solvent (a), benzene, or solvent (b), benzene-ethanol (9:1, v/v), or solvent (c), butan-1-ol-propan-1-ol-aq. 2*M*-NH₃ (2:1:1, by vol.), and the Chromogram sheets with solvent (d), cyclohexane-dioxan (9:1, v/v), or solvent (e), cyclohexane-dioxan (3:1, v/v), or solvent (f), cyclohexane-dioxan (1:1, v/v). The products were detected by examining the plates in u.v. light both before and after exposure to NH₃. Chromatograms developed in solvent (c) were sometimes treated with a 0.2% solution of ninhydrin in acetone, applied by means of a brush, and heated in an oven to 110°C for 5 min. Material separated by preparative t.l.c. was eluted from the silica gel with diethyl ether. The properties

Scheme 1. Metabolic reactions of 7,12-dimethylbenz[a]anthracene 5,6-oxide (II) and 7-hydroxymethyl-12-methylbenz[a]anthracene 5,6-oxide (VII)

For details see the text.

of the compounds examined by t.l.c. are recorded in Table 1.

U.v.-absorption spectra. These were measured as described by Waterfall & Sims (1972). GSH conjugates were eluted from the silica gel with methanol containing 5% (v/v) of aq. NH_3 soln. (sp.gr. 0.88). The u.v. spectra of some of the compounds used in this work have been described previously (Boydland & Sims, 1965a, 1967).

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 Wistar rats as previously described.

In the experiments with homogenates, the oxides

(II and VII) (each 50mg) in acetone (5ml) were each incubated at 37°C for 30min with homogenate prepared from rat liver (40g). The incubates were extracted with equal volumes of ethyl acetate and the extracts were dried over Na_2SO_4 and evaporated under reduced pressure. The aqueous layers were filtered to remove protein and the filtrates were acidified to pH4 with acetic acid. Charcoal (2g) was added to each filtrate; the mixtures were filtered and the charcoal was washed with water (250ml). In each experiment the absorbed material was eluted from the charcoal with methanol (150ml) containing 5% (v/v) of aq. NH_3 soln. (sp.gr. 0.88). The solvent was removed under reduced pressure and the residues

Table 1. Properties of 7,12-dimethylbenz[a]anthracene derivatives on t.l.c. plates

Details are given in the text. —, R_f not measured. The fluorescences recorded are those of the compounds chromatographed on silica gel-coated glass plates; the compounds were detected on Chromogram sheets by the formation in u.v. light of dark or fluorescent spots against an orange fluorescent background.

Compound	Fluorescence		R_f on silica gel on glass plates			R_f on Chromogram sheets			
			Solvent (a)	Solvent (b)	Solvent (c)	Solvent (d)	Solvent (e)	Solvent (f)	
	Immediate	After exposure to NH_3							
7,12-Dimethylbenz[a]anthracene 5,6-oxide (II)	Violet	Violet	0.54	0.83	—	0.68	0.86	—	
5-Hydroxy-7,12-dimethylbenz[a]anthracene	Violet	Yellow	0.53	0.80	—	0.46	0.73	—	
7-Hydroxymethyl-12-methylbenz[a]anthracene 5,6-oxide (VII)	Violet	Violet	0.13	0.45	—	0.02	0.44	—	
5-Hydroxy-7-hydroxymethyl-12-methylbenz[a]anthracene	Violet	Yellow	0.12	0.38	—	0.00	0.28	—	
7-Acetoxyethyl-12-methylbenz[a]anthracene 5,6-oxide	Violet	Violet	0.32	0.70	—	0.50	0.73	—	
7-Acetoxyethyl-5-hydroxy-12-methylbenz[a]anthracene	Violet	Yellow	0.23	0.68	—	0.24	0.62	—	
<i>cis</i> -5,6-Dihydro-5,6-dihydroxy-7,12-dimethylbenz[a]anthracene	Dark violet	Dark violet	0.00	0.32	—	0.08	0.53	—	
<i>trans</i> -5,6-Dihydro-5,6-dihydroxy-7,12-dimethylbenz[a]anthracene (III)	Dark violet	Dark violet	0.00	0.21	—	0.07	0.37	—	
<i>cis</i> -5,6-Dihydro-5,6-dihydroxy-7-hydroxymethyl-12-methylbenz[a]anthracene	Dark violet	Dark violet	0.00	0.09	—	0.00	0.00	0.79	
<i>trans</i> -5,6-Dihydro-5,6-dihydroxy-7-hydroxymethyl-12-methylbenz[a]anthracene (VI)	Dark violet	Dark violet	0.00	0.08	—	0.00	0.00	0.71	
S-(5,6-Dihydro-6-hydroxy-7,12-dimethylbenz[a]anthracen-5-yl)glutathione (V)*	Dark violet	Dark violet	—	—	0.25	—	—	—	
S-(7,12-Dimethylbenz[a]anthracen-5-yl)-glutathione*	Violet	Violet	—	—	0.28	—	—	—	
N-Acetyl-S-(5,6-dihydro-6-hydroxy-7,12-dimethylbenz[a]anthracen-5-yl)cysteine	Dark violet	Dark violet	—	—	0.50	—	—	—	
7,12-Dimethylbenz[a]anthracen-5-ylmercapturic acid	Violet	Violet	—	—	0.52	—	—	—	
S-(5,6-Dihydro-6-hydroxy-7-hydroxymethyl-12-methylbenz[a]anthracen-5-yl)glutathione (VIII)*	Dark violet	Dark violet	—	—	0.20	—	—	—	
S-(7,12-Dimethylbenz[a]anthracen-5-yl)-glutathione*	Violet	Violet	—	—	0.22	—	—	—	

