Metabolism of Polycyclic Compounds

19. THE METABOLISM OF PHENANTHRENE IN RABBITS AND RATS: PHENOLS AND SULPHURIC ESTERS*

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In this paper the formation of monohydroxy- and dihydroxy-phenanthrenes and their sulphuric esters from phenanthrene in rabbits and rats is described. Boyland & Sims (1962*a, b, c*) describe the formation of mercapturic acids and related compounds and of the glucosiduronic acids of the monohydroxy-, dihydroxy- and dihydrodihydroxy-phenanthrenes.

Although Elson, Goulden & Warren (1945) showed that when phenanthrene was given to rats an increase in ethereal sulphate output occurred, indicating the formation of sulphuric esters of hydroxyphenanthrenes, nothing was known about the structures of these esters. The present work has shown the presence of sulphuric esters of 1-, 2-, 3- and 4-hydroxyphenanthrene and of 1,2- and 9,10-dihydroxyphenanthrene in the urine of both rabbits and rats treated with phenanthrene. Small amounts of 1-, 2-, 3-, 4- and 9-hydroxyphenanthrene were also present in these urines.

MATERIALS AND METHODS

Melting points. These are uncorrected.

Chromatography. Paper chromatography was carried out on Whatman no. 1 paper by downward development for 18 hr. in 1, butan-1-ol-propan-1-ol-aq. 2N-NH₃ (2:1:1, by vol.), or 2, butan-1-ol-acetic acid-water (2:1:1, by vol.); or by upward development for 6 hr. in 3, aq. 2N-NH₃-propan-1-ol (9:1, v/v). The dried chromatograms were examined under u.v. light and sprayed with one of: (i) a 0.5% solution of 2,6-dichloroquinonechloroimide in ethanol (Gibbs, 1927) followed by aq. 10% (w/v) Na₂CO₃; (ii) aq. 0.1M-K₂Cr₂O₇-acetic acid (1:1, v/v) followed by 0.1M-AgNO₃ (Knight & Young, 1958); (iii) a freshly diazotized solution of *p*-nitroaniline (0.20% in 0.1N-HCl); (iv) a solution of diazotized *p*-nitroaniline (0.2% in 4N-HCl), after which the papers were heated at 80° until colours appeared (5–10 min.). After being treated with reagents (iii) or (iv), the papers were sprayed with aq. Na₂CO₃ (10%, w/v). Other chromatograms were dipped in a 0.2% (w/v) solution of ninhydrin in acetone and heated at 70° for 10 min. or they were dipped in the platinic iodide reagent of Toennies & Kolb (1951). The properties of the hydroxyphenanthrenes and their sulphuric esters are listed in Table 1.

Although most of the hydroxyphenanthrenes could be separated from one another on paper chromatograms, a better separation was carried out with the thin-layer chromatographic technique as described by Stahl (1958). Glass plates were coated with films of silica gel G (E. Merck A-G., Darmstadt, West Germany) of 0.25 mm. thickness and the chromatograms were developed with benzene and sprayed with reagent (i) above. This system was also used in the identification of the monomethyl ethers of the dihydroxy-phenanthrenes (see Table 2).

Materials. Phenanthrene (98–100%) was obtained from Rutgerswerke-Aktiengesellschaft, Castrop-Rauxel, West Germany. ($\pm$)-*trans*-9,10-Dihydro-9,10-dihydroxyphenanthrene, prepared by the reduction of 9,10-phenanthraquinone with lithium aluminium hydride (lithium tetrahydroaluminate) as described by Booth, Boyland & Turner (1950), formed silky needles (from benzene), m.p. 186–187°. 9,10-Dihydroxyphenanthrene, prepared by reducing the quinone with sodium dithionite (Grandmougin, 1906), formed grey plates, m.p. 146–147°. The diacetate separated from ethanol in prisms, m.p. 202°.

2- and 3-Hydroxyphenanthrene were prepared by the sulphonation of phenanthrene and the subsequent fusion of the separated barium sulphonates with KOH as described by Fieser (1929*d*). When recrystallized from aq. ethanol, the 2-isomer formed plates, m.p. 168–169°, and the 3-isomer needles, m.p. 120–122°. The phenols yielded methoxy derivatives separating from ethanol in plates, m.p. 100° and 61° respectively.

1- and 4-Hydroxyphenanthrene were formed from the corresponding amino compounds [prepared by the method of Langenbeck & Weissenborn (1939)] by diazotization and decomposition of the diazonium salts in boiling water by the method of Bachmann & Boatner (1936). The phenols separated from light petroleum (b.p. 80–100°) in needles, m.p. 156° and 110–112° respectively, and yielded methyl ethers in plates (from aq. ethanol), m.p. 105° and 68°.

9-Hydroxyphenanthrene was prepared by the acid decomposition of *trans*-9,10-dihydro-9,10-dihydroxyphenanthrene in hot acetic acid-H₂SO₄, as described by Moriconi, Wallenberger & O'Connor (1959), and formed pale-pink prisms, m.p. 152°. The methoxy derivative separated from methanol in needles, m.p. 96°.

Potassium 2- and 3-phenanthryl sulphate were prepared by heating the corresponding phenols (1 g.) with sulphamic acid (1 g.) in pyridine (100 ml.) under reflux for 18 hr. The solutions were poured into water (250 ml.) containing KOH (2 g.), and the mixtures were washed twice with ether (50 ml.) and evaporated to small volume under reduced pressure, when the esters separated. *Potassium 2-phenanthryl sulphate* (1.65 g.) separated from a large volume of

* Part 18: Boyland, Ramsay & Sims (1961).

