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Metabolism of Polycyclic Compounds

THE METABOLISM OF 9,10-EPOXY-9,10-DIHYDROPHENANTHRENE IN RATS

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1. 9,10-Epoxy-9,10-dihydrophenanthrene is converted by rats into *trans*-9,10-dihydro-9,10-dihydroxyphenanthrene and its conjugates and into *N*-acetyl-*S*-(9,10-dihydro-9-hydroxy-10-phenanthryl)-L-cysteine. Small amounts of 9-phenanthryl sulphate and glucosiduronate are also formed, together with 9-hydroxy-10-phenanthryl sulphate. 2. The epoxide readily rearranges to 9-hydroxyphenanthrene and reacts with water to give the dihydrodihydroxy compound and with *N*-acetylcysteine to give the mercapturic acid. One of the diastereoisomeric forms of the methyl ester of the mercapturic acid is identical with the ester of the mercapturic acid excreted by rats dosed with the epoxide or by rats and rabbits dosed with phenanthrene. 3. A rat-liver homogenate converts the epoxide into *trans*-9,10-dihydrodihydroxyphenanthrene and *S*-(9,10-dihydro-9-hydroxy-10-phenanthryl)glutathione. The latter compound was not formed when boiled rat-liver homogenate was used, but was formed chemically from the epoxide and glutathione under more vigorous conditions. 4. The results provide further indications that the metabolism of aromatic hydrocarbons involves the intermediary formation of epoxides.

As a result of an investigation into the metabolism of phenanthrene in rats and rabbits (Sims, 1962; Boyland & Sims, 1962*a*,*b*), it was suggested that the initial step in the metabolism of the hydrocarbon was an addition of oxygen across the 1,2-, the 3,4- and the 9,10-bonds to form epoxydihydro compounds. These could then either rearrange to yield phenols or react with water to yield dihydrodihydroxy compounds or with glutathione by an enzymic process to yield glutathione conjugates that are further metabolized in the body to yield finally the mercapturic acids found in the urine. 9,10-Epoxy-9,10-dihydrophenanthrene (I), which has recently become available through the work of Newman & Blum (1964), is one such postulated intermediate and the metabolism of this compound in rats is described below.

EXPERIMENTAL

Light-absorption spectra. These were measured on either a Unicam SP. 500 spectrophotometer or on a Perkin–Elmer model 137 ultraviolet spectrophotometer.

Melting points. These are uncorrected.

Chromatography. Paper chromatography was carried out by downward development for 18hr. on Whatman no. 1 chromatography paper (3MM paper for preparative chromatography), with butan-1-ol–propan-1-ol–aq. 2N-NH_3 (2:1:1, by vol.). The dried chromatograms were examined in u.v. light and either (1) sprayed with a solution of diazotized *p*-nitroaniline (0·2% in 0·1N-HCl) or (2) sprayed with a solution of diazotized *p*-nitroaniline (0·2% in 4N-HCl) and heated for 5min. to 80°. Papers were then sprayed with aq. 10% (w/v) Na_2CO_3. Other papers were dipped in the platinic iodide reagent of Toennies & Kolb (1951) or in 0·2% ninhydrin in acetone.

Thin-layer chromatograms were prepared by coating glass plates with a layer of silica gel G (E. Merck A.-G., Darmstadt, Germany) of 0·25mm. thickness. Plates were developed for 10cm. with (*a*) light petroleum (b.p. 60–80°)–benzene (19:1, v/v), (*b*) benzene, (*c*) benzene–ethanol (19:1, v/v) or (*d*) benzene–ethanol (9:1, v/v). The chromatograms were sprayed with a 0·5% solution of 2,6-dichloroquinonechloroimide in ethanol, either immediately or after spraying with conc. HCl and heating to 80° for 10min. The methyl esters of the mercapturic acids were detected by spraying the plates with the $K_2Cr_2O_7$–$AgNO_3$ reagent of Knight & Young (1958). Two-dimensional thin-layer chromatograms were developed in the first direction with solvent (*c*) or (*d*), sprayed with conc. HCl and heated at 80° for 10min. and developed in the second direction with solvent (*a*) or (*b*). In this way products formed by the acid-decomposition of metabolites were identified.

For the properties on paper and thin-layer chromatograms of the metabolites described in this paper see Sims (1962) and Boyland & Sims (1962*a*,*b*,*c*).

Materials. cis- and *trans*-9,10-Dihydro-9,10-dihydroxyphenanthrene (IV, R = H) were prepared by the methods of Criegee, Marchand & Wannowius (1942) and Booth, Boyland & Turner (1950) respectively, and had m.p. 176° and 187°.

Potassium 9-phenanthryl sulphate (II, R=SO_3K) and ammonium 9-hydroxy-10-phenanthryl sulphate were prepared as described by Sims (1962), and potassium 9,10-dihydro-9-hydroxy-10-phenanthryl sulphate (IV, R=SO_3K) as described by Boyland & Sims (1962*c*).

The (−)-methyl ester of *N*-acetyl-*S*-(9,10-dihydro-9-hydroxy-10-phenanthryl)-L-cysteine was prepared from the mercapturic acid (III) isolated from the urines of both rats and rabbits dosed with phenanthrene (Boyland & Sims, 1962*b*): the (−)-methyl ester from each source had m.p. 94°, not depressed when the two samples were mixed. (+)- and (−)-Methyl (9-acetoxy-9,10-dihydro-10-phenanthryltri-*O*-acetyl-*O*-glucosid)uronate were prepared from the parent glucosiduronic acids (IV, R=$C_6H_9O_6$) obtained from the urine of rats dosed with *trans*-9,10-dihydro-9,10-dihydroxyphenanthrene (IV, R=H) (Boyland & Sims, 1962*c*). They had m.p. 247° and 167° respectively.

Biphenyl-2,2′-dicarboxaldehyde was prepared in about 60% yield by the oxidation of *trans*-9,10-dihydro-9,10-dihydroxyphenanthrene (IV, R=H) with sodium periodate under the conditions described by Hadler & Kryger (1960) for the oxidation of *cis*-5,6-dihydro-5,6-dihydroxy-7,12-dimethylbenz[*a*]anthracene. The dialdehyde, cyclized in benzene with tri(dimethylamino)phosphine, as described by Newman & Blum (1964), yielded 9,10-epoxy-9,10-dihydrophenanthrene (I). The crystals that separated from cyclohexane had m.p. 103–104°, $\lambda_{max.}$, in cyclohexane, at 277 and 290 mμ (ϵ 19500 and 12700 respectively) and inflexions at 269 and 302 mμ. *trans*-9,10-Dihydro-9,10-dihydroxyphenanthrene (IV, R=H) has $\lambda_{max.}$, in ethanol, at 268 mμ and inflexions at 224, 230 and 300 mμ (Boyland & Sims, 1962*b*). Newman & Blum (1964) give m.p. 104–105° for the epoxide (I).

The epoxide (I) had R_F 0·53 and 0·83 on thin-layer chromatograms in solvents (*b*) and (*c*) respectively, but some decomposition usually occurred, particularly on chromatograms developed with solvent (*b*), to give spots, with a violet fluorescence in u.v. light, indistinguishable from 9-hydroxyphenanthrene. The epoxide spots often developed 'tails' with a violet fluorescence in u.v. light, which were due to the rearrangement of the epoxide (I) to the phenol (II, R=H) (with which it is isomeric) during the running of the chromatograms. The epoxide spot was not at first visible when the chromatograms were examined in u.v. light, but after a few minutes a violet fluorescence appeared and the spots gave an immediate blue–green colour with the 2,6-dichloroquinonechloroimide–Na_2CO_3 reagent, characteristic of that given by 9-hydroxyphenanthrene. A spot indistinguishable from that of *trans*-9,10-dihydro-9,10-dihydroxyphenanthrene was often found during the chromatography of the epoxide, presumably arising from the reaction of the epoxide with water bound to the silica gel. In experiments described below, in which the reactions of the epoxide were studied and in which unchanged epoxide was detected, the formation of the phenol and the dihydroxy compound in the reactions was indicated on thin-layer chromatograms by increases in the sizes of their spots as compared with those formed when the pure epoxide was chromatographed.