* These compounds gave purple colours with the ninhydrin reagent.

were examined by t.l.c. to give the results described below.

The oxides (II) and (VII) (5mg) in acetone (1ml) were each incubated at 37°C for 30min with microsomal fractions prepared from rat liver (4g). The mixtures were each extracted with equal volumes of ethyl acetate; the extracts were dried over Na₂SO₄ and evaporated and the residues examined by t.l.c.

In experiments with rat liver soluble fractions, the oxides (II and VII) (each 5mg) in acetone (1ml) were each incubated at 37°C for 30min with soluble fractions prepared from rat liver (4g) and GSH (5mg). The mixtures were extracted with equal volumes of ethyl acetate and the products thus extracted examined by t.l.c. as described above. The material present in the aqueous layer was recovered by means of charcoal as described above and examined by t.l.c.

The metabolism of 7-hydroxymethylbenz[a]anthracene (IV) by a rat liver microsomal fractions was also investigated. The incubation mixture consisted of the hydroxymethyl derivative (IV) (5mg) in acetone (1ml), the microsomal fraction obtained from rat liver (20g), and NADP⁺ (70.0mg), glucose 6-phosphate (500mg) and glucose 6-phosphate dehydrogenase (5.5 units) [all obtained from Boehringer Corp. (London) Ltd., London W.5, U.K.]. The incubation was carried out at 37°C for 30min and the ethyl acetate-soluble and the aqueous fractions were isolated and examined as described above.

Control experiments in which the oxides (II and VII) were incubated with rat liver homogenates and microsomal and soluble fractions that had been boiled for 5min were also carried out.

Materials

7,12-Dimethylbenz[a]anthracene (I) was obtained from Eastman-Kodak Co., Rochester, N.Y., U.S.A. *cis*- and *trans*-5,6-Dihydro-5,6-dihydroxy-7,12-dimethylbenz[a]anthracene (Cook & Schoental, 1948; Boyland & Sims, 1967), 7-hydroxymethyl-12-methylbenz[a]anthracene (IV) (Boyland & Sims, 1965a), *trans*-5,6-diacetoxy-5,6-dihydro-7,12-dimethylbenz[a]anthracene, *cis*-5,6-dihydro-5,6-dihydroxy-7-hydroxymethyl-12-methylbenz[a]anthracene and *cis*-7-acetoxymethyl-5,6-dihydro-5,6-dihydroxy-12-methylbenz[a]anthracene (Boyland & Sims, 1967), 1,4-dimethyl-2-phenylnaphthalene 3,2'-dicarboxaldehyde (Hadler & Kryger, 1960), and benz[a]anthracene 5,6-oxide and 7-methylbenz[a]anthracene 5,6-oxide (Newman & Blum, 1964) were prepared as described in the references.

Preparation of 7,12-dimethylbenz[a]anthracene 5,6-oxide (5,6-epoxy-5,6-dihydro-7,12-dimethylbenz[a]anthracene) (II). 1,4-Dimethyl-2-phenylnaphthalene 3,2'-dicarboxaldehyde (1g) in benzene (2ml) was heated for 5min on a steam bath with hexamethylphosphorus triamide [tris(dimethylamino)phosphine]

(Ralph N. Emanuel, Ltd., Alpertton, Middx., U.K.) (1ml). An examination of the u.v.-absorption spectrum of the material in solution showed that the spectrum had changed from that typical of the dialdehyde ($\lambda_{\max}$ at 233, 258, 297 and 350nm) to that expected for the oxide (II) (see below). The oxide failed to separate from the solution even after the solution was diluted with 3vol. of cyclohexane and kept at 0°C for some days. The solution was evaporated under reduced pressure and the residue chromatographed on an alumina (Spence type H) column (3cm×8cm) in cyclohexane containing 4% (v/v) dioxan. Fractions (25ml) were collected and the u.v.-absorption spectra of the material in the solutions were measured. The measurements showed that the oxide was present in fractions 3–8. These were combined and evaporated and the pale-yellow gum was dissolved in diethyl ether (2ml). The solution was kept overnight at 0°C and the product that separated recrystallized from benzene-cyclohexane to yield 7,12-dimethylbenz[a]anthracene 5,6-oxide (II) (370mg) in rhombs, m.p. 130–132°C (Found: C, 88.0; H, 5.9; C₂₀H₁₆O requires C, 88.2; H, 5.9%), $\lambda_{\max}$ at 225, 249 (inflexion), 257.5, 266, 275.5, 298 (inflexion), 309, 318 and 352nm (log ϵ 4.22, 4.25, 4.51, 4.69, 4.74, 4.02, 4.11, 4.05 and 3.02 respectively).

In subsequent preparations it was sufficient to dilute the reaction mixture with 3vol. of cyclohexane and add one crystal of the oxide (II): crystallization of the oxide (II) present in the solution soon began and was complete after about 3h at 0°C. Yields of the oxide (II) of about 50% were obtained in this manner, and chromatography on alumina as described above, of the mixture in the mother liquors, gave more product, so that overall yields of the oxide (II) of about 75% were finally obtained.

The oxide (II) was rapidly decomposed when its solution in dioxan was treated with a few drops of conc. HCl at room temperature. A number of products were detected when the reaction mixture was examined by t.l.c., the major one of which appeared to be a phenol that was either 5- or, less likely, 6-hydroxy-7,12-dimethylbenz[a]anthracene. The chromatographic properties of the phenol are recorded in Table 1 and its u.v.-absorption spectrum showed $\lambda_{\max}$ at 218, 262, 268, 298, 317 (inflexion), 352, 372 and 416nm.

Preparation of 7-acetoxymethyl-12-methylbenz[a]anthracene 5,6-oxide (7-acetoxymethyl-5,6-epoxy-5,6-dihydro-12-methylbenz[a]anthracene). *cis*-7-Acetoxymethyl-5,6-dihydro-5,6-dihydroxy-12-methylbenz[a]anthracene (2g) in methanol (750ml) was stirred overnight with a solution of NaIO₄ (5g) in water (20ml). The solution was filtered and the methanol distilled off under reduced pressure. The mixture was extracted with diethyl ether (100ml) and the extract was washed with water (100ml) and dried over Na₂SO₄. Removal of the ether yielded a pale-yellow

oil (1.8g) that failed to crystallize: it is presumed to be mainly 1-acetoxymethyl-4-methyl-3-phenyl-naphthalene 2,2'-dicarboxaldehyde. Its u.v.-absorption spectrum was similar to that of 1,4-dimethyl-2-phenylnaphthalene 3,2'-dicarboxaldehyde and showed $\lambda_{\max}$ at 223, 254, 297 and 347nm.

The dialdehyde (1g) in benzene (2ml) was heated on a steam bath for 5min with hexamethylphosphorus triamide (1ml). The solution was kept overnight at 0°C and the product that separated recrystallized from benzene-cyclohexane. 7-Acetoxymethyl-12-methylbenz[a]anthracene 5,6-oxide (600mg) was obtained as pale-yellow flat needles, m.p. 132–133°C (Found: C, 79.6; H, 5.2; $C_{22}H_{18}O_3$ requires C, 80.0; H, 5.5%). $\lambda_{\max}$ at 225, 248 (inflexion), 258, 266.5, 275.5, 300 (inflexion), 310 and 320 (inflexion) nm ($\log \epsilon$ 4.29, 4.31, 4.42, 4.59, 4.63, 3.97, 4.04 and 3.99 respectively).

When a solution of the oxide in dioxan was treated with a few drops of conc. HCl, the oxide was rapidly decomposed to yield a mixture of products, the major one of which appeared to be 7-acetoxymethyl-5(or 6)-hydroxy-12-methylbenz[a]anthracene. Its u.v.-absorption spectrum was similar to that of the phenol obtained in the acid decomposition of the oxide (II) and showed $\lambda_{\max}$ at 218, 261.5, 269, 292, 300 (inflexion), 348 (inflexion), 369 and 417nm. Its chromatographic properties are recorded in Table 1.

Preparation of 7-hydroxymethyl-12-methylbenz[a]anthracene 5,6-oxide (5,6-epoxy-5,6-dihydro-7-hydroxymethyl-12-methylbenz[a]anthracene) (VII). 7-Acetoxymethyl-12-methylbenz[a]anthracene 5,6-oxide (1g) in a mixture of acetone (100ml) and water (10ml) was kept at room temperature for 3h with tetraethylammonium hydroxide solution (25%, w/v; BDH Chemicals Ltd., Poole, Dorset, U.K.) (2.5ml). At the end of this time an examination by t.l.c. of the products in the solution showed that hydrolysis of the acetoxo derivative was complete. The reaction mixture was diluted with water (500ml) to yield a pale-yellow oil (650mg). From its properties on thin-layer chromatograms and from its u.v.-absorption spectrum, which was almost identical with that of the acetoxo derivative described above and showed $\lambda_{\max}$ at 225, 248 (inflexion), 257, 266.5, 275, 300 (inflexion), 309 and 319 (inflexion) nm, the product appeared to consist mainly of 7-hydroxymethyl-12-methylbenz[a]anthracene 5,6-oxide (VII). In some preparations the product separated from the reaction mixture as a pale-yellow amorphous powder. Attempts to obtain a crystalline product by crystallization from any one of a number of solvents, either of the crude product or of the product obtained by column chromatography on alumina as described above by using cyclohexane-dioxan (3:1, v/v) as developing solvent, were unsuccessful. The material obtained from the column was examined by t.l.c. and appeared to be essentially free of impurities, since only one spot was present on

the chromatogram: this material, which formed a pale-yellow gum, was used in the experiments described below. When the gum (10mg) was treated overnight with a few drops of acetic anhydride in pyridine (0.1ml), 7-acetoxymethyl-12-methylbenz[a]anthracene 5,6-oxide was obtained, m.p. and mixed m.p. 132–133°C.

Reactions of the oxides (II) and (VII) with water

The oxides (II) and (VII) (50mg) were each heated under reflux for 24h with acetone (50ml) and water (5ml). The acetone was removed under reduced pressure and the products that separated were applied to the base lines of preparative chromatograms that were developed with solvent (b).

The oxide (II) yielded two major products, the first of which was identical in its chromatographic properties and u.v.-absorption spectrum with 5-hydroxy-7,12-dimethylbenz[a]anthracene. The second was a dihydrodiol that was identical both in its chromatographic properties and in its u.v.-absorption characteristics with *trans*-5,6-dihydro-5,6-dihydroxy-7,12-dimethylbenz[a]anthracene. In agreement with earlier observations (Boyland & Sims, 1967), the *trans*-dihydrodiol could not be crystallized. The gum was treated with acetic anhydride in pyridine to yield *trans*-5,6-diacetoxy-5,6-dihydro-7,12-dimethylbenz[a]anthracene (12mg) in plates, m.p. and mixed m.p. 205–207°C.

The oxide (VII) similarly yielded a phenol that was indistinguishable from 5-hydroxy-7-hydroxymethyl-12-methylbenz[a]anthracene and a dihydrodiol, the u.v. spectrum of which was similar to that of *cis*-5,6-dihydro-5,6-dihydroxy-7-hydroxymethyl-12-methylbenz[a]anthracene (Boyland & Sims, 1967) and showed $\lambda_{\max}$ at 220, 252 (inflexion), 260, 267, 292 (inflexion), 300, 310 (inflexion) and 344 (inflexion) nm. The R_F of the product differed from that of the *cis*-dihydrodiol (see Table 1): the product is therefore assumed to be the *trans*-isomer. Insufficient material was obtained to allow further characterization to be carried out.

Reactions of the oxides (II) and (VII) with GSH and N-acetyl-L-cysteine

The oxides (II) and (VII) (50mg) were each heated under reflux for 4h with GSH (50mg) and $NaHCO_3$ (100mg) in aq. 50% (v/v) acetone (250ml). The acetone was distilled off under reduced pressure and the aqueous residues were each extracted with diethyl ether (100ml). The residues remaining when the ether was removed were examined by t.l.c. The aqueous layers were each acidified to pH4 with acetic acid and activated charcoal (2g) was added. The charcoal was immediately filtered off and washed with water (250ml). The absorbed material

was eluted from the charcoal with methanol (500ml) containing 5% (v/v) of aq. NH_3 soln. (sp.gr. 0.88). The solvent was removed under reduced pressure and the residues were examined as described below.

The oxide (II) (50mg) was similarly allowed to react with *N*-acetyl-L-cysteine (50mg) in the presence of NaHCO_3 (100mg) and the products were examined as described below.

Reaction of 7,12-dimethylbenz[a]anthracene 5,6-oxide (II) with GSH. The material extracted from the reaction mixture with diethyl ether contained a product with the chromatographic properties and u.v.-absorption spectrum of 5-hydroxy-7,12-dimethylbenz[a]anthracene. The product obtained from the aqueous layer formed a clear gum that appeared to consist mainly of the ammonium salts of the diastereoisomeric forms of *S*-(5,6-dihydro-6-hydroxy-7,12-dimethylbenz[a]anthracen-5-yl)glutathione (V) (or possibly the 5-hydroxy-benz[a]anthracen-6-yl derivative). The chromatographic properties of the conjugate (V) are shown in Table 1. The u.v.-absorption spectrum [λ_{max} . at 221, 254 (inflexion), 262, 270, 292, 302, 310 (inflexion) and 345 (inflexion) nm] resembled that of the dihydrodiol (III) except that the peaks were shifted to slightly longer wavelengths in the conjugate.

The conjugate was not decomposed when a solution in 2M-HCl was kept for some hours at room temperature, but it was completely decomposed to yield three products when the solution was heated on a steam bath for 30min. The first, which could be extracted with diethyl ether from the reaction mixture after the mixture was made alkaline with 2M-NaOH, was identified by t.l.c. and by its u.v.-absorption spectrum as 7,12-dimethylbenz[a]anthracene (I). The second product, which remained in the aqueous layer, appeared to be *S*-(7,12-dimethylbenz[a]anthracen-5-yl)glutathione (or the isomeric 6-substituted derivative). Its properties on chromatograms are given in Table 1 and its u.v.-absorption spectrum showed λ_{max} . at 226, 266, 284 (inflexion), 296, 307, 354 (inflexion), 370 and 386 (inflexion) nm. From a comparison of the heights of the peaks in the absorption spectra it appears that less than 30% of the parent GSH conjugate (V) is decomposed by the pathway leading to the hydrocarbon (I). The third product was identical in its chromatographic properties with GSSG, but GSH was not detected. There was no evidence for the formation of phenols in the acid decomposition of the conjugate (V).

Reaction of 7,12-dimethylbenz[a]anthracene 5,6-oxide (II) with *N*-acetyl-L-cysteine. The diethyl ether extract contains a product with the chromatographic characteristics and u.v.-absorption spectrum of 5-hydroxy-7,12-dimethylbenz[a]anthracene. The product obtained from the aqueous layer appeared to be mainly the ammonium salts of the diastereoisomeric forms of the mercapturic acid, *N*-acetyl-*S*-(5,6-

dihydro-6-hydroxy-7,12-dimethylbenz[a]anthracen-5-yl)-L-cysteine. Its u.v.-absorption spectrum was identical with that of the GSH conjugate (V) recorded above. It was not decomposed when its solution in 2M-HCl was kept at room temperature for some hours, but it was completely decomposed when its solution was heated on a steam bath for 15min. Two products, detected as described above, appeared to be 7,12-dimethylbenz[a]anthracene (I) and 7,12-dimethylbenz[a]anthracen-5-ylmercapturic acid. The u.v.-absorption spectrum of the mercapturic acid was identical with that of the arylglutathione conjugate described above. In the acid decomposition of the parent conjugate less than 40% appeared to be decomposed by the pathway leading to the parent hydrocarbon (I). Phenols were not detected as products of the acid decomposition of the *N*-acetyl derivative.

Reaction of 7-hydroxymethyl-12-methylbenz[a]anthracene 5,6-oxide (VII) with GSH. Extraction of the reaction mixture with diethyl ether yielded a product with the chromatographic properties and u.v. spectrum of 5-hydroxy-7-hydroxymethyl-12-methylbenz[a]anthracene. The product obtained from the aqueous fractions formed a clear gum that appeared to consist mainly of the ammonium salts of the diastereoisomeric forms of *S*-(5,6-dihydro-6-hydroxy-7-hydroxymethyl-12-methylbenz[a]anthracen-5-yl)glutathione (VIII). The chromatographic properties of the conjugate (VIII) are shown in Table 1. The u.v.-absorption spectrum was identical with that of the conjugate (V) and showed λ_{max} . at 221, 254 (inflexion), 262, 270, 292, 302, 310 (inflexion) and 345 (inflexion) nm.

The conjugate was only slowly decomposed when its solution in 1M-HCl was kept at room temperature, but it was completely decomposed when the solution was heated on a steam bath for 45min. Three products were detected, one of which was identified by t.l.c. and by its u.v.-absorption spectrum as 7-hydroxymethyl-12-methylbenz[a]anthracene (IV). The other products appeared to be *S*-(7-hydroxymethyl-12-methylbenz[a]anthracen-5-yl)glutathione and GSSG.

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

This was done as described by Grover & Sims (1970) by using 7,12-dimethylbenz[a]anthracene 5,6-oxide (II), 7-methylbenz[a]anthracene 5,6-oxide and benz[a]anthracene 5,6-oxide as substrates. The results are shown in Fig. 1.

Results

Metabolism of 7,12-dimethylbenz[a]anthracene 5,6-oxide (II)

When the oxide (II) was incubated with rat liver homogenate as described above, two products were

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 7,12-dimethylbenz[*a*]anthracene 5,6-oxide (II) (○), 7-methylbenz[*a*]anthracene 5,6-oxide (Δ) and benz[*a*]anthracene 5,6-oxide (□) with 4-(*p*-nitrobenzyl)pyridine.

detected in the ethyl acetate-soluble fraction. The minor product was identical in its chromatographic properties with 5-hydroxy-7,12-dimethylbenz[*a*]anthracene, and the major product was identical both in its chromatographic characteristics and in its u.v.-absorption spectrum with *trans*-5,6-dihydro-5,6-dihydroxy-7,12-dimethylbenz[*a*]anthracene (III). There was no evidence for the formation of the *cis*-dihydrodiol in these incubations.

The aqueous fraction contained a product with the chromatographic characteristics and u.v.-absorption spectrum of the GSH conjugate (V). When the metabolite was heated on a steam bath with 2M-HCl, products with the chromatographic properties of the hydrocarbon (I) and *S*-(7,12-dimethylbenz[*a*]anthracen-5-yl)glutathione were obtained.

Only one product was detected when the oxide (II) was incubated with a rat liver microsomal fraction, and it was identified both by t.l.c. and by its u.v.-absorption spectrum as *trans*-5,6-dihydro-5,6-dihydroxy-7,12-dimethylbenz[*a*]anthracene (III).

When the oxide (II) was incubated with GSH in the presence of rat liver soluble fraction as described above, a small amount of a product with the chromatographic properties of 5-hydroxy-7,12-dimethylbenz[*a*]anthracene was detected in the ethyl acetate-soluble fraction. The aqueous fraction contained large amounts of a product with the chromato-

graphic properties and u.v.-absorption spectrum of the GSH conjugate (V), which was decomposed on heating with 2M-HCl into the hydrocarbon (I) and *S*-(7,12-dimethylbenz[*a*]anthracen-5-yl)glutathione.

In the experiments in which the oxide (II) was incubated with liver preparations that were previously boiled, neither the dihydrodiol (III) nor the GSH conjugate (V) was detected. Small amounts of 5-hydroxy-7,12-dimethylbenz[*a*]anthracene were usually formed in these incubations.

*Metabolism of 7-hydroxymethyl-12-methylbenz[*a*]anthracene 5,6-oxide (VII)*

The ethyl acetate-soluble fraction obtained when the oxide (VII) was incubated with rat liver homogenate contained two products. The minor product was identical in its chromatographic properties and u.v.-absorption spectrum with 5-hydroxy-7-hydroxymethyl-12-methylbenz[*a*]anthracene, and the major product was similarly identical with *trans*-5,6-dihydro-5,6-dihydroxy-7-hydroxymethyl-12-methylbenz[*a*]anthracene (VI).

The aqueous fraction from this incubation contained a product with the chromatographic properties and u.v.-absorption spectrum of the GSH conjugate (VIII).

The only product formed when the oxide (VII) was incubated with a rat liver microsomal fraction was identified both by t.l.c. and by its absorption spectrum as the *trans*-dihydrodiol (VI).

The aqueous fraction obtained from the incubation of the oxide (VII) with GSH in the presence of rat liver soluble fraction contained large amounts of a product with the chromatographic characteristics and u.v.-absorption spectrum of the GSH conjugate (VIII).

Neither the dihydrodiol (VI) nor the GSH conjugate (VIII) was detected when the oxide (VII) was incubated with rat liver preparations that had been previously boiled.

*Metabolism of 7-hydroxymethyl-12-methylbenz[*a*]anthracene (IV)*

A number of products were detected when the hydroxymethyl derivative (IV) was incubated with a rat liver microsomal preparation containing the co-factors indicated above. Of the dihydrodiols formed, two were identified by their u.v.-absorption spectra as the 8,9-dihydro-8,9-dihydroxy and the 10,11-dihydro-10,11-dihydroxy derivatives of the hydroxymethyl compound (IV) (Sims, 1970b). A third dihydrodiol, which was present only in small amounts, was identical in its chromatographic characteristics and u.v.-absorption spectrum with the *trans*-dihydrodiol (VI).

Discussion

The structures of 7,12-dimethylbenz[*a*]anthracene 5,6-oxide (II) and 7-hydroxymethyl-12-methylbenz[*a*]anthracene 5,6-oxide (VII) follow from their method of synthesis, since they were prepared from precursors that were obtained from the oxidation of the 'K-region' bonds of 7,12-dimethylbenz[*a*]anthracene (I) and 7-acetoxymethylbenz[*a*]anthracene respectively with OsO₄. Thus the oxides (II) and (VII) are 'K-region' epoxides, as distinct from non-'K-region' epoxides such as benz[*a*]anthracene 8,9-oxide (Sims, 1971) and benzo[*a*]pyrene 7,8-oxide and 9,10-oxide (Waterfall & Sims, 1972). The u.v.-absorption spectra of the oxides are those expected from the 'K-region' epoxides.

Like other arene oxides, the oxides (II) and (VII) readily rearrange to phenols with mineral acid. The small amounts of phenols detected when the oxides are incubated with rat liver preparations apparently arise through a non-enzymic rearrangement, since they are formed in incubations with both fresh and boiled liver preparations. Other arene oxides undergo similar non-enzymic rearrangements (Jerina *et al.*, 1968, 1970; Boyland & Sims, 1965c; Sims, 1966, 1967). In the rearrangement of benzene oxide and naphthalene 1,2-oxide, Kasperek & Bruce (1972) distinguished two types of reaction, an acid-catalysed aromatization and, in the pH 7–12 region, a spontaneous aromatization. Presumably similar mechanisms are involved in the rearrangements of the oxides (II) and (VII).

The oxides (II) and (VI) are both metabolized by rat liver preparations into the related *trans*-dihydrodiols and GSH conjugates. In this respect they resemble other 'K-region' epoxides, such as those of phenanthrene (Boyland & Sims, 1965b), benz[*a*]anthracene and dibenz[*a,h*]anthracene (Boyland & Sims, 1965c), 7-methylbenz[*a*]anthracene (Sims, 1967) and 3-methylcholanthrene (Sims, 1966). 7-Hydroxymethylbenz[*a*]anthracene is also converted by rat liver preparations into a *trans*-dihydrodiol and a GSH conjugate (Sims, 1972). The conversion of the epoxides (II) and (VII) into *trans*-dihydrodiols is presumably carried out by a microsomal 'epoxide hydrase' similar to those previously described by Jerina *et al.* (1968), Brooks *et al.* (1970), Oesch & Daly (1971) and Pandov & Sims (1970). The enzyme catalysing the conjugation of the oxides (II) and (VII) with GSH is present in the soluble fraction and is presumably the 'glutathione *S*-epoxide transferase' described by Boyland & Williams (1965).

In the incubation of 7,12-dimethylbenz[*a*]anthracene (I) with rat liver microsomal fractions, the epoxide (II) is formed as a metabolite (Keysell *et al.*, 1972), and presumably the small amounts of *trans*-5,6-dihydro-5,6-dihydroxy-7,12-dimethylbenz[*a*]anthracene that were formed when the hydrocarbon (I)

was incubated with rat liver microsomal preparations (Sims & Grover, 1968) arise from the enzymic hydration of the epoxide (II). Similarly, the small amounts of dihydrodiol (VI) detected in the metabolism of the hydroxymethyl compound (IV) also probably arise through an epoxide intermediate, since a metabolite that is probably the oxide (VII) was formed when the hydroxymethyl compound (IV) was incubated with a rat liver microsomal preparation (G. R. Keysell, unpublished work). In the incubations of the hydrocarbon (I) (Sims, 1970a) and its hydroxymethyl derivative (IV) (Sims, 1970b) with rat liver homogenates, the 'K-region' dihydrodiols (III) and (IV) were not detected, although both benz[*a*]anthracene and 7-methylbenz[*a*]anthracene form 'K-region' dihydrodiols (Sims, 1970a): possibly the epoxide intermediates (II) and (VII) react preferentially with other constituents in the crude liver homogenates. The results shown in Fig. 1 indicate that, in the alkylation of 4-(*p*-nitrobenzyl)pyridine, 7,12-dimethylbenz[*a*]anthracene 5,6-oxide (II) is more active than either 7-methylbenz[*a*]anthracene 5,6-oxide or benz[*a*]anthracene 5,6-oxide. The oxide (II) might therefore be expected to react more readily with cellular constituents than either of the other oxides.

The stability of the GSH conjugates (V) and (VIII) in the presence of cold mineral acid was unexpected, since related conjugates such as those derived from benz[*a*]anthracene 5,6-oxide (Boyland & Sims, 1965c) and 7-methylbenz[*a*]anthracene 5,6-oxide (Sims, 1967) were immediately decomposed under the same conditions. All the conjugates, however, yielded mixtures of the parent hydrocarbon, the corresponding arylglutathione conjugates and GSSG as decomposition products. Phenols were not produced in these reactions.

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.

References

- Booth, J., Boyland, E., Sato, T. & Sims, P. (1960) *Biochem. J.* **77**, 182–186
- Boyland, E. & Sims, P. (1965a) *Biochem. J.* **95**, 780–787
- Boyland, E. & Sims, P. (1965b) *Biochem. J.* **95**, 788–792
- Boyland, E. & Sims, P. (1965c) *Biochem. J.* **97**, 7–16
- Boyland, E. & Sims, P. (1967) *Biochem. J.* **104**, 394–403
- Boyland, E. & Williams, K. (1965) *Biochem. J.* **94**, 190–197
- Brooks, G. T., Harrison, A. & Lewis, S. E. (1970) *Biochem. Pharmacol.* **19**, 255–273
- Cook, J. W. & Schoental, R. (1948) *J. Chem. Soc. London* 170–173
- Grover, P. L. & Sims, P. (1970) *Biochem. Pharmacol.* **19**, 2251–2259
- Grover, P. L., Hewer, A. & Sims, P. (1971a) *FEBS Lett.* **18**, 76–80

- Grover, P. L., Sims, P., Huberman, E., Marquardt, H., Kuroki, T. & Heidelberger, C. (1971*b*) *Proc. Nat. Acad. Sci. U.S.* **68**, 1098–1101
- Grover, P. L., Hewer, A. & Sims, P. (1972) *Biochem. Pharmacol.* **21**, 2713–2726
- Hadler, H. I. & Kryger, A. C. (1960) *J. Org. Chem.* **25**, 1896–1901
- Jerina, D., Daly, J., Witkop, B., Zaltzman-Nirenberg, P. & Udenfriend, S. (1968) *Arch. Biochem. Biophys.* **128**, 176–183
- Jerina, D. M., Daly, J. W., Witkop, B., Zaltzman-Nirenberg, P. & Udenfriend, S. (1970) *Biochemistry* **9**, 147–155
- Kasperek, G. J. & Bruice, T. C. (1972) *J. Amer. Chem. Soc.* **94**, 198–202
- Keysell, G. R., Booth, J., Sims, P., Grover, P. L. & Hewer, A. (1972) *Biochem. J.* **129**, 41P–42P
- Newman, M. S. & Blum, S. (1964) *J. Amer. Chem. Soc.* **86**, 5598–5600
- Oesch, F. & Daly, J. (1971) *Biochim. Biophys. Acta* **227**, 692–697
- Pandov, H. & Sims, P. (1970) *Biochem. Pharmacol.* **19**, 299–303
- Selkirk, J. K., Huberman, E. & Heidelberger, C. (1971) *Biochem. Biophys. Res. Commun.* **43**, 1010–1016
- Sims, P. (1966) *Biochem. J.* **98**, 215–228
- Sims, P. (1967) *Biochem. J.* **105**, 591–598
- Sims, P. (1970*a*) *Biochem. Pharmacol.* **19**, 795–818
- Sims, P. (1970*b*) *Biochem. Pharmacol.* **19**, 2261–2275
- Sims, P. (1971) *Biochem. J.* **125**, 159–168
- Sims, P. (1972) *Xenobiotica* **2**, 469–477
- Sims, P. & Grover, P. L. (1968) *Biochem. Pharmacol.* **17**, 1751–1758
- Waterfall, J. F. & Sims, P. (1972) *Biochem. J.* **128**, 265–277