Table 1. *Properties of the hydroxyphenanthrenes and their sulphuric esters on paper chromatograms*

Experimental details are given in the text.

Compound	R_F in solvent			Fluorescence	Colour		
	1	2	3		With 2,6-dichloro-quinonechloroimide and Na_2CO_3	With diazotized <i>p</i> -nitroaniline and Na_2CO_3	With diazotized <i>p</i> -nitroaniline in 4 <i>N</i> -HCl, heating at 80°, and Na_2CO_3
1-Hydroxyphenanthrene	0.91	0.96	0.45	Blue	Blue	Blue	Blue
2-Hydroxyphenanthrene	0.91	0.96	0.34	Violet	Purple-red	Brown	Brown
3-Hydroxyphenanthrene	0.91	0.96	0.38	Violet	Grey	Purple-brown	Purple-brown
4-Hydroxyphenanthrene	0.91	0.96	0.46	Violet	Blue	Blue	Blue
9-Hydroxyphenanthrene	0.91	0.96	0.45*	Blue	Blue-green	Orange	Orange
1-Phenanthryl sulphate	0.72	0.90	0.55	Violet	None	None	Blue
2-Phenanthryl sulphate	0.68	0.90	0.51	Dark-violet	None	None	Brown
3-Phenanthryl sulphate	0.70	0.90	0.58	Dark-violet	None	None	Purple-brown
4-Phenanthryl sulphate	0.76	0.90	0.65	Violet	None	None	Blue
9-Phenanthryl sulphate	0.76	0.90	0.63	Violet	None	None	Orange
Unidentified product from 9-hydroxyphenanthrene and sulphamic acid	0.72	0.83	—	Violet	Blue-grey	Pale-yellow	Pale-orange
2-Hydroxy-1-phenanthryl sulphate	0.70	0.82	0.65	Bright-violet	Purple-brown	Pale-brown	Pale-pink
Product, probably 1-hydroxy-2-phenanthryl sulphate	0.68	0.80	0.64	Violet	Blue	Blue	Pale-pink
3-Hydroxy-4-phenanthryl sulphate	0.73	0.85	0.68	Violet	Grey	Purple-brown	Purple
Second product from the oxidation of 3-hydroxyphenanthrene with $\text{K}_2\text{S}_2\text{O}_8$	0.60	0.85	0.70	Violet	Grey	Purple-brown	Purple
9-Hydroxy-10-phenanthryl sulphate	0.75	0.85	0.70	Bright-blue	Pale-blue-green	Pale-green	Pale-green, then yellow

* 9-Hydroxyphenanthrene was partly oxidized in this solvent to give a yellow streak on the paper.

Table 2. *Properties of the hydroxyphenanthrenes and related compounds on thin-layer chromatograms*

Experimental details are given in the text. The compounds were run on plates coated with silica gel G and developed with benzene.

Compound	R_F	Colour with 2,6-dichloroquinone-chloroimide and Na_2CO_3
1-Hydroxyphenanthrene	0.32	Blue
2-Hydroxyphenanthrene	0.22	Purple-red
3-Hydroxyphenanthrene	0.38	Grey
4-Hydroxyphenanthrene	0.45	Violet
9-Hydroxyphenanthrene	0.35	Blue-green
1-Hydroxy-2-methoxyphenanthrene	0.57	Violet
Compound, probably 2-hydroxy-1-methoxyphenanthrene	0.52	Purple-red
4-Hydroxy-3-methoxyphenanthrene	0.75	Blue
9-Hydroxy-10-methoxyphenanthrene	0.35	Blue-green
9,10-Phenanthraquinone	0.17	Forms a yellow spot on the untreated chromatograms

water as a powder (Found: C, 53.65; H, 2.9; K, 12.5; S, 9.8. $\text{C}_{14}\text{H}_9\text{KO}_4\text{S}$ requires C, 53.8; H, 2.9; K, 12.5; S, 10.3%). *Potassium 3-phenanthryl sulphate* (1.5 g.) formed plates (from water) (Found: C, 53.9; H, 3.2; S, 10.3%).

Potassium 1-phenanthryl sulphate (85 mg.), separating from water in plates (Found: C, 53.9; H, 2.9; S, 10.5%), and *potassium 4-phenanthryl sulphate* (48 mg.), separating from water in plates (Found: S, 9.6%), were similarly prepared from 1- and 4-hydroxyphenanthrene (100 mg.) respectively. All four sulphuric esters yielded the parent phenols (shown by mixed m.p.) and inorganic sulphate (tested with aq. BaCl_2) after being heated to 100° with 5N-HCl for 5 min. They were hydrolysed by the sulphatases of Taka-diastrase as described below to compounds indistinguishable on thin-layer chromatograms from the parent phenols.

When 9-hydroxyphenanthrene (1 g.) was treated with sulphamic acid as described above, *potassium 9-phenanthryl sulphate* (0.5 g.) was obtained, separating from water in plates (Found: C, 53.8; H, 3.1; S, 10.4%). The ester was hydrolysed with HCl and with Taka-diastrase as described above to 9-hydroxyphenanthrene and inorganic sulphate. When the mother liquors from the preparation of the ester were acidified with acetic acid, a second product (1.1 g.) separated, which was recrystallized from water in pink plates (Found, after drