Metabolism of Polycyclic Compounds

21. THE METABOLISM OF PHENANTHRENE IN RABBITS AND RATS: DIHYDRODIHYDROXY COMPOUNDS AND RELATED GLUCOSIDURONIC ACIDS

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It was shown by Young (1947) and by Boyland & Wolf (1950) that rats convert phenanthrene into trans-9,10-dihydro-9,10-dihydroxyphenanthrene; the latter workers further showed that rats convert phenanthrene into this compound and also into trans-1,2-dihydro-1,2-dihydroxyphenanthrene. Bergel & Pachor (1903) had found that phenanthrene causes an increased excretion of glucuronic acid conjugates. In the present work it was found that the above compounds, together with trans-3,4-dihydro-3,4-dihydroxyphenanthrene, are excreted both free and conjugated with glucuronic acid in animals of both species. Glucuronic acid conjugates of hydroxyphenanthrenes and of dihydroxyphenanthrenes are also present.

MATERIALS AND METHODS

Melting points. These are uncorrected.

Materials. The preparations of 1-, 2-, 3-, 4- and 9-hydroxyphenanthrene and their methyl ethers, of 1,2- and 3,4-dihydroxyphenanthrene and their monomethyl ethers, and of (+)-trans-9,10-dihydro-9,10-dihydroxyphenanthrene, have been described by Sims (1962). cis-9,10-Dihydro-9,10-dihydroxyphenanthrene was prepared by the oxidation of phenanthrene with osmic acid by the method of Criegee, Marchand & Wannowius (1942).

Chromatography. Paper chromatography (in solvents 1 and 2, Table 1) and the thin-layer chromatography used in the detection of the hydroxyphenanthrene and its monomethyl ethers of the dihydroxyphenanthrenes were carried out as described by Sims (1962). The dihydrodihydroxy compounds (Table 2) were detected on thin-layer chromatograms, prepared from silica gel G and developed with benzene containing 5% (v/v) of ethanol, by spraying the chromatograms with 10N-HCl, heating at 85° for 15 min. and then spraying with 2,6-dichloroquinonechlorimide and Na2CO3 (Sims, 1962). The dihydrodihydroxy compounds were also examined on two-dimensional thin-layer chromatograms which were developed in the first direction with benzene containing 5% (v/v) of ethanol, sprayed with 10N-HCl and heated at 85° for 15 min. and then developed in the second direction with benzene. Phenanthrene was identified on thin-layer chromatograms, prepared from silica gel G, which were developed with n-hexane containing 5% (v/v) of benzene, by its violet fluorescence in u.v. light and by the purple colour given with the tetracyanoethylene reagent of Tarbell & Huang (1959).

Hydrolysis of dihydrodihydroxy compounds and glucosiduronic acids. The dihydrodihydroxy compounds were heated with 5N-HCl at 100° for 10 min., and the products were extracted from the cooled solutions with ether and examined for hydroxyphenanthrenes on thin-layer chromatograms. Glucosiduronic acids were heated with 5N-HCl at 100° for 30 min. and the acetyl derivatives of these acids were heated under reflux with ethanol-HCl (1:1, v/v) for 30 min. The products were extracted and chromatographed.

The glucosiduronic acids were hydrolysed with β-glucuronidase, by using either Ketodase (Warner–Chilcott Laboratories) in 0.1M-acetate buffer (pH 5.0) or bacterial β-glucuronidase (Sigma Chemical Co.) in 0.1M-phosphate buffer (pH 6.0). The mixtures were incubated overnight at 37° and extracted with ether, and the products were either examined on thin-layer chromatograms or treated as described below.

The acetyl methyl ester derivatives of the glucosiduronic acids were hydrolysed to the parent acids as described by Bollenback, Long, Benjamin & Lindquist (1955), by suspension in a 10% excess of 0.1N-barium methoxide in methanol and keeping the mixtures overnight at room temperature. The precipitated barium salts were filtered off, washed with methanol and treated as described below.

Animal experiments. The fractions containing the dihydrodihydroxy compounds and the glucosiduronic acids obtained from rabbits and rats dosed with phenanthrene (Sims, 1962) were used.

RESULTS

Examination of the dihydrodihydroxyphenanthrene fractions from rabbits

The combined fractions formed a brown, partly crystalline mass (520 mg.) which, by two-dimensional acid-treated thin-layer chromatograms, was found to contain three substances that gave rise to compounds indistinguishable from 9-hydroxyphenanthrene, 1- and 2-hydroxyphenanthrene, and 3- and 4-hydroxyphenanthrene respectively. The first of these substances was indistinguishable on paper and thin-layer chromatograms from synthetic trans-9,10-dihydro-9,10-dihydroxyphenanthrene, but the corresponding cis-compound could not be detected.

The mixture of crystals and gum was dissolved in the minimum amount of boiling benzene and treated with charcoal. The filtered solution was allowed to cool, when (+) · trans · 9,10-dihydro · 9,10-dihydroxyphenanthrene (200 mg.) separated. It was recrystallized from benzene in oily needles, m.p. 187°, and yielded, on
acetylation with acetic anhydride in pyridine, a diacetate which separated from ethanol in plates, m.p. and mixed m.p. 173°.

The mother liquors were passed through a column prepared from silica gel G (60 g.) and Hyflo Super-Cel (30 g.) which was developed with benzene containing 3% (v/v) of ethanol. Fractions of 30 ml. were collected and examined for dihydrodihydroxy compounds on thin-layer chromatograms. Fractions 4–10, containing trans-9,10-dihydro-9,10-dihydroxyphenanthrene, were combined and evaporated, and the residue was recrystallized from benzene to yield the (±)-isomer in needles (35 mg.), m.p. and mixed m.p. 187°. The filtrate was evaporated and the residue acetylated with acetic anhydride in pyridine to yield a substance (15 mg.), separating from ethanol in plates, m.p. 126–128°, [α]D25 + 106 ± 2° (c 0.5 in CHCl3) (Found: C, 72.6; H, 5.6. Calc. for C21H18O4: C, 72.9; H, 5.4%). This appeared to be a mixture of (+)- and (−)-trans-9,10-diacetoxy-9,10-dihydrophenanthrene since only 9-hydroxyphenanthrene could be detected on thin-layer chromatograms after hydrolysis with acid.

Fractions 11 and 12 contained trans-9,10-dihydro-9,10-dihydroxyphenanthrene and trans-1,2-dihydro-1,2-dihydroxyphenanthrene, but fractions 13–23 contained only the 1,2-isomer. These were combined and evaporated, and the residue was recrystallized three times from benzene to yield a product (65 mg.) which appeared to be a mixture of (−)- and (±)-trans-1,2-dihydro-1,2-dihydroxyphenanthrene, forming thick needles, m.p. 156–158°, [α]D25 + 33 ± 6° (c 0.5 in ethanol) (Found: C, 79; H, 5.8. Calc. for C21H18O4: C, 79.2; H, 5.7%). The product was acetylated with acetic anhydride to yield a diacetate which separated from ethanol in plates, m.p. 96–98°, [α]D25 ± 90 ± 3° (c 0.5 in ethanol). The mother liquors from the recrystallization of the dihydrodihydroxy compound were combined and evaporated and the residue was acetylated as above to yield a diacetate (85 mg.) separating from ethanol in needles, m.p. 99–101°, [α]D25 + 255 ± 3° (c 0.5 in ethanol) (Found: C, 72.6; H, 5.1%). The two acetates also appear to mixtures of the (−) and (±)-isomers and it is probable that

Table 2. Properties of dihydrodihydroxyphenanthrenes on thin-layer chromatograms

The chromatograms were prepared and compounds were detected as described in the text.

<table>
<thead>
<tr>
<th>Compound</th>
<th>( R_F )</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-9,10-Dihydro-9,10-dihydroxyphenanthrene</td>
<td>0.23</td>
<td>Orange</td>
</tr>
<tr>
<td>trans-9,10-Dihydro-9,10-dihydroxyphenanthrene*</td>
<td>0.27</td>
<td>Orange</td>
</tr>
<tr>
<td>trans-1,2-Dihydro-1,2-dihydroxyphenanthrene*</td>
<td>0.17</td>
<td>Blue</td>
</tr>
<tr>
<td>Compound, probably trans-3,4-dihydro-3,4-dihydroxyphenanthrene</td>
<td>0.13</td>
<td>Violet</td>
</tr>
<tr>
<td>Compound, possibly 9,10-dihydro-9-hydroxyphenanthrene†</td>
<td>0.50</td>
<td>None</td>
</tr>
</tbody>
</table>

* The optical isomers of these compounds could not be separated on these chromatograms.
† This compound had \( R_F 0.12 \) in benzene. It could only be detected by its decomposition to phenanthrene when two-dimensional chromatograms were treated with HCl between the first and second developments as described in the text.
the products described by Boyland & Wolf (1950) are also mixtures. The dihydrodihydroxy compound and the diacetates all yielded compounds indistinguishable from 1- and 2-hydroxyphenanthrene on thin-layer chromatograms after hydrolysis with acid.

Fractions 24–27 contained trans-1,2-dihydro-1,2-dihydroxyphenanthrene and a product believed to be trans-3,4-dihydro-3,4-dihydroxyphenanthrene, but attempts to separate the products by fractional crystallization were unsuccessful. Fractions 28–37 were combined and evaporated to a small volume when a substance (85 mg.) separated. The substance was recrystallized from benzene in light-brown plates, m.p. 152° (Found: C, 42-3; H, 5-4%). Calc. for C_{18}H_{16}O_4: C, 42-5; H, 5-4%. Since it showed no light-absorption in ethanol above 230 mp it was presumed not to be a phenanthrene metabolite and was not examined further.

The mother liquors contained the compound believed to be trans-3,4-dihydro-3,4-dihydroxyphenanthrene but attempts to obtain a crystalline product were unsuccessful. The gummy product yielded compounds indistinguishable from 3- and 4-hydroxyphenanthrene on thin-layer chromatograms after hydrolysis with acid, and from the size of the spots on the chromatogram there appeared to be more of the 3- than of the 4-isomer. The absorption spectrum of the dihydrodihydroxy compound resembled that of a product, described below, which is believed to be a derivative of a glucuronic acid conjugate of the dihydrodihydroxy compound.

**Examination of the dihydrodihydroxyphenanthrene fractions from rats**

The combined fractions were evaporated to yield a mixture (800 mg.) of a crystalline solid and a dark-brown gum. The crystals were separated, washed several times with benzene and recrystallized twice from benzene to yield (±)-trans-9,10-dihydro-9,10-dihydroxyphenanthrene (600 mg.) in silky needles, m.p. and mixed m.p. 187°, which formed a diacetate in plates from ethanol, m.p. and mixed m.p. 173°. The gum and the benzene washings were combined and chromatographed on a silica gel G–Hyflo Super-Cel column as above. Fractions 3–11, which contained trans-9,10-dihydro-9,10-dihydroxyphenanthrene, were combined and evaporated to a jelly which was dissolved in pyridine and acetylated with acetic anhydride. The product was fractionally crystallized from acq. ethanol to yield a less soluble fraction (28 mg.) in plates, m.p. 127–129°, [α]_D^24 + 84±2° (c 0.5 in CHCl_3) (Found: C, 72.8; H, 5.4. Calc. for C_{18}H_{16}O_4: C, 72.9; H, 5.4%). The product appeared to be a mixture of the (+)- and (±)-isomers of the dihydrodihydroxy compound: a mixture with the product of m.p. 126–128° obtained from rabbit urine had m.p. 127–129°. 9-Hydroxyphenanthrene was detected on thin-layer chromatograms when the product was hydrolysed with acid. The more soluble fraction was recrystallized from acq. ethanol to yield (+)-trans-9,10-diacetoxy-9,10-dihydrophenanthrene (35 mg.) in prismatic needles, m.p. 119°, [α]_D^24 + 292±8° (c 0.5 in acetone) (Found: C, 72; H, 5-6%). The m.p. was undepressed in admixture with the diacetate of the (−)-dihydrodihydroxy compound of m.p. 121–122° obtained by Booth, Boyland & Turner (1950) through the resolution of the (±)-dihydrodihydroxy compound. Fractions 14–26 were combined and recrystallized from benzene to yield (±)-trans-1,2-dihydro-1,2-dihydroxyphenanthrene (28 mg.) in needles, m.p. 138°, [α]_D^24 0±2° (c 0.5 in CHCl_3) (Found: C, 79-2; H, 5-65. C_{18}H_{16}O_4 requires C, 79-2; H, 5-7%). The (±)-diacetate separated from acq. ethanol in plates, m.p. 82° (Found: C, 72-8; H, 5-55. C_{18}H_{16}O_4 requires C, 72-9; H, 5-4%). Both compounds yielded substances indistinguishable from 1- and 2-hydroxyphenanthrene on thin-layer chromatograms after acid hydrolysis.

Fractions 30–36 contained small amounts of the product believed to be trans-3,4-dihydro-3,4-dihydroxyphenanthrene. It formed a gum which was indistinguishable on a thin-layer chromatogram from the product obtained from rabbit urine and yielded compounds indistinguishable from 3- and 4-hydroxyphenanthrene on thin-layer chromatograms.

**Examination of the glucosiduronic acid fractions from rabbits**

The combined glucosiduronic acid fractions formed a dark-brown gum (10 g.) which was dissolved in methanol and treated with an excess of diazomethane in ether. The solvent was removed under reduced pressure, and the residue was dissolved in pyridine (250 ml) and the solution cooled to 0° while acetic anhydride (25 ml) was added with stirring during 30 min. The mixture was kept overnight and poured into water (3 l). The solid mixture of acetyl derivatives was dried at room temperature and fractionally crystallized from ethanol as outlined in Scheme 1 to give the following products.

**Product A.** This was identified as (+)-methyl (9-acetoxy-9,10-dihydro-10-phenanthryltri-O-acetyl-d-glucosiduronate), which crystallized from ethanol in long needles (1.8 g.), m.p. 247°, [α]_D^24 + 65±8° (c 0.5 in CHCl_3) (Found: C, 60-8; H, 5-1. C_{18}H_{16}O_{12} requires C, 61-05; H, 5-3%). Only 9-hydroxyphenanthrene was detected in thin-layer chromatograms after hydrolysis with ethanol–HCl. The derivative (1.5 g.) was hydrolysed with barium methoxide in methanol to yield (+)-trans-9,10-dihydro-9-hydroxy-10-phenanthrylglucosiduronic acid (0.35 g.), which was dissolved in the minimum amount of acq. 2N-NaOH. The solution was washed with ether, and the free acid was precipitated with HCl and crystallized from water in small colourless needles, which turned pink on exposure to air, m.p. 222° (decomp.). [α]_D^24 + 185±6° (c 0.5 in 0.1N-NaOH) (Found, after drying at 100° and 10 mm. for 1 hr.: C, 59-3; H, 5-6; C_{18}H_{16}O_4.H_2O requires C, 59-1; H, 5-5%). The acid (250 mg.) was hydrolysed with gluconic acid to yield a compound presumed to be (−)-trans-9,10-dihydro-9,10-dihydroxyphenanthrene as a yellow powder (45 mg.), m.p. 160–162°, which separated from water, ethanol and benzene in jellies as described by Booth et al. (1950) for the synthetic isomer. The product gave a spot with the same properties as those of the synthetic racemate on thin-layer chromatograms and yielded a compound indistinguishable from 9-hydroxyphenanthrene after acid hydrolysis. Acetylation of the isomer with acetic anhydride in pyridine yielded (−)-9,10-diacetyl-9,10-dihydrophenanthrene, which separated from acq. ethanol in prismatic needles, m.p. 119°, undepressed after admixture with the isomer described above, [α]_D^24 + 250±6° (c 0.5 in ethanol) (Found: C, 72-7; H, 5-6. Calc. for C_{18}H_{16}O_4: C, 72-9; H, 5-4%).

**Product B.** This appeared to consist mainly of (−)-methyl (9-acetoxy-9,10-dihydro-10-phenanthryltri-O-acetyl-D-glucosiduronate) (1.1 g.), which after four recrystallizations from ethanol formed clusters of plates, m.p. 162–163°,
The properties of the pure compound are described by Boyland & Sims (1962b). After hydrolysis with ethanol-HCl a compound was detected which was indistinguishable from 9-hydroxyphenanthrene on thin-layer chromatograms, and the presence of glucuronic acid in the hydrolysate was shown by the naphtharesorcinol test. The compound (1 g) was hydrolysed with barium methoxide in methanol to yield (−)-trans-9,10-dihydro-9-hydroxy-10-phenanthrylglucosiduronic acid (0.4 g.), which was purified as described for the (+)-isomer and was recrystallized from water in plates, m.p. 170° (decomp.), [α]D20 + 245 ± 6° (c 0.5 in ethanol) (Found: C, 72.6; H, 5.4%). A mixture with the (−)-diacetate of m.p. 121–122° obtained by Booth et al. (1950) had m.p. 119–120°, whereas a mixture with an equal amount of the (+)-isomer had m.p. 166–168°.

Both of the isomeric glucosiduronic acids gave rise to 9-hydroxyphenanthrene and a product which appeared to be 9-phenanthrylglicosiduronic acid when heated at 60° for 10 min. with 2X-HCl. The product was obtained as a gum by eluting with methanol containing 5% (v/v) of aqueous NH3 (sp.gr. 0.88) the appropriate spots from paper chromatograms developed with solvent 1 and it yielded a compound indistinguishable from 9-hydroxyphenanthrene both on hydrolysis with acid and with glucuronidase. (Product C) This was recrystallized from ethanol to yield (+)-methyl (2-acetoxyl-1,2-dihydro-1-phenanthryltri-O-acetyl-n-glucosid)uronate (350 mg) in needles, m.p. 220°, [α]D20 + 150 ± 2° (c 0.5 in CHCl3) (Found: C, 61.2; H, 5.0. C39H30012 requires C, 61.05; H, 5.3%). After hydrolysis with ethanol-HCl, compounds indistinguishable from 1- and 2-hydroxyphenanthrene were detected on thin-layer chromatograms and the mother liquors gave a positive naphtharesorcinol test for glucuronic acid. The derivative (700 mg.) was hydrolysed with barium methoxide in methanol to yield (+)-trans-1,2-dihydro-2-hydroxy-1-phenanthrylglicosiduronic acid as a gum (220° mg.) which could not be crystallized. The ammonium salt of the glucosiduronic acid formed a light-brown powder from methanol–ether (Found: C, 59.1; H, 6.0. C39H30012 requires C, 59.25; H, 5.7%). The acid (100 mg.) was hydrolysed with glucuronidase as

\[
[x]_{D}^{H} - 180° ± 4° (c 0.5) in CHCl3
\] (Found: C, 60.9; H, 5.4%).

The glucosiduronic acid (200 mg.) was hydrolysed with glucuronidase to yield a compound (30 mg.), presumed to be (+)-trans-9,10-dihydro-9,10-dihydroxyphenanthrene, which formed a yellow amorphous powder, m.p. 158–162°. The compound had the same properties on thin-layer chromatograms as both the (±)- and the (−)-isomer and yielded only 9-hydroxyphenanthrene on acid hydrolysis. As with the (−)-isomer, attempts to crystallize the (+)-isomer from water, ethanol or benzene yielded only jellies; but when about equal amounts of the jellies of the two isomers in benzene were mixed, crystallization rapidly occurred to yield the (±)-isomer in needles, m.p. 183–185°, undepressed on admixture with the synthetic compound. The (+)-isomer was acetylated with acetic anhydride in pyridine to yield (−)-9,10-diacetoxy-9,10-dihydrophenanthrene, which separated from aq. ethanol in prismatic needles, m.p. 119°, [α]D20 + 245 ± 6° (c 0.5 in ethanol) (Found: C, 72.6; H, 5.4%).

Mixed acetyl derivatives

<table>
<thead>
<tr>
<th>1st crop, needles, m.p. 215°</th>
<th>2nd crop, needles, m.p. 218°</th>
<th>3rd crop, crystals, m.p. 190–195°</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Product A)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st crop, needles, m.p. 210–212°</td>
<td>2nd crop, crystals, m.p. 182–185°</td>
<td>(Product E)</td>
</tr>
<tr>
<td>(Product C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st crop, needles, m.p. 208–210°</td>
<td>2nd crop, crystals, m.p. 170–180°</td>
<td>Residual gum</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual gum</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Scheme 1. Fractional crystallization from ethanol of the methylated and acetylated glucosiduronic acid fraction from rabbit urine. The subsequent treatments of the products in parentheses are described in the text.
described above to yield (+)-trans-1,2-dihydro-1,2-dihydroxyphenanthrene (22 mg.), separating from benzene in plates, m.p. 164°, (c 0.5 in CHCl₃) (Found: C, 79.1; H, 5.7). The dihydrodihydroxy compound yielded compounds indistinguishable from 1- and 2-hydroxyphenanthrene on thin-layer chromatograms after acid hydrolysis. From the sizes of the spots on the chromatograms, about equal amounts of the isomers appeared to have been formed.

**Product D.** This was identified as (→)-methyl (2-acetoxy-1,2-dihydro-1-phenanthryltri-O-acetyl-D-glucosiduronic acid (230 mg.) as a brown amorphous powder which separated from water as a jelly. The *ammonium salt* formed a brown powder from methanol-ether (Found: C, 68.9; H, 5.8%). When the glucosiduronic acid (150 mg.) was hydrolysed with glucuronidase, (→)-trans-1,2-dihydro-1,2-dihydroxyphenanthrene (150 mg.) was obtained, separating from benzene in plates, m.p. 164°, (c 0.5 in CHCl₃) (Found: C, 79.3; H, 5.8%). The dihydrodihydroxy compound gave compounds indistinguishable from 1- and 2-hydroxyphenanthrene on thin-layer chromatograms after acid hydrolysis. When heated at 60° for 10 min. with 2N-HCl, both glucosiduronic acids gave rise to 2- and a small amount of 1-hydroxyphenanthrene, together with a glucosiduronic acid which was obtained as a gum by eluting the appropriate areas from chromatograms of the hydrolysate. The gum appeared to be mainly 1-phenanthrylg glucosiduronic acid because it yielded a compound indistinguishable from 1-hydroxyphenanthrene on thin-layer chromatograms after hydrolysis with glucuronidase or with acid and gave a positive napththaresorcinol test.

**Product E.** This yielded compounds indistinguishable from 1-, 2-, 3- and 4-hydroxyphenanthrene on acid hydrolysis with ethanol-HCl. The product was recrystallized twice from ethyl acetate-light petroleum (b.p. 80-100°) and three times fromaq. ethanol to yield one of the isomers of methyl (3-acetoxyl-3,4-dihydro-4-phenanthryltri-O-acetyl-D-glucosiduronic acid (11 mg.) in needles, m.p. 224-225° (Found: C, 61.2; H, 5.2%). When a few milligrams of the derivative were hydrolysed with barium methoxide in methanol the product was presumed to be the corresponding glucosiduronic acid and gave compounds indistinguishable from 3- and 4-hydroxyphenanthrene on thin-layer chromatograms after hydrolysis with acid. When the product was hydrolysed by heating with 2N-HCl at 60° for 10 min., 3-hydroxyphenanthrene was formed together with a product which from its properties on paper chromatograms appeared to be 4-phenanthrylglucosiduronic acid.

**Product F.** This appeared to be methyl (2-methoxy-1-phenanthryltri-O-acetyl-D-glucosiduronic acid and it separated from ethanol in needles (210 mg.), m.p. 222°, (c 0.5 in CHCl₃) (Found: C, 62.9; H, 5.25. C₂₇H₂₇O₁₃ requires C, 62.2; H, 5.9%). The derivative (100 mg.) was hydrolysed by heating with ethanol-HCl and the product which separated on cooling was recrystallized fromaq. ethanol to yield 1-hydroxy-2-methoxyphenanthrene (25 mg.) in grey plates, m.p. 113°, undepressed in admixture with the product obtained by Sims (1962). Methylation with methyl sulphate in 2N-NaOH gave 1,2-dimethoxyphenanthrene, m.p. and mixed m.p. 100-101°. The methylation of the 2-hydroxy group in the glucosiduronic acid was presumably brought about by the action of diazomethane in the preparation of the crude mixtures of derivatives. There was no evidence that the methylation occurred in the body.

**Product G.** This was shown to be methyl (2-phenanthryltri-O-acetyl-D-glucosiduronic acid, which separated fromaq. ethanol in needles (88 mg.) m.p. 162°, (c 0.5 in CHCl₃) (Found: C, 63.1; H, 4.9. C₂₉H₂₉O₁₃ requires C, 63.5; H, 5.1%). A compound indistinguishable from 2-hydroxyphenanthrene was detected on thin-layer chromatograms after hydrolysis in ethanol-HCl, and the mother liquors gave a positive test for glucuronic acid with napththaresorcinol. The derivative was hydrolysed with barium methoxide in methanol to yield 2-phenanthrylg glucosiduronic acid separating from water in flat needles, m.p. 189° (decomp.) (Found: C, 64.6; H, 5.1. C₂₇H₂₉O₁₃ requires C, 64.9; H, 4.9%). With glucuronidase the acid yielded a compound indistinguishable from 2-hydroxyphenanthrene on thin-layer chromatograms. When the acid was hydrolysed with HCl, 2-hydroxyphenanthrene was obtained, m.p. and mixed m.p. 183-184°, which yielded a methyl ether in plates from ethanol, m.p. and mixed m.p. 100°.

**Examination of the non-crystalline residues from the fractionation**

The gums were combined and dissolved in excess of methanol containing barium methoxide and kept at room temperature. After 24 hr. the precipitated barium salts were filtered off, washed with methanol, suspended in water (600 ml.), and an excess of HCl was added. The glucosiduronic acids were separated from the solution with charcoal to yield a mixture of ammonium salts. A little of the mixture was hydrolysed by heating with acid and the hydrolysis products were examined on thin-layer chromatograms, when compounds indistinguishable from 1-, 2-, 3- and 4-hydroxyphenanthrene, 1-hydroxy-2-methoxyphenanthrene and 9,10-phenanthraquinone were detected. About 5 g. of the mixture was hydrolysed with glucuronidase to yield a gum (800 mg.). The gum was dissolved in benzene and chromatographed on a silica gel G-Hyflo Super-Cel column, fractions of 30 ml. being collected. Fractions 3 and 4 were combined and evaporated to yield 1-hydroxy-2-methoxyphenanthrene (20 mg.) in grey plates (from water), m.p. and mixed m.p. 113°; fractions 8 and 9 were combined and evaporated, and the residue was treated with methyl sulphate in 2N-NaOH to yield 4-methoxyphenanthrene (30 mg.) in plates fromaq. ethanol, m.p. and mixed m.p. 67-68°; fractions 11-14 were combined and evaporated, and the residue was methylated to yield 1-methoxyphenanthrene (108 mg.) which separated from ethanol in needles, m.p. and mixed m.p. 104-105°; fractions 16-18 yielded, after evaporation and methylation, 3-methoxyphenanthrene (35 mg.) as plates fromaq. ethanol, m.p. and mixed m.p. 60°; and fractions 21-23 were combined and evaporated and methylated to yield 2-methoxyphenanthrene (96 mg.) as plates fromaq. ethanol, m.p. and mixed m.p. 100°.
At this point the solvent was changed to benzene containing 0-5% (v/v) of ethanol and fractions 26-28 were combined and evaporated to yield 9,10-phenanthroquinone (22 mg.), m.p. and mixed m.p. 204-205°. After 36 fractions had been collected, the solvent was changed to benzene containing 5% (v/v) of ethanol and fractions of 40 ml. were collected. Fraction 39, which contained the material forming a dark band on the column, yielded a number of unidentified substances which gave blue colours on thin-layer chromatograms sprayed with the 2,6-dichloroquinonechlorimide and acq. Na2CO3 reagent. Fractions 40 and 41 yielded (+)-trans-9,10-dihydro-9,10-dihydroxyphenanthrene (15 mg.), which separated from benzene in needles, m.p. and mixed m.p. 187°; fractions 42 and 43 yielded a mixture of (+) and (-)-trans-1,2-dihydro-1,2-dihydroxyphenanthrene (32 mg.) as needles, m.p. 150-154°; and fractions 45 and 46 yielded a gum (10 mg.) which appeared to be mainly trans-3,4-dihydro-3,4-dihydroxyphenanthrene when examined on thin-layer chromatograms. The acid hydrolysis of the last three products yielded compounds indistinguishable from 9-hydroxyphenanthrene, 1- and 2-hydroxyphenanthrene, and 3- and 4-hydroxyphenanthrene respectively.

Examination of the urine of rabbits for products released by the action of hydrolytic reagents

About 100 mg. of the gum obtained by treating the urine of rabbits dosed with phenanthrene with charcoal as described by Sims (1962) was applied to four sheets of Whatman no. 3MM chromatography paper and the chromatograms were developed with solvent 1. The dried chromatograms were each cut into ten strips each of about the same width, the actual position of the cuts being determined by the positions of fluorescent bands seen in u.v. light. The absorbed materials were eluted with methanol containing 5% ofaq. NH3 (sp.gr. 0.88). The eluates were evaporated under reduced pressure and examined on paper chromatograms. Fraction I contained 1-, 2-, 3-, 4- and 9-hydroxyphenanthrene and fraction 7 no metabolites. Samples from the other fractions were dissolved in water and treated with one or more of the following reagents: cold 5N-HCl; HCl at 100° for 15 min.; sulphatase (Sims, 1962); glucuronidase; diazomethane in ether, followed by the hydrolysis of the products with HCl at 100° for 15 min. The solutions were extracted with ether and the ether-soluble products examined on thin-layer chromatograms to give the results shown in Table 3. These confirm the presence of the metabolites described above and indicate the presence of small amounts of 3-hydroxy-4-phenanthrylglycoursiduronic acid in the glucouridonic acid fractions. Although most of the phenanthrene liberated in the urine by mineral acid arises from the decomposition of mercapturic acids, a small amount appears to arise from the decomposition of a substance present in the glucoursiduronic acid fractions. When the products from the hydrolyses of these fractions with glucuronidase were chromatographed on two-dimensional thin-layer chromatograms developed first with either benzene or benzene containing 5% of ethanol, sprayed with 2N- HCl and allowed to dry at room temperature, and then developed in the second direction with n-hexane containing 5% (v/v) of benzene, phenanthrene could be detected, evidently arising from the acid decomposition of a precursor.

Examination of the glucursiduronic acid fractions from rats

The combined glucouridonic acid fractions formed a dark-brown gum (12 g.) which was methylated and acetylated and the products fractionally crystallized from ethanol to yield: (1) (+)-methyl (9-acetoxy-9,10-dihydro-9,10-phenanthryliri-O-acetyl-n-glucuronic)urate (21 g.), as long needles from ethanol, m.p. alone and when mixed with the product from rabbits, 247°, [α]D20 +71± 2° (c 0-5 in CHCl3) (Found: C, 60-0; H, 5-1; Calc. for C35H29O12: C, 61-05; H, 5-3%); (2) (-)-methyl (9-acetoxy-9,10-dihydro-phenanthrylri-O-acetyl-n-glucuronic)urate, which was recrystallized three times from ethanol, when it separated in clusters of plates (1-2 g.), m.p. and mixed m.p. 164°, [α]D20 +285 ± 2° (c 0-5 in CHCl3) (Found: C, 61-45; H, 5-3%); (3) (+)-methyl (2-acetoxy-1,2-dihydro-1-phenanthrylri-O-acetyl-n-glucuronic)urate (800 mg.) which separated from ethanol in needles, m.p. and mixed m.p. 200°, [α]D20 +148 ± 4° (c 0-5 in CHCl3) (Found: C, 61-15; H, 5-3%); (4) (-)-methyl (2-acetoxy-1,2-dihydro-1-phenanthrylri-O-acetyl-n-glucuronic)urate (850 mg.) which was recrystallized from ethanol in needles, m.p. and mixed m.p. 222°, [α]D20 +285 ± 5° (c 0-5 in CHCl3) (Found: C, 60-9; H, 5-1%). When these products were hydrolysed with barium methoxide in methanol, glucoursiduronic acids were obtained, indistinguishable in their chemical and chromatographic properties from the products obtained by the hydrolyses of the corresponding derivatives from rabbits. The hydrolyses of the glucoursiduronic acids with glucuronidase similarly yielded the corresponding dihydrodihydroxy compounds.

The gummy residues from the fractional crystallizations were hydrolysed with barium methoxide in methanol and the resultant mixture of glucoursiduronic acids was treated with glucuronidase. Examination of the products on thin-layer chromatograms revealed compounds indistinguishable from 1-, 2-, 3- and 4-hydroxyphenanthrene, trans-9,10-dihydro-9,10-dihydroxy, trans-1,2-dihydro-1,2-dihydroxy and trans-3,4-dihydro-3,4-dihydroxy-phenanthrene, 1-hydroxy-2-methoxyphenanthrene, and small amounts of 2-hydroxy-1-methoxy- and 4-hydroxy-3-methoxy-phenanthrene, and 9,10-phenanthroquinone.

On one occasion, a product was isolated during the fractional crystallization of the methylated and acetylated mixture of glucoursiduronic acids from the urine of rats which appeared to be (+)-methyl (9,10-dihydro-9-methoxy-10-phenanthryliri-O-acetyl-n-glucuronic)urate (85 mg.), separating from ethanol in needles, m.p. 178°, [α]D20 +36 ± 5° (c 0-5 in CHCl3) (Found: C, 62-0; H, 5-6). C29H26O11 requires C, 62-0; H, 5-6%). The derivatives yielded a product indistinguishable from 9-hydroxyphenanthrene on thin-layer chromatograms and the mother liquors gave a positive naphthaesercuricin test for glucuronic acid. It presumably arose from the corresponding glucoursiduronic acid during the methylation of the crude glucouridonic acid fraction with diazomethane.

DISCUSSION

The products of the metabolism of phenanthrene, isolated or identified in the present work, are set out in Scheme 2. The light-absorption data (Table 4) support the proposed structures of the dihydrodihydroxy compounds and their derivatives.
Table 3. *Products formed from the metabolites of phenanthrene by the action of hydrolytic reagents*

The products were detected on thin-layer chromatograms as described in the text.

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Type of metabolite present</th>
<th>Products from the hydrolysates</th>
<th>Action of diazomethane followed by heating with HCl at 100° for 15 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Dihydriodihydroxy compounds (the 1,2-, 3,4- and 9,10-isomers)</td>
<td>With cold 5N-HCl</td>
<td>With HCl at 100° for 15 min.</td>
</tr>
<tr>
<td>3 and 4</td>
<td>Sulphuric esters</td>
<td>None</td>
<td>1-, 2-, 3-, 4-, and 9-hydroxyphenanthrene</td>
</tr>
<tr>
<td>5 and 6</td>
<td>Mercapturic acids and related compounds</td>
<td>1-, 2-, 3-, 4- and 9-hydroxyphenanthrene†</td>
<td>1-, 2-, 3-, 4- and 9-hydroxyphenanthrene; 9,10-phenanthraquinone*</td>
</tr>
<tr>
<td>8, 9 and 10</td>
<td>Glucuronic acid conjugates</td>
<td>Phenanthrene; 1-, 2-, 3-, 4- and 9-hydroxyphenanthrene†</td>
<td>Phenanthrene; 1-, 2-, 3-, 4- and 9-hydroxyphenanthrene</td>
</tr>
</tbody>
</table>

* This product is presumably derived from 9,10-dihydroxyphenanthrene by oxidation.
† Present only in traces.
All the metabolites could have arisen in the body through three intermediates of the type suggested by Boyland (1950), 9,10-epoxy-9,10-dihydro-, 1,2-epoxy-1,2-dihydro- and 3,4-epoxy-3,4-dihydro-phenanthrene. The formation of these intermediates in the body has yet to be proved with phenanthrene, but previous work (Boyland & Sims, 1960) indicates that, for 1,2-dihydronaphthalene, epoxidation of the olefinic 3,4-bond is the initial metabolic step. The insecticide, Heptachlor, is converted into a chemically inactive epoxide in dogs and rats (Davidow & Radomski, 1953). If, in phenanthrene metabolism, the postulated epoxide intermediates undergo one of the four types of reaction described below, the formation of the observed pattern of metabolic products can be explained.

(1) **Reactions with the thiol groups of glutathione.** The reaction would presumably be catalysed by the enzyme found in the livers of rats and other animals (Booth, Boyland & Sims, 1961; Combes & Stakelum, 1961). The conversion of glutathione conjugates into mercapturic acids by liver slices and homogenates (Bray, Franklin & James, 1959) and by kidney slices and homogenates (Booth, Boyland & Sims, 1960), and the presence of the intermediate cysteinylglycine and cysteine conjugates in the bile of animals treated with naphthalene (Boyland, Ramsay & Sims, 1961) and with phenanthrene (Boyland & Sims, 1962a), have been described. The fact that rabbits and rats treated with phenanthrene excrete only one of the diastereoisomeric forms of the mercapturic acid is further evidence that the glutathione conjugate is formed in an enzymic reaction.

(2) **Reactions with water to yield dihydrodihydroxy compounds.** There is, as yet, no direct evidence to show whether or not the dihydrodihydroxy compounds are formed in enzymic reactions. Although a non-enzymic reaction of an epoxide with water should yield the (±)-dihydrodihydroxy compound, it has been found that with phenanthrene, as with
Table 4. Ultraviolet absorption of some dihydrodihydroxy compounds and related glucosiduronic acids derived from phenanthrene

Measurements were made in ethanol solution except for the free glucosiduronic acids which were made in water with a Perkin-Elmer model 137 ultraviolet spectrophotometer. With compounds where more than one optically active form was available, the measurements are those made on the (+)-isomer; these were identical with those on the (-)- and, where available, the (±)-isomer.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\lambda_{\text{max}}$ (m$\mu$)</th>
<th>$\epsilon_{\text{max}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-trans-9,10-Dihydro-9,10-dihydroxyphenanthrene*</td>
<td>288</td>
<td>16 000</td>
</tr>
<tr>
<td>(+)-trans-9,9-Diacetoxy-9,10-dihydrophenanthrene*</td>
<td>288</td>
<td>18 000</td>
</tr>
<tr>
<td>(+)-9,10-Dihydro-9-hydroxy-10-phenanthrylglucosiduronic acid*</td>
<td>298</td>
<td>17 700</td>
</tr>
<tr>
<td>(+)-Methyl (9-acetoxy-9,10-dihydro-10-phenanthryltri-O-acetyl-D-glucosid)uronate*</td>
<td>288</td>
<td>17 800</td>
</tr>
<tr>
<td>(+)-Methyl (9,10-dihydro-9-methoxy-10-phenanthryltri-O-acetyl-D-glucosid)uronate*</td>
<td>267</td>
<td>15 200</td>
</tr>
<tr>
<td>(+)-trans-1,2-Dihydro-9,10-dihydroxyphenanthrene</td>
<td>237</td>
<td>57 500</td>
</tr>
<tr>
<td>(-)-trans-1,2-Diacetoxy-1,2-dihydrophenanthrene</td>
<td>315</td>
<td>7 200</td>
</tr>
<tr>
<td>(+)-trans-1,2-Dihydro-2-hydroxy-1-phenanthrylglucosiduronic acid</td>
<td>327</td>
<td>58 000</td>
</tr>
<tr>
<td>Substance, probably trans-3,4-dihydro-3,4-dihydroxyphenanthrene</td>
<td>315</td>
<td>7 500</td>
</tr>
<tr>
<td>Methyl (3-acetoxy-3,4-dihydro-4-phenanthryltri-O-acetyl-D-glucosid)uronate*</td>
<td>249</td>
<td>8 100</td>
</tr>
<tr>
<td>Methyl (2-methoxy-1-phenanthryltri-O-acetyl-D-glucosid)uronate†</td>
<td>256</td>
<td>50 000</td>
</tr>
<tr>
<td>Methyl (2-phenanthryltri-O-acetyl-D-glucosid)uronate</td>
<td>259</td>
<td>58 500</td>
</tr>
<tr>
<td></td>
<td>293</td>
<td>5 200</td>
</tr>
<tr>
<td>* These compounds all showed pronounced shoulders in the absorption curves at 224, 230 and 300 m$\mu$.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>† This compound showed pronounced shoulders in the absorption curve at 230 and 292 m$\mu$.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Other hydrocarbons, both optically active isomers of the dihydrodihydroxy compounds are normally present in the urine of treated animals, although there is often a small excess of one of the forms. Thus, with naphthalene, rabbits excrete an excess of the (+)-form (Booth & Boyland, 1949), rats an excess of the (-)-form (Young, 1947) and guinea pigs (Corner & Young, 1954) only the (-)-form of trans-1,2-dihydro-1,2-dihydroxynaphthalene. With phenanthrene, both rabbits and rats excrete small excesses of the (-)-form of trans-9,10-dihydro-9,10-dihydroxyphenanthrene. The dihydroxy compounds can, however, undergo other reactions in the body. Thus, for example, they (or, less likely, their sulphuric acid or glucuronic acid conjugates) are dehydrogenated to dihydroxy compounds. The dehydrogenation of trans-9,10-dihydro-9,10-dihydroxyphenanthrene is described by Boyland & Sims (1962), who also showed that although the (±)-isomer was administered to rats more of the (-)- than of the (+)-isomer and their derivatives were recovered from the urine. This suggests that the (+)-isomer is more readily dehydrogenated than the (-)-isomer, and would account for the presence of an excess of the latter form in the urine of animals given phenanthrene. The present results show that relatively large amounts of 1,2-dihydroxyphenanthrene conjugates, presumably arising from the dehydrogenation of trans-1,2-dihydro-1,2-dihydroxyphenanthrene, are formed, whereas only traces of 3,4-dihydroxyphenanthrene conjugates could be detected, probably because only relatively small amounts of the precursor, trans-3,4-dihydro-3,4-dihydroxyphenanthrene, are formed in the body.

Positional isomerism in the conjugates of the dihydrodihydroxy and of the dihydroxy compounds is possible. Thus, for example, although the glucuronic acid conjugates of trans-1,2-dihydro-1,2-dihydroxyphenanthrene isolated are believed to be the 1-conjugates, it is probable that 2-conjugates are also present: the 2-glucuronic acid conjugate of trans-1,2-dihydro-1,2-dihydroxyphenanthrene has been isolated (Sims, 1959). It has been postulated (Corner & Young, 1955; Sims, 1959) that, with naphthalene, some of the naphthols and their conjugates present in the urine of treated animals arise from the breakdown of conjugates (probably the sulphuric esters) of the dihydrodihydroxy compound, the nature of the products depending on the position of the conjugating group. With the exception of trans-9,10-dihydro-9,10-dihydroxyphenanthrene, the dihydrodihydroxy compounds derived from phenanthrene yield mixtures of hydroxyphenanthrenes on acid hydrolysis, but it is possible that the breakdown of the...
conjugates of the dihydrodihydroxy compounds would yield mixtures containing different proportions of the hydroxy compounds. Although 1-naphthol and its conjugates are present in the urine of animals dosed with naphthalene, only free 2-naphthol has been detected in these urines, and, in experiments in which the hydrocarbon was incubated with the microsomal hydroxylating system, 1-, but not 2-naphthol, was formed (Mitoma, Posner, Reitz & Udenfriend, 1956; Booth & Boyland, 1958). With phenanthrene, however, sulphuric acid and glucuronic acid conjugates of 1-, 2-, 3- and 4-hydroxyphenanthrene were found, which suggests that these hydroxy compounds arise, at least in part, by a different process from that yielding 2-naphthol, possibly by the mechanism discussed below.

The dihydrodihydroxy compounds derived from the metabolism of aromatic hydrocarbons usually have the trans configuration, which is not unexpected if they arise from the opening of an epoxide ring. With phenanthrene, no evidence could be obtained for the formation of cis-9,10-dihydro-9,10-dihydroxyphenanthrene or of its conjugates in the body. From the urine of the animals dosed with indene, however, Brooks & Young (1956) isolated both cis- and trans-isomers of 1,2-dihydro-1,2-dihydroxyindene: indene epoxide is exceptional in that it yields a mixture of the two isomers with water.

(3) Reductions to yield dihydrohydroxy compounds. 1,2-Dihydro-1-naphthylglucosiduronic acid, which yields naphthalene with mineral acid, is present in the urine of rabbits and rats dosed with naphthalene (Boyland & Solomon, 1955), and its triacetetyl methyl ester derivative is the only example of a compound of this type to be isolated, although Chang & Young (1943) showed that the urines of animals dosed with anthracene or with phenanthrene also contained compounds from which the parent hydrocarbon was liberated with mineral acid. Although it has now been shown that most of the phenanthrene which is liberated when the urine of treated animals is acidified arises from the breakdown of mercapturic acids and related compounds, at least one other acid-labile precursor of phenanthrene has been detected. This is present in the glucosiduronic acid fraction from the urine of treated animals, from which it is liberated by glucuronidase, and both the parent compound and its glucuronic acid conjugate are readily broken down by cold mineral acid to yield phenanthrene just as the product obtained by Boyland & Solomon (1955) yields naphthalene. It seems likely, therefore, that both naphthalene and phenanthrene metabolites have analogous structures, the phenanthrene metabolite possibly being 9,10-dihydro-9-phenanthrylglucosiduronic acid. Although it was originally postulated that 1,2-dihydro-1-hydroxy-naphthalene arose by the hydroxylation of 1,2-dihydrornaphthalene formed by the reduction of naphthalene in the body (Boyland & Solomon, 1955), the work of Posner, Mitoma, Rothberg & Udenfriend (1961), who found that 1,2-dihydro-naphthalene is not hydroxylated to the dihydrodihydroxy compound in the microsomal hydroxylating-enzyme system, makes this unlikely. The postulated formation of dihydrodihydroxy compounds in the body by the reduction of epoxides is an alternative explanation. If the reductions are enzymic, then this could explain why, with the naphthalene metabolites, different isomers are produced by rabbits and rats. Only a small proportion of the administered hydrocarbons is metabolized in this way.

(4) Rearrangement to phenols. Although the rearrangement of aliphatic epoxides to ketones can be brought about under drastic conditions, little is known about the analogous rearrangements of epoxides of the type suggested above (in these cases the ketones which would be expected are tautomeric with the corresponding phenols), although in the oxidation of phenanthrene with perbenzoic acid, a reaction which could yield 9,10-epoxy-9,10-dihydrornaphthalene, 9-hydroxyphenanthrene has been detected in the reaction products (E. Boyland & P. Sims, unpublished work). It is likely that the 1-naphthol and trans-1,2-dihydro-1,2-dihydroxynaphthalene formed when naphthalene is oxidized in the microsomal enzyme system arise from the same intermediate (J. Booth, personal communication; Posner et al. 1961). The work of Posner et al. (1961), which showed that in the hydroxylation of acetanilide in the microsomal enzyme system the oxygen introduced is derived from molecular oxygen, whereas the hydrogen is not derived from the water of the hydroxylating system, is also in accord with the suggested rearrangement.

If the phenols formed in the metabolism of the aromatic hydrocarbons do, in fact, arise by this type of rearrangement, then the relative amount of the phenol as compared with the dihydrodihydroxy compound and the mercapturic acid formed should depend on the ease with which the rearrangement takes place, and this should depend on the gain in resonance energy in the change from the non-aromatic to the aromatic state. Although no such values are available for the reactions where epoxydihydro compounds rearrange to the corresponding phenols, Pullman (1954) and Pullman & Pullman (1955) have calculated the gain in resonance energy in a number of cases when the dihydrodihydroxy compounds are dehydrated to the corresponding phenols. These show that between trans - 9,10 - dihydro - 9,10 - dihydroxyphenan-
threne and 9-hydroxyphenanthrene the energy gain is relatively small, whereas that between the corresponding benzene derivatives is high and those between trans-1,2-dihydro-1,2-dihydroxy- naphthalene and 1-naphthol and between trans-1,2-dihydro-1,2-dihydroxyphenanthrene and 1-hydroxyphenanthrene have intermediate values. The energy gains with respect to the rearrangement of the epoxides to the corresponding phenols should be higher than those described above, but it is reasonable to suppose that in both cases the relative orders of magnitude between the derivatives of the various hydrocarbons are the same. Boyland & Wolf (1950) have shown that, in the dehydration reaction, cis-9,10-dihydro-9,10-dihydroxyphenanthrene is more reactive than the trans-isomer, which suggests that the values for the energy gains related to the cis-isomers should lie between the epoxides and the trans-isomers. The stabilities of the epoxides with respect to their rearrangements to phenols should be reflected in the nature of the metabolic products observed. Thus, with benzene, where the related epoxide should be relatively labile, phenol but no dihydrodihydroxy compounds are formed either by rabbits (Tomida & Nakajima, 1960) or by the microsomal hydroxylating system (Posner, Mitoma & Udenfriend, 1961), and only small amounts of a mercapturic acid appear (Zbarsky & Young, 1943; Parke & Williams, 1953). No values of the energy differences are available for the halobenzenes, but the presence of the halogen atoms should have a profound effect on the stabilities of any epoxides formed. The metabolic reactions on the 1,2-bonds of naphthalene and of phenanthrene yield the expected mixtures of phenols, dihydrodihydroxy compounds and mercapturic acids. 9,10-Epoxy-9,10-dihydrophenanthrene should be relatively stable so that the observed absence of appreciable amounts of the conjugates of 9-hydroxyphenanthrene is not unexpected.

The values calculated by Pullman & Pullman (1955) for the bonds which are associated with hydroxylation in the body of the carcinogenic hydrocarbons are similar to that of benzene, so that mainly phenols would be expected. No values have been calculated for the bonds of the 'K regions'; chemically these bonds are equivalent to the 9,10-bond of phenanthrene, and hydroxylations of the 'K regions' bonds of the higher aromatic hydrocarbons in the body have not been observed. Epoxides formed on these bonds may be relatively stable and could react with other tissue constituents such as the amino groups of proteins rather than with water to form dihydrodihydroxy compounds which would be excreted. The calculations also indicate that the sulphuric ester of trans-9,10-dihydro-9,10-dihydroxyphenanthrene should be more stable than that of trans-1,2-dihydro-1,2-dihydroxynaphthalene with respect to their breakdown to aromatic compounds: this is so (Boyland & Sims, 1962b).

If the phenols do arise by the rearrangement discussed above, then it is possible in many cases for two phenols to be formed from one epoxide, depending on the direction in which the oxirane ring opens. Thus, with naphthalene, where it appears (for the reasons discussed above) that 1-naphthol is the only phenolic product formed initially in the body, the oxirane ring must open in one direction only, whereas, with phenanthrene, where 1-, 2-, 3- and 4-hydroxyphenanthrene are formed, opening of the oxirane ring in both directions must be postulated. The fact that with naphthalene only one mercapturic acid is formed in the body, whereas phenanthrene yields a mixture of isomeric mercapturic acids, is in accord with this hypothesis. Whereas trans-1,2-dihydro-1,2-dihydroxynaphthalene yields mainly 1-naphthol in the dehydration reaction, trans-1,2-dihydro-1,2-dihydroxyphenanthrene yields about equal amounts of 1- and 2-hydroxyphenanthrene, and trans-3,4-dihydro-3,4-dihydroxyphenanthrene more 3- than 4-hydroxyphenanthrene.

If the initial step in the metabolism of the aromatic hydrocarbon involves the enzymic addition of oxygen then the ease with which this occurs should depend on the bond order of the bond under attack and on steric factors which might affect the positioning of the aromatic molecule on the enzyme. With phenanthrene, metabolic reaction has been shown to occur on the 9,10-, the 1,2- and the 3,4-bonds, which are in fact the bonds with the highest bond orders in the molecule (see, for example, Badger, 1954). There is no evidence of any reaction on the 2,3-bond although 2- and 3-hydroxyphenanthrene could be formed by the rearrangement of an epoxide on this bond. Because of the diverse reactions described above which the primary products undergo, it is difficult to assess the relative amounts of these products formed from phenanthrene in the body, but the results indicate that more reaction occurs on the 9,10- and 1,2-bonds than on the 3,4-bond. If bond order were the only factor involved, then more reaction on the 9,10- than on the 1,2- and 3,4-bonds with about equal amounts of reactions on the last two bonds might be expected. The free-valence number of the 9-position is higher than those of the other positions, so that if the hydroxyphenanthrenes and their conjugates found in the urine of treated animals arose from reactions involving direct hydroxylation of the phenanthrene nucleus, 9-hydroxyphenanthrene and its conjugates should be the major products.
are differences in phenanthrene metabolism between rabbits and rats, the present work does not confirm this. Any differences are likely to be quantitative rather than qualitative and, if they occur, could be due to differences in the later stages of the metabolic processes described above. Rats are, however, much more sensitive than rabbits to the carcinogenic action of polycyclic hydrocarbons. The fact that the metabolism of phenanthrene in the two species is the same indicates that metabolic processes such as those described in the present reports may not be involved in carcinogenesis. The formation of complexes between polycyclic hydrocarbons and purines of nucleic acids (cf. Booth & Boyland, 1953; Boyland & Green, 1960) would change nucleic acids without hydroxylation of the hydrocarbons. Such complex formation could be the basic biochemical lesion in carcinogenesis by polycyclic hydrocarbons.

Preliminary studies on the hydroxylation of phenanthrene in the microsomal enzyme system (E. Boyland & P. Sims, unpublished work) show that 1-, 2-, 3- and 4-hydroxyphenanthrene and \textit{trans}-9,10-dihydro-9,10-dihydroxyphenanthrene and \textit{trans}-1,2-dihydro-1,2-dihydroxy- and \textit{trans}-3,4-dihydro-3,4-dihydroxy-phenanthrene are formed. 9-Hydroxy-phenanthrene was not detected. These results, which are in agreement with those obtained from the examination of the urine of animals given phenanthrene, would be expected if the ideas expressed above are correct.

**SUMMARY**

1. Phenanthrene is converted by rabbits and rats into the (+)- and (−)-isomers of \textit{trans}-9,10-dihydro-9,10-dihydroxyphenanthrene and \textit{trans}-1,2-dihydro-1,2-dihydroxyphenanthrene. The glucuronic acid conjugates of these compounds were isolated together with a conjugate of \textit{trans}-3,4-dihydro-3,4-dihydroxyphenanthrene.

2. The glucuronic acid conjugate of 2-hydroxyphenanthrene was isolated from these urines, and evidence was obtained for the presence of similar conjugates of 1-, 3- and 4-hydroxyphenanthrene. The glucuronic acid conjugate of 9-hydroxyphenanthrene was not detected.

3. The presence of 1,2-dihydroxyphenanthrene and small amounts of 3,4-dihydroxyphenanthrene in conjugation with glucuronic acid was demonstrated.

4. A substance, present as a glucuronic acid conjugate, which yielded phenanthrene on acidification with mineral acid, was detected in the urine of rabbits dosed with phenanthrene.

5. A possible mechanism for the formation of the known phenanthrene metabolites in the body is discussed.

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