Anthracene 1,2-Oxide: Synthesis and Role in the Metabolism of Anthracene by Mammals

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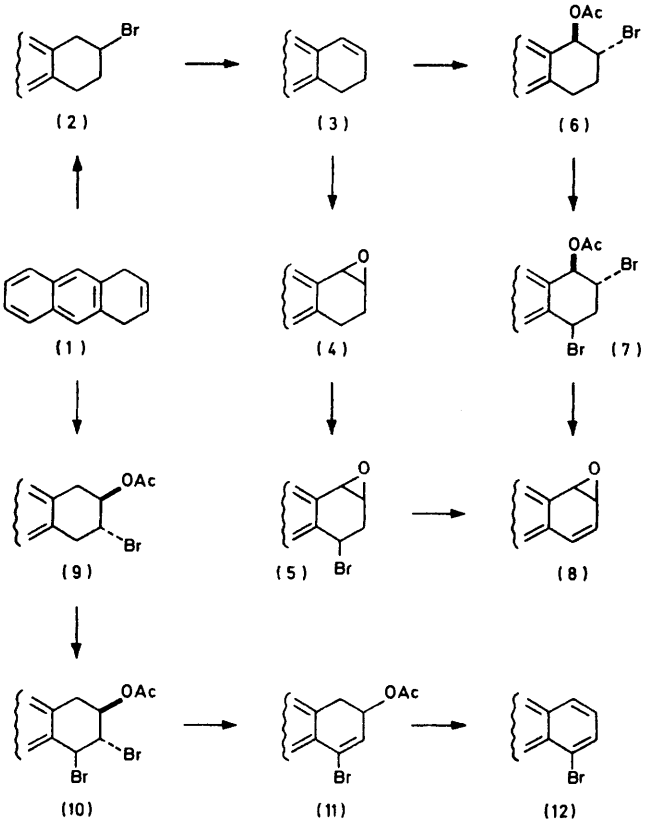
Synthesis of anthracene 1,2-oxide proceeds in good yield *via* dehydrobromination of 1-acetoxy-2,4-dibromo-1,2,3,4-tetrahydroanthracene. In contrast, dehydrobromination of 2-acetoxy-3,4-dibromo-1,2,3,4-tetrahydroanthracene produced 1-bromoanthracene instead of the desired anthracene 2,3-oxide or its oxepin tautomer. Nucleophilic attack of thioethoxide on anthracene 1,2-oxide yields *trans*-1-hydroxy-2-ethylthio-1,2-dihydroanthracene which readily eliminates water with concomitant migration of the thioethoxide group to produce 1-anthryl ethyl sulphide. *In vitro* metabolism of anthracene with liver microsomes forms predominantly *trans*-1,2-dihydroxy-1,2-dihydroanthracene with little evidence of metabolism at the 9,10-position.

ALTHOUGH hydroxylation had long been recognized as a major detoxication pathway of aromatic hydrocarbons,[1] about one hundred years was to elapse before arene oxides were established as intermediates in the process.[2] Detailed mechanistic studies were greatly facilitated by the observation of migration and retention of hydrogen isotope to a neighbouring carbon atom during hydroxylation by mono-oxygenase enzymes from plants, animals, and micro-organisms (NIH Shift) [3,4] and by the availability of a wide range of arene oxides for biochemical studies.[4-7]

Among the lower members of the polycyclic aromatic hydrocarbon (PAH) series (mono ⟶ tetracyclic), whose metabolism by mammals has previously been studied, anthracene is particularly interesting in that a wide range of metabolites has been detected.[8,9] Although the major metabolite was reported to be *trans*-1,2-dihydroxy-1,2-dihydroanthracene [10] (both free and as a glucuronic acid conjugate), additional products which included *N*-acetyl-*S*-(1,2-dihydro-2-hydroxy-1-anthryl)cysteine, 1-anthryl mercapturic acid, 9,10-anthraquinone, *cis*- and *trans*-9,10-dihydroanthracenes, 9,10-dihydroxyanthracene, 2,9,10-trihydroxyanthracene, and 2-hydroxy-9,10-anthraquinone were also isolated.[9] In contrast to the behaviour of the other lower members of the PAH series on metabolism in mammals, monophenolic products were not reported. Thus, a major goal of the present study was the synthesis of anthracene 1,2-oxide, a probable metabolic intermediate, in order to allow a comparison of its *in vitro* metabolism by hepatic enzymes with results obtained on anthracene.

Previous synthetic routes to naphthalene 1,2-oxide proceeded by the interconversion of 1,2-dihydronaphthalene *via* the corresponding dibromide and bromohydrin [7] (or directly by peracid oxidation) [11] to 1,2-epoxy-1,2,3,4-tetrahydronaphthalene, followed by benzylic bromination (*N*-bromosuccinimide) and base-promoted dehydrobromination. Application of a similar epoxidation route (Scheme 1) to 1,2-dihydroanthracene (3) produced 1,2-epoxy-1,2,3,4-tetrahydroanthracene (4) in good yield. However (as was found in the synthesis of phenanthrene oxides),[12] the *N*-bromosuccinimide (NBS) step produced a complex range of products probably resulting from polymerization and decomposition of the starting material, in addition to the desired 4-bromo-1,2-epoxy-1,2,3,4-tetrahydroanthracene (5). On treatment with 1,5-diazabicyclo[4,3,0]non-5-



SCHEME 1

ene, the bromo-epoxide (5) (1 minor component of the crude product mixture) was converted to anthracene 1,2-oxide (8). The proportion of (8) among the products was small but identifiable on the basis of its t.l.c. characteristics, its formation of anthrol when acidified, and its n.m.r. spectrum. Purification of the anthracene

1,2-oxide prepared by this route was found to be impracticable.

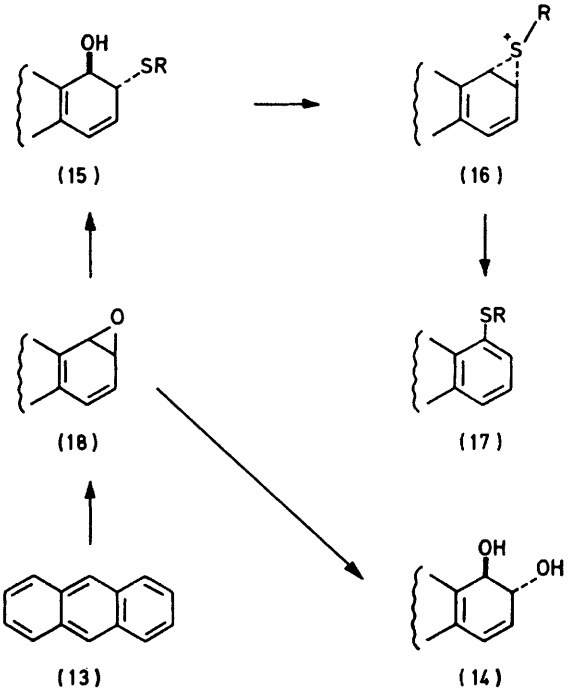
An alternative route which avoids NBS bromination of epoxides [12] was followed in which 1-acetoxy-2-bromo-1,2,3,4-tetrahydroanthracene (6) was the key intermediate. This was formed from 1,2-dihydroanthracene (3) directly by reaction with *N*-bromoacetamide and lithium acetate in glacial acetic acid. The 1-acetoxy-2,4-dibromo-1,2,3,4-tetrahydroanthracene (7) (formed by benzylic bromination with NBS) proved to be stable to these reaction conditions and was isolated in moderate yield (49%) after recrystallization. Dehydrobromination with freshly prepared dry sodium methoxide gave anthracene 1,2-oxide as a crystalline solid whose spectroscopic data and rearrangement to 1-anthrol (plus a trace of 2-anthrol) in the presence of acid was consistent with the structure assigned.

An attempt was made to synthesize the tautomeric oxepin form of anthracene 2,3-oxide based on previous observations that the bromoacetate (9) can be converted to 2,3-epoxy-1,2,3,4-tetrahydroanthracene,[13] and that 1-bromo-2,3-epoxy-1,2,3,4-tetrahydronaphthalene can be converted to naphth[2,3-*d*]oxepin;[7] both reactions occur in the presence of alkoxide base. Chromatography of the crude product obtained after reaction between potassium t-butoxide and the dibromo-ester (10) yielded a viscous oil which on exposure to air at room temperature rapidly darkened and evolved acetic acid. Chromatography then yielded 1-bromoanthracene (12) which was identified by microanalysis, n.m.r. spectrum, and analogy with 1-bromonaphthalene (obtained from similar treatment of 2-acetoxy-3,4-dibromo-1,2,3,4-tetrahydronaphthalene [13]). The unstable oil which eliminated acetic acid has been tentatively identified as (11) on the basis of the n.m.r. spectrum. Structure (11) is of a similar diene type to t-butyl 3-hydroxy-5-bromo-2,3-dihydrobenzoate, formed by treatment of a bromo-epoxide with alkoxide.[14]

In contrast to other non-K-region arene oxides which have previously been synthesized,[4,5] anthracene 1,2-oxide proved relatively stable at ambient temperature; only a slight trace of decomposition was observed after several weeks. Presumably this stability is associated with the decrease in aromaticity (and increase in olefinic character) with increasing molecular weight in the aromatic hydrocarbon series benzene ⟶ naphthalene ⟶ anthracene.

The isolation of 1-anthrylmercapturic acid (17a) and *trans*-1,2-dihydroxy-1,2-dihydroanthracene (14) from the metabolism of anthracene by mammals [8,9] may be rationalized in terms of the enzyme-catalyzed synthesis and ring-opening reactions of the intermediate anthracene 1,2-oxide (Scheme 2). Thus, the 1-anthrylmercapturic acid (17a) may be formed from the premercapturic acid (15a) by dehydration [possibly involving (16a)] and migration of the SR group to the 1-position. Evidence for a similar sequence has previously been presented for the mammalian metabolism of naphthalene.[15] In support of the latter studies, nucleophilic attack of thioethoxide on naphthalene 1,2-oxide to yield *trans*-1-hydroxy-2-ethylthio-1,2-dihydronaphthalene (18),[16] followed by acid-catalyzed dehydration and migration of thioethoxide, gave 1-naphthyl ethyl sulphide.

Nucleophilic attack of EtS^- on anthracene 1,2-oxide (8) under identical conditions to those used for naphthalene 1,2-oxide [15,16] yielded a product (15b) whose n.m.r. spectrum was virtually identical (excluding the aromatic region) to (18). This component appeared to constitute >90% of the crude product. Purification was not possible since (15b) readily decomposed in solution to 1-anthryl ethyl sulphide (17b), identical with a sample prepared by treatment of 1-anthrol with acidic ethanethiol.[17] This evidence is consistent with the formation of intermediates (15b) and (16b) during the inter-



SCHEME 2 a, R = glutathionyl; b, R = Et

conversion of (8) to (17b) and concurs with the view that (17a) is formed from mammalian metabolism of anthracene *via* intermediates (8) and (15a). Thus, the structure which was tentatively assigned [9] to the acid-sensitive mercapturic acid [*N*-acetyl-*S*-(1,2-dihydro-2-hydroxy-1-anthryl)cysteine] should now probably be reassigned as *N*-acetyl-*S*-(1,2-dihydro-1-hydroxy-2-anthryl)cysteine (15a).

Attempts to hydrate anthracene 1,2-oxide using aqueous acetone (which proved successful for K-region arene oxides) [18] produced anthrols with no trace of the *trans*-1,2-dihydrodiol (14). This observation shows that in this respect anthracene 1,2-oxide behaves more like a non-K-region than a K-region arene oxide. However, it was possible to catalyze the hydration of (8) with the liver microsomal enzyme epoxide hydrase. The stereospecificity of epoxide hydrase toward anthracene 1,2-oxide was tested by a preparative scale incubation with

rabbit-liver microsomes. The resultant diol (14) was isolated in *ca.* 80% yield after preparative t.l.c. of the diacetate. The specific rotation of the diacetate ($[\alpha]_D$ −10.5°) corresponds to an optical purity of <3% based upon configurational correlation with optically pure 1,2,3,4-tetrahydro-2-acetoxyanthracene. The negative rotation corresponds to 1*R* : 2*R* absolute stereochemistry in the dihydrodiol. The low optical purity may in part be due to the very high conversion of the substrate. Enzymatic hydration of cyclohexene oxide, benzene oxide, naphthalene 1,2-oxide, and phenanthrene 1,2-oxide [19,20] by rabbit-liver microsomes also favours the 1*R* : 2*R* enantiomer. Conclusions with regard to the specificity of epoxide hydrase *in vivo* must be drawn with caution since dihydrodiols are known to resolve readily on crystallization and since other enzymatic pathways such as dehydrogenation to catechols [19] may occur with enantiomeric preference.

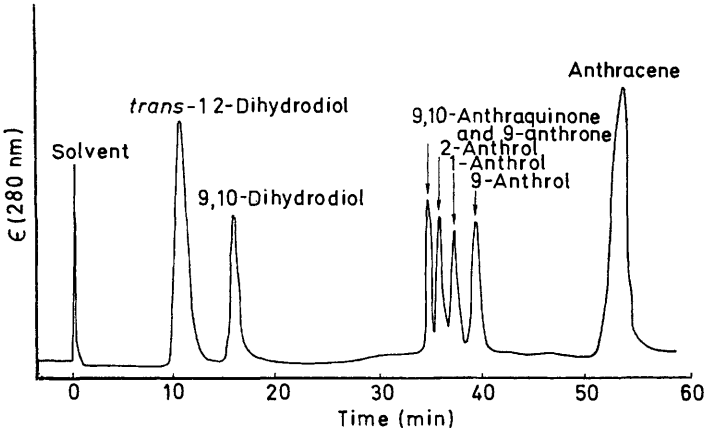
A study of the *in vitro* metabolism of [^{14}C]-labelled anthracene with liver microsomes from rates was undertaken to investigate further the mechanism of formation, and range of metabolites of anthracene. Sprague–Dawley rats were used either without pretreatment or after injection of phenobarbital or 3-methylcholanthrene to induce drug-metabolizing enzymes. In order to ensure detection of even minor metabolites, extracts of the incubation mixture were examined by high pressure liquid chromatography (h.p.l.c.) (Du Pont ODS column, eluted with a linear gradient of methanol in water, Figure). Marker quantities of non-radioactive *trans*-1,2-dihydroxy-1,2-dihydroanthracene, 9,10-dihydroxy-9,10-dihydro-anthracene, 9-anthrone, 9,10-anthraquinone, and 1-, 2-, and 9-anthrol were added to a portion of the extracts prior to chromatography. With all three types of microsomes, >95% of the total metabolism-induced radioactivity was associated with the *trans*-1,2-dihydroxy-1,2-dihydroanthracene.

Trace amounts of radioactivity were occasionally observed in the phenol region. Induction factors for the untreated and induced microsomes are similar to those observed for other hydrocarbons such as benzo[*a*]-pyrene [21] (Table). The virtual absence of metabolites at the 9,10-position of anthracene in the present *in vitro* studies (Figure) suggests that such *in vivo* metabolites [9] may not be hepatic in origin.

Metabolism of [^{14}C]anthracene to *trans*-1,2-dihydroxy-1,2-dihydroanthracene by rat liver microsomes

Preparation	Dihydrodiol/mmol^{-1} mg protein^{-1} min^{-1}
Untreated	0.2
Phenobarbital-induced	0.3
3-Methylcholanthrene-induced	1.1

The highly efficient conversion of anthracene into the *trans*-1,2-dihydrodiol (14) by the microsomal enzymes, without the formation of anthrols, requires that anthracene 1,2-oxide be a good substrate for epoxide hydrase. This point was established by observing a moderately high rate (1 000 nmol diol mg^{-1} min^{-1}) for hydration of anthracene 1,2-oxide to diol (14) by a purified epoxide hydrase preparation.[22] Assay of enzyme activity by h.p.l.c. indicated the anthracene 1,2-oxide (8) is a better substrate than styrene oxide which is commonly used to assay the enzyme. The high specificity of epoxide hydrase towards anthracene 1,2-oxide, combined with the unusual stability of this non-K-region arene oxide towards isomerization to phenols, adequately accounts for the inability of the present and previous [8,9] attempts to detect any quantities of 1- and 2-anthrol as metabolites.



Separation by h.p.l.c. of potential metabolites of anthracene. Chromatographic reference standards consisted of *trans*-1,2-dihydroxy-1,2-dihydroanthracene (14) and 1- and 2-anthrol (D. M. Jerina, H. Selander, H. Yagi, M. C. Wells, J. F. Davey, V. Mahadevan, and D. T. Gibson (*J. Amer. Chem. Soc.*, 1976, **98**, 5988), 9,10-dihydroxy-9,10-dihydroanthracene (E. Boyland and D. Manson, *J. Chem. Soc.*, 1951, 1837), and 9-anthrone, 9-anthrol, and 9,10-anthraquinone (Aldrich). Chromatography was conducted by injecting samples onto two coupled 1 m × 2.1 mm Du Pont permaphase ODS columns equilibrated with 0.1% phosphoric acid in water at a flow rate of 0.5 ml min^{-1}. At the solvent breakthrough, a linear gradient was initiated from 0—60% methanol in the aqueous phase at a rate of change of 2% min^{-1}. Elution of the column was then continued at 60% methanol until the anthracene had emerged

EXPERIMENTAL

M.p.s were determined using a Kofler block. Optical rotations were obtained using a Perkin-Elmer 141 automatic polarimeter. N.m.r. spectra were obtained with a Varian A-60 or a Bruker WH-90 spectrometer for solutions in

$CDCl_3$. Chemical shifts are reported as δ values. Compounds (1)—(4) were synthesised by routes reported previously from these laboratories.[10]

4-Bromo-1,2-epoxy-1,2,3,4-tetrahydroanthracene (5).—1,2-Epoxy-1,2,3,4-tetrahydroanthracene (0.43 g; 0.002 mol), *N*-bromosuccinimide (0.38 g, 0.002 mol), and α,α′-azoisobutyronitrile (0.01 g) were heated in CCl_4 under N_2 until refluxing occurred. After heating for 5 min the mixture was cooled and filtered to remove succinimide. Concentration under vacuum yielded the bromide (5) as a viscous oil (*ca.* 0.5 g), *m/e* 156 and 158.

The crude 4-bromo-1,2-epoxy-1,2,3,4-tetrahydroanthracene (5) (0.5 g) was dissolved in tetrahydrofuran (5 ml) and added dropwise to a stirred and cooled (*ca.* 0 °C) solution of 1,5-diazabicyclo[4,3,0]non-5-ene (0.4 g). The mixture was stirred in an ice-bath for 12 h, then diluted with ether, and filtered. The solution was washed (5% KOH), dried (K_2CO_3), and concentrated to a dark solid (0.1 g). T.l.c. (silica gel washed with triethylamine; chloroform–benzene–ethyl acetate–triethylamine 4 : 2 : 4 : 1 as eluant) showed a grey spot (R_F 0.6) upon spraying with Gibbs reagent–ammonia. A considerable quantity of material of lower R_F was also detected. Treatment with acetic acid gave a product whose t.l.c. lacked the original grey spot but showed a new purple spot (R_F 0.32) of identical colour and R_F to that obtained from 1-anthrol.

The n.m.r. spectrum showed a large proportion of unidentified impurity. Peaks at δ 7—8 (aromatic) and a characteristic doublet of doublets at δ 6.60 ($J_{3,4}$ 10, $J_{2,3}$ 3.5 Hz) and at δ 6.89 ($J_{3,4}$ 10 Hz) corresponded closely to those previously reported for naphthalene 1,2-oxide [7] and were assigned to anthracene 1,2-oxide.

1-Acetoxy-2-bromo-1,2,3,4-tetrahydroanthracene (6).—A mixture of *N*-bromoacetamide (1.65 g, 0.012 mol), 1,2-dihydroanthracene (1.98 g, 0.01 mol), and lithium acetate (4 g, 0.04 mol) in glacial acetic acid (50 ml) was stirred at room temperature for 1.5 h, and then poured into water (250 ml). The precipitate was filtered off, dissolved in ether, washed with water, and dried ($MgSO_4$). Concentration yielded the *acetate* (6) as crystals (2.5 g, 70%), m.p. 108—109° (from ether–hexane), δ 2.1 (3 H, s), 2.4 (2 H, m), 3.1 (2 H, m), 5.5 (1 H, m), 6.35 (1 H, d), and 7.8—7.4 (6 H, m) (Found: C, 60.0; H, 4.8; Br, 24.9. $C_{16}H_{15}O_2Br$ requires C, 60.2; H, 4.7; Br, 25.1%).

1-Acetoxy-2,4-dibromo-1,2,3,4-tetrahydroanthracene (7).—*N*-Bromosuccinimide (1.25 g, 0.007 mol) and α,α′-azoisobutyronitrile (0.02 g) were added to a solution of 1-acetoxy-2-bromo-1,2,3,4-tetrahydroanthracene (2.3 g, 0.007 mol) in CCl_4 (30 ml), and bromination was carried out as for epoxide (4). The dark residue was recrystallized from ether–light petroleum (b.p. 40—60°) to provide the *dibromide* (7) as crystals (1.35 g, 49%), m.p. 128—130° (decomp.), δ 2.25 (3 H, s), 3.0 (2 H, m), 4.8 (1 H, m), 5.75 (1 H, t), 7.55 (1 H, d), and 7.5—7.9 (6 H, m) (Found: C, 48.2; H, 3.4. $C_{16}H_{14}Br_2O_2$ requires C, 48.4; H, 3.5%).

Anthracene 1,2-Oxide (8).—Sodium methoxide (0.6 g) was added to a solution of the dibromide (7) (2.0 g, 0.011 mol) in tetrahydrofuran at 0 °C, and the mixture was stirred at this temperature for 24 h. The precipitated sodium bromide was filtered off, and the filtrate was diluted with ether, washed (5% KOH), dried (Na_2CO_3), and concentrated at 0 °C to provide a crystalline solid. Recrystallization from ether–pentane gave the *oxide* (8) as crystals (0.3 g, 33%), m.p. 130—134° (decomp.), δ 4.07 (1 H, dd, $J_{1,2}$ 4.0, $J_{2,3}$ 3.5 Hz), 4.53 (1 H, d, $J_{1,2}$ 4.0 Hz), 6.40 (1 H, dd, $J_{2,3}$ 3.5, $J_{3,4}$ 10 Hz), 6.89 (1 H, d, $J_{3,4}$ 10 Hz), and 7.20—8.00 (6 H, m) (Found: C, 85.7; H, 5.1. $C_{14}H_{10}O$ requires C, 86.5; H, 5.2%).

2-Acetoxy-3-bromo-1,2,3,4-tetrahydroanthracene (9).—Lithium acetate (4.0 g, 0.06 mol) and *N*-bromoacetamide (2.4 g, 0.01 mol) were added at room temperature to a stirred solution of 1,4-dihydroanthracene (2.8 g, 0.01 mol) in glacial acetic acid (70 ml). The mixture was stirred for 2 h and treated as described for the synthesis of (6) to yield the *acetate* (9) (4.2 g, 83%), m.p. 99—104° [from ether–light petroleum (b.p. 40—60°)], δ 2.07 (3 H, s), 3.07 (1 H, dd), 3.24—3.87 (3 H, m), 4.44 (1 H, dd), 4.50 (1 H, dd), and 7.18—7.82 (6 H, m) (Found: C, 60.5; H, 4.6. $C_{16}H_{15}BrO_2$ requires C, 60.2; H, 4.7%).

2-Acetoxy-3,4-dibromo-1,2,3,4-tetrahydroanthracene (10).—Benzylic bromination was carried out as described for the synthesis of (7). *N*-Bromosuccinimide (4.7 g, 0.026 mol), α,α′-azoisobutyronitrile (0.03 g), and 2-acetoxy-3-bromo-1,2,3,4-tetrahydroanthracene (6.0 g, 0.018 mol) in CCl_4 (180 ml) thus produced a viscous oil which recrystallized from ether–light petroleum (b.p. 40—60°) as pale yellow crystals (2.5 g, 34%), m.p. 128—130°, δ 2.13 (3 H, s), 3.72—3.15 (2 H, m), 5.06 (1 H, dd), 5.54 (1 H, dd), and 5.92 (1 H, d) (Found: C, 48.0; H, 3.5. $C_{16}H_{14}Br_2O_2$ requires C, 48.3; H, 3.6%).

1-Bromoanthracene (12).—2-Acetoxy-3,4-dibromo-1,2,3,4-tetrahydroanthracene (10) (2.0 g, 0.005 mol) in ether (275 ml) was stirred with potassium t-butoxide (2.0 g, 0.018 mol) for 5 h at ambient temperature. The precipitate was filtered off, and the yellow filtrate was washed (H_2O), dried ($MgSO_4$), and concentrated to give a yellow oil which was chromatographed on silica gel. Elution with light petroleum (b.p. 40—60°) and recrystallization from ether–pentane gave *1-bromoanthracene* (0.8 g, 63%), m.p. 97—100° (Found: C, 65.4; H, 3.5. $C_{14}H_9Br$ requires C, 65.4; H, 3.5%).

After a shorter reaction time (1.5 h) a dark viscous oil was formed which evolved acetic acid spontaneously at ambient temperature. Chromatography of this oil also provided 1-bromoanthracene in addition to a second oil which decomposed into acetic acid and 1-bromoanthracene. The latter unstable oil was identified as 2-acetoxy-4-bromo-1,2-dihydroanthracene (11) on the basis of its n.m.r. spectrum [δ 1.95 (3 H, s), 3.0—3.5 (2 H, m), 4.2—5.8 (1 H, m), 6.57 (1 H, d), and 7.0—8.0 (6 H, m)].

trans-*2-Ethylthio-1-hydroxy-1,2-dihydroanthracene* (15b) *and 1-Anthryl Ethyl Sulphide* (17b).—Anthracene 1,2-oxide (0.1 g) was stirred with ethanethiol (0.5 g) in aqueous NaOH (10 ml; 1%) at room temperature under nitrogen for 5 h. Multiple extraction with ether followed by drying ($MgSO_4$) and concentration gave an unstable yellow oil, δ 1.20 (3 H, t), 2.50 (2 H, q), 3.72 (1 H, dd), 4.93 (1 H, d), 6.05 (1 H, dd), 6.72 (1 H, d), and 7.33—7.83 (4 H, m). This adduct readily decomposed during attempted purification. It was identified as *trans*-2-ethylthio-1-hydroxy-1,2-dihydroanthracene (15b) on the basis of (i) the close similarity between the n.m.r. spectrum of (15b) and the previously identified structure (18) and (ii) decomposition in either chloroform or in trifluoroacetic acid–methanol (1 : 9) to 1-anthryl ethyl sulphide.

Passage of dry HCl gas for 30 min through a solution of 1-anthrol (0.1 g) in ethanethiol (1 ml) followed by heating in a sealed tube (180 °C, 72 h) gave a quantitative yield of 1-anthryl ethyl sulphide, yellow crystals, m.p. 43—45° (from ether–pentane), δ 1.37 (3 H, t, *J* 7 Hz), 3.05 (2 H, q,

J 7 Hz), and 7.20—8.98 (9 H, m) (Found: C, 80.9; H, 5.95. $C_{16}H_{16}S$ requires C, 80.7; H, 5.9%).