Reaction of 9,10-*epoxy*-9,10-*dihydrophenanthrene* (*I*) *with water*. The epoxide (I) (100 mg.) was heated under reflux with aq. 60% (v/v) acetone (25 ml.) for 36 hr. The acetone was removed under reduced pressure and the mixture extracted with ether (50 ml.). The ether solution was extracted with 2 N-NaOH (20 ml.) and washed with water and dried over Na_2SO_4. The residue was recrystallized from benzene to yield *trans*-9,10-dihydro-9,10-dihydroxyphenanthrene (45 mg.) in needles, m.p. and mixed m.p. 187°. A mixture with the *cis*-isomer had m.p. 156–157°. The diacetate separated from ethanol in plates, m.p. 173°, undepressed on admixture with *trans*-9,10-diacetoxy-9,10-dihydrophenanthrene.

The aqueous layer was acidified with conc. HCl and extracted with ether. The presence of 9-hydroxyphenanthrene in the extracts was demonstrated by thin-layer chromatography.

In other experiments in which the epoxide was kept for 1 and 7 days at room temperature in aq. 50% (v/v) acetone, unchanged epoxide together with 9-hydroxyphenanthrene and *trans*-9,10-dihydro-9,10-dihydroxyphenanthrene were found when the mixtures were examined on thin-layer chromatograms.

Reaction of 9,10-*epoxy*-9,10-*dihydrophenanthrene* (*II*) *with* N-*acetyl*-L-*cysteine*, L-*cysteine and glutathione*. 9,10-Epoxy-9,10-dihydrophenanthrene (I) (300 mg.), *N*-acetyl-L-cysteine (250 mg.) (British Drug Houses Ltd., Poole, Dorset) and $NaHCO_3$ (400 mg.) were heated under reflux with aq. 50% (v/v) acetone (50 ml.) for 4 hr. The acetone was distilled off under reduced pressure and the aqueous residue extracted twice with ether (25 ml.). The residue obtained on evaporation of the ether was examined on thin-layer chromatograms, when unchanged epoxide, 9-hydroxyphenanthrene and *trans*-9,10-dihydro-9,10-dihydroxyphenanthrene were detected.

The aqueous layer was acidified to pH 4 with acetic acid and activated charcoal (5 g.) (British Drug Houses Ltd.) was added. The charcoal was filtered off and washed with water and the absorbed material eluted with methanol (500 ml.) containing 5% (v/v) of aq. NH_3 (sp.gr. 0·88). The solvent was evaporated and the residue chromatographed on paper. The dark-violet-fluorescent bands, seen when the chromatograms were examined in u.v. light, were cut out and the absorbed material was eluted from the paper with methanol containing 5% (v/v) of aq. NH_3 (sp.gr. 0·88). Evaporation of the solvent under reduced pressure yielded about 200 mg. of a colourless gum that had R_F 0·40 on paper chromatograms, gave a positive reaction with the platinic iodide reagent and was indistinguishable from *N*-acetyl-*S*-(9,10-dihydro-9-hydroxy-10-phenanthryl)-L-cysteine (III), previously detected in the urines of rats and rabbits dosed with phenanthrene (Boyland & Sims, 1962*a*). The gum, which could not be crystallized, is presumed to be a mixture of the diastereoisomeric forms of the mercapturic acid (III). Its light-absorption spectrum, measured in methanol, showed $\lambda_{max.}$ at 270 mμ. When a little of the gum in water was treated with a few drops of conc. HCl at room temperature, a product was detected on paper chromatograms that was indistinguishable from 9-phenanthrylmercapturic acid, and a second was detected on thin-layer chromatograms developed with solvent (*a*) that was indistinguishable from phenanthrene.

The gum, in methanol, was esterified with diazomethane in ether to yield a gummy product after evaporation of the solvents. This was dissolved in a small volume of methanol, and water was added dropwise until the oily product that separated began to crystallize. When the crystallization was complete the solid was filtered off and recrystallized twice from aq. methanol to yield the (−)-methyl ester of *N*-acetyl-*S*-(9,10-dihydro-9-hydroxy-10-phenanthryl)-L-

cysteine (80mg.), m.p. 91–92°, $[\alpha]_D^{25}$ $-397\pm5°$ (*c* 0·5 in methanol). The melting point was not depressed on admixture with the ester samples obtained from rats and rabbits treated with phenanthrene.