Metabolism of Anthracene.—Male Sprague–Dawley rats (*ca.* 175 g) were used with or without interperitoneal injections of phenobarbital (75 mg kg^{-1} in 0.9% aqueous NaCl) or 3-methylcholanthrene (25 mg kg^{-1} in cotton seed oil) for 4 days and were sacrificed on day 5. The livers from three animals in each group were pooled, homogenized in 0.1M-phosphate buffer (pH 7.4) and microsomes were prepared as reported previously.[23] Protein concentrations were determined by the method of Lowry.[24] Incubation mixtures contained 0.5 μmol NADPH, 3 μmol $MgCl_2$, 80 nmol [^{14}C]anthracene (3.64 mCi $mmol^{-1}$; Amersham Searle) added in 15 μl of acetone, and varying amounts of microsomal protein in a final volume of 1.0 ml of 0.1M-phosphate buffer at pH 7.4. All incubations were carried out at 37° and were terminated by extraction of the products into acetone (1 ml) and ethyl acetate (2 ml). Incubation with boiled microsomes served as controls. The organic phase was dried (Na_2SO_4) and evaporated at ambient temperature under a stream of nitrogen. The residues were dissolved in methanol (30 μl) which contained u.v.-detectable quantities of the structures shown in the Figure. Aliquots were injected onto the h.p.l.c. column, fractions were collected at intervals of 30 s, and radioactivity was determined in Aquasol. In all experiments >95% of the radioactivity due to metabolites was associated with the *trans*-1,2-dihydroxy-1,2-dihydroanthracene fractions; the remainder of the radioactivity emerged in the phenol region. A preliminary experiment established that metabolism was linear for up to 30 min with 400 μg ml^{-1} of microsomal protein from the phenobarbital-treated rats. All other incubations were carried out over a 30 min period. Metabolism was linear up to 300 μg ml^{-1} of microsomal protein from the 3-methylcholanthrene-treated animals and to 800 μg ml^{-1} for phenobarbital-treated and control animals. Data on turnover numbers for the three preparations in the Table are based on 2—3 determinations at each of 3—4 different concentrations of protein.

Epoxide Hydrase Studies.—Kinetic studies on the enzymatic hydration of anthracene 1,2-oxide were conducted with a nearly homogeneous preparation of epoxide hydrase [22] with a specific activity of 509 nmol styrene glycol mg $protein^{-1}$ min^{-1}. Incubations were conducted at 37 °C in a final volume of 1.0 ml of 0.17M-Tris buffer (pH 9.0) containing 0.033% Tween 80. Reactions were terminated and worked up as for metabolism of anthracene except that *trans*-9,10-dihydroxy-9,10-dihydrophenanthrene was the only marker compound added. The latter was used as an internal standard in order to obtain a quantitative estimate of the anthracene diol metabolite. Chromatography was conducted as described for metabolism of anthracene (Figure) except that the gradient was run from 0 ⟶ 30% methanol at a rate of change of 1% min^{-1}. Peak areas were measured with an Autolab System IV automatic integrator which had been checked against mixtures of known ratio using standard solutions. *trans*-1,2-Dihydroxy-1,2-dihydroanthracene was estimated spectrophotometrically using ε(268 nm) 14 000 l mol^{-1}.

The effect of substrate concentration was examined in the range 0.20—0.55mM with 17.5 μg of enzyme ml^{-1} and 5 min incubations. Since substrate concentrations in excess of 0.4—0.5mM showed inhibition, further incubations were conducted at 0.33mM which was near the optimal rate. With this concentration of substrate, reactions were linear for 6 min with up to 20 μg of enzyme ml^{-1}. Values of apparent K_m (0.34mM) and $V_{max.}$ (1 053 nmol diol mg $protein^{-1}$ min^{-1}) were measured with 17.5 μg of enzyme ml^{-1} and were incubated for 5 min.

For the preparative incubation, liver microsomes were prepared from female New Zealand white rabbits. The incubation mixture contained 80 mg of microsomal protein and 20 mg of anthracene 1,2-oxide in 0.6 ml of tetrahydrofuran in a final volume of 50 ml of 0.1M-Tris buffer (pH 9.0). After incubation for 2 h at 37 °C the solution was saturated with NaCl and the diol was extracted into ethyl acetate. The diol was purified by t.l.c., acetylated with acetic anhydride–pyridine, and repurified by t.l.c. to provide 23.5 mg of diacetate ($[\alpha]_D$ −10.5°, dioxan).

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