The mother liquors from which the solid first separated were evaporated under reduced pressure to yield a gum that could not be crystallized. It is presumed to consist mainly of the (+)-methyl ester of *N*-acetyl-*S*-(9,10-dihydro-9-hydroxy-10-phenanthryl)-L-cysteine, $[\alpha]_D^{25}$ $+261\pm7°$ (*c* 0·5 in methanol). The light-absorption curves, measured in methanol, of both the esters showed $\lambda_{max.}$ at 269mμ. Both esters had R_F 0·33 on thin-layer chromatograms developed with solvent (*d*) and both were indistinguishable on these chromatograms from the esters of the mercapturic acid obtained from the urine of animals dosed with phenanthrene. On two-dimensional, thin-layer chromatograms developed with solvent (*d*), acid-treated, and then developed with solvent (*a*), it was shown that both esters yielded phenanthrene as acid-decomposition products.

The epoxide (I) (50mg.), L-cysteine hydrochloride (41mg.) and $NaHCO_3$ (44mg.) were heated under reflux with aq. 50% (v/v) acetone (10ml.) for 3hr. The mixture was worked up as before and the aqueous layer yielded a gum that appeared to be a mixture of the diastereoisomers of *S*-(9,10-dihydro-9-hydroxy-10-phenanthryl)-L-cysteine. It formed a spot on paper chromatograms, R_F 0·28, that gave a positive reaction with the platinic iodide reagent and a purple colour with ninhydrin. It was indistinguishable from the corresponding metabolite detected in the bile of rats treated with phenanthrene (Boyland & Sims, 1962*a*) and from the cysteine derivatives obtained when the products of the oxidation of phenanthrene with perbenzoic acid were treated with cysteine (Boyland & Sims, 1961). Its light-absorption curve, measured in methanol, showed $\lambda_{max.}$ at 270mμ. When treated with acid the conjugate yielded compounds indistinguishable from *S*-(9-phenanthryl)cysteine and phenanthrene.

9,10-Epoxy-9,10-dihydrophenanthrene (20mg.), glutathione (20mg.) and $NaHCO_3$ (20mg.) in aq. 50% (v/v) acetone (2ml.) were heated under reflux for 3hr. The solution was treated as before to yield an ether-soluble fraction that contained compounds indistinguishable on thin-layer chromatograms from the epoxide (I), 9-hydroxyphenanthrene and *trans*-9,10-dihydro-9,10-dihydroxyphenanthrene. The aqueous fraction formed a gum that appeared to be a mixture of the diastereoisomers of *S*-(9,10-dihydro-9-hydroxy-10-phenanthryl)glutathione. It formed a spot on paper chromatograms, R_F 0·12, that gave a positive reaction with the platinic iodide reagent and a purple colour with ninhydrin. The light-absorption, in methanol, had $\lambda_{max.}$ at 270mμ. The product was indistinguishable from the glutathione conjugate detected in the bile of rats treated with phenanthrene (Boyland & Sims, 1962*a*). On acidification with conc. HCl, a product was detected on thin-layer chromatograms indistinguishable from phenanthrene and a product was detected on paper chromatograms, R_F 0·16, that had an orange fluorescence in u.v. light, gave a purple colour with ninhydrin and appeared to be *S*-(9-phenanthryl)-glutathione.

Toxicity. Phenanthrene and 9,10-epoxy-9,10-dihydrophenanthrene were given to mice by intraperitoneal injection in arachis oil. Mice tolerated doses of 1g./kg. but died after doses of 2g. of each compound/kg.

Metabolism of 9,10-epoxy-9,10-dihydrophenanthrene (I). (*a*) In rats. Four rats of the Chester Beatty strain (body wt. approx. 250g.) were each given 9,10-epoxy-9,10-dihydrophenanthrene (I) (75mg.) in arachis oil (1ml.) by intraperitoneal injection on two successive days and the urine was collected up to the third day. The pooled urines were filtered and the filtrate was acidified to pH4 with acetic acid. Charcoal (15g.) was added and the mixture filtered. The charcoal was washed with water (250ml.) and the adsorbed materials were eluted with methanol (500ml.) containing 5% (v/v) of aq. NH_3 (sp.gr. 0·88). The gum that was obtained on evaporation of the solvent was chromatographed on paper. The dried chromatograms were examined in u.v. light, and five bands with R_F values 0·90, 0·75, 0·50, 0·40 and 0·20 were cut out and the absorbed materials eluted from the paper with methanol containing 5% (v/v) of aq. NH_3 (sp.gr. 0·88). Evaporation of the solvent under reduced pressure afforded five fractions, numbered from 1 to 5 in descending order of R_F values.

Fraction 1 contained a compound that had R_F 0·27 on thin-layer chromatograms developed with solvent (*c*) and gave an orange colour with the 2,6-dichloroquinonechloroimide–Na_2CO_3 reagent after the plate was heated with acid: it was indistinguishable from *trans*-9,10-dihydro-9,10-dihydroxyphenanthrene. On acid-treated two-dimensional thin-layer chromatograms it yielded a compound indistinguishable from 9-hydroxyphenanthrene.

Fraction 2 contained two compounds that were separated by chromatography on Whatman no. 1 chromatography paper. The faster-moving fraction formed a spot, R_F 0·78, that had a violet fluorescence in u.v. light, and gave an orange colour with reagent (2). It yielded a compound indistinguishable from 9-hydroxyphenanthrene on thin-layer chromatograms after being heated to 100° with aq. 5N-HCl for 15min. and its light-absorption spectrum, measured in methanol, showed $\lambda_{max.}$ at 251, 274 and 284mμ. The second product, R_F 0·72, had a bright-blue fluorescence when paper chromatograms were examined in u.v. light. It gave pale-green colours with reagents (1) and (2) and, after hydrolysis with hot 5N-HCl, yielded a compound indistinguishable from 9,10-phenanthrenequinone. Its light-absorption spectrum had $\lambda_{max.}$ at 251, 274 and 284mμ. It is concluded that the two metabolites were 9-phenanthryl sulphate and 9-hydroxy-10-phenanthryl sulphate respectively.

Fraction 3 contained a compound, R_F 0·52 on paper chromatograms, that had a dark-violet fluorescence in u.v. light and gave an orange colour with reagent (2). Its light-absorption curve, measured in methanol, showed $\lambda_{max.}$ at 267mμ. The product yielded a compound indistinguishable from 9-hydroxyphenanthrene on thin-layer chromatograms after being heated with 5N-HCl. In all these properties the compound was indistinguishable from 9,10-dihydro-9-hydroxy-10-phenanthryl sulphate.

Fraction 4 contained a compound, R_F 0·39 on paper chromatograms, that had a dark-violet fluorescence in u.v. light and gave a positive reaction with the platinic iodide reagent. Its light-absorption curve had $\lambda_{max.}$ at 270mμ. It was decomposed by cold HCl to yield compounds indistinguishable from 9-phenanthrylmercapturic acid and phenanthrene.

The compound, in methanol, was esterified with diazomethane in ether and the product applied to the base lines of two thin-layer chromatograms. The portions of the chromatograms that appeared as translucent bands, seen

when the wet chromatograms were examined in daylight, were removed and the absorbed material was eluted from the silica gel with ether. The ether was removed and the residue was recrystallized from aq. methanol to yield an ester (15mg.) in flat needles, m.p. 89–90°, undepressed on admixture with the (−)-methyl ester of *N*-acetyl-*S*-(9,10-dihydro-9-hydroxy-10-phenanthryl)-L-cysteine obtained both from the epoxide and from the urine of rats or rabbits dosed with phenanthrene. The light-absorption spectrum, measured in methanol, had $\lambda_{max.}$ at 269mμ, and the four esters were indistinguishable on thin-layer chromatograms developed with solvent (*d*). It therefore appears that the mercapturic acid formed by rats treated with 9,10-epoxy-9,10-dihydrophenanthrene is *N*-acetyl-*S*-(9,10-dihydro-9-hydroxy-10-phenanthryl)-L-cysteine (III) and is the same stereoisomer as that excreted by rats and rabbits treated with phenanthrene.

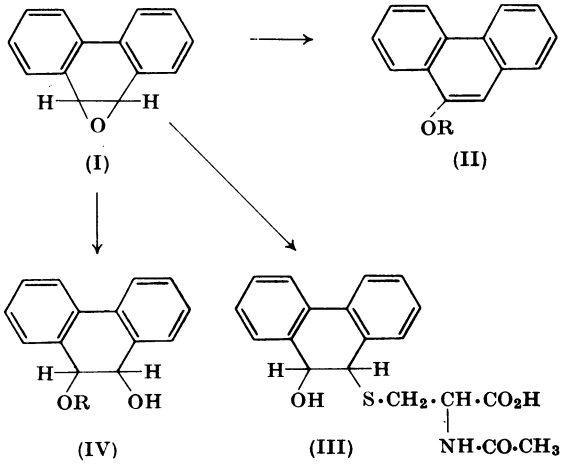
Fraction 5 contained a compound that was indistinguishable on paper chromatograms from 9,10-dihydro-9-hydroxy-10-phenanthrylglucosiduronic acid, and its light-absorption curve, measured in methanol, had $\lambda_{max.}$ at 268mμ. A little of the material was incubated in 0·1M-phosphate buffer, pH 5·0, with β-glucuronidase (Ketodase; Warner Chilcott Laboratories, Morris Plains, N.J., USA.) at 37° overnight. The mixture was extracted with ether and the residue obtained by evaporating the solution examined on thin-layer chromatograms, when a compound indistinguishable from *trans*-9,10-dihydro-9,10-dihydroxyphenanthrene and a small amount of a compound indistinguishable from 9-hydroxyphenanthrene were detected.

The fraction, in methanol, was esterified with diazomethane in ether, and the residue that remained on removal of the solvent was dissolved in pyridine and the solution treated with acetic anhydride and kept overnight. The mixture was poured into water and the solid that separated was fractionally recrystallized from ethanol. The least soluble fraction formed needles (21mg.), m.p. 246–247°, undepressed on admixture with (+)-methyl (9-acetoxy-9,10-dihydro-10-phenanthryltri-*O*-acetyl-D-glucosid)uronate. The least soluble fraction formed plates (10mg.), m.p. 163–164°, undepressed on admixture with (−)-methyl (9-acetoxy-9,10-dihydro-10-phenanthryltri-*O*-acetyl-D-glucosid)uronate.

(*b*) By rat-liver homogenate. The homogenate, prepared from the livers of four rats of the Chester Beatty strain (body wt. approx. 200g.) as described by Boyland & Sims (1965), was divided into two equal portions, one of which was heated to 100° for 5min. Nicotinamide (880mg.), $NADP^+$ (15mg.) and glucose 6-phosphate (125mg.), obtained from the sources previously described, were added to each portion and the mixtures were each incubated at 37° for 1hr. in the presence of 9,10-epoxy-9,10-dihydrophenanthrene (25mg.), added as a solution in ethanol (0·5ml.). The mixtures were each treated as described by Boyland & Sims (1965) to give an ethyl acetate extract and a fraction containing water-soluble material.

The ethyl acetate extracts were examined on thin-layer chromatograms. That from the fresh preparation contained a compound indistinguishable from *trans*-9,10-dihydro-9,10-dihydroxyphenanthrene, whereas that from the boiled preparation contained compounds indistinguishable from the epoxide, from 9-hydroxyphenanthrene and from *trans*-9,10-dihydro-9,10-dihydroxyphenanthrene. There was no marked difference in the sizes of the dihydrodihydroxy compound spots formed in the two preparations.

The fraction containing the water-soluble products was examined on paper chromatograms. The material from the fresh preparation contained a product, R_F 0·12, that gave a positive reaction with the platinic iodide reaction and a purple colour with ninhydrin. It was indistinguishable from the synthetic *S*-(9,10-dihydro-9-hydroxy-10-phenanthryl)-glutathione described above. The light-absorption curve had $\lambda_{max.}$ at 270mμ. With conc. HCl the product yielded compounds indistinguishable on chromatograms from phenanthrene and *S*-(9-phenanthryl)glutathione. The glutathione conjugate was not detected when the water-soluble fraction from the boiled preparation was examined on paper chromatograms.



Scheme 1. Metabolic reactions of 9,10-epoxy-9,10-dihydrophenanthrene.

DISCUSSION

The products formed in the metabolism of 9,10-epoxy-9,10-dihydrophenanthrene (I) in rats are, with two exceptions, those formed by metabolic reactions at the 9,10-bond of phenanthrene. The exceptions are the sulphuric acid and glucuronic acid conjugates of 9-hydroxyphenanthrene (II, R = SO_3H and C_6H_9O), neither of which were detected as products of phenanthrene metabolism (Boyland & Sims, 1962*b*). 9,10-Epoxy-9,10-dihydrophenanthrene (I), however, readily rearranges to 9-hydroxyphenanthrene (II, R = H), both on heating (Newman & Blum, 1964) and in aqueous media at room temperature, so that it is possible that the phenol was formed before the material injected reached the liver. 9-Hydroxyphenanthrene (II, R = H) was not detected as a product of the metabolism of the epoxide (I) with fresh homogenate, presumably because the enzymic conjugation with glutathione was faster than the rearrangement reaction leading to the phenol. The phenol, however,

was formed in the experiments with heated homogenates.

9-Hydroxy-10-phenanthryl sulphate presumably arises from 9,10-dihydroxyphenanthrene formed by the dehydrogenation of *trans*-9,10-dihydro-9,10-dihydroxyphenanthrene (IV, R = H), a reaction known to occur in rats (Boyland & Sims, 1962*c*). *trans*-9,10-Dihydro-9,10-dihydroxyphenanthrene, excreted free and in conjugation with sulphuric acid and glucuronic acid (IV, R = SO_3H and $C_6H_9O_6$), is presumably formed by the reaction of water and the epoxide (I). It seems likely that this reaction is non-enzymic, because derivatives of both the (+)- and the (−)-forms of the dihydrodihydroxy compound (IV, R = H) were formed and because there was no apparent difference between the amounts of the dihydrodihydroxy compound formed from the epoxide by fresh and boiled rat-liver preparations. In both the chemical and biological reactions, the *trans*- and not the *cis*-form of the dihydrodihydroxy compound (IV, R = H) is formed: the *trans*-isomer is also formed in phenanthrene metabolism.

The mercapturic acid formed (III) from the epoxide had the same chemical properties and the same stereochemical configuration as that formed from phenanthrene by both rats and rabbits. In this, the hydrocarbon and the related epoxide (I) resemble 1,2-dihydronaphthalene and 1,2-epoxy-1,2,3,4-tetrahydronaphthalene, both of which yield the same stereochemical form of *N*-acetyl-*S*-(1,2,3,4-tetrahydro-2-hydroxy-1-naphthyl)-L-cysteine in rats (Boyland & Sims, 1960). The initial conjugation of 9,10-epoxy-9,10-dihydrophenanthrene (I) is evidently an enzymic process, since it occurs with fresh, but not with boiled, rat-liver preparations; the isolation of an optically active mercapturic acid confirms this. The enzyme is probably that which catalyses the conjugations of a number of epoxides with glutathione (Boyland & Williams, 1964, 1965), since the reaction of 9,10-epoxy-9,10-dihydrophenanthrene (I) with glutathione is catalysed by this enzyme (K. Williams, personal communication).

The synthesis of this mercapturic acid is the first unambiguous synthesis of a dihydrohydroxy mercapturic acid of the type formed as metabolites of aromatic hydrocarbons, and it provides additional evidence that these acids possess structures analogous to that postulated for the naphthalene metabolite (Boyland & Sims, 1958).

The results provide further indications that the metabolism of aromatic hydrocarbons involves the intermediary formation of epoxides.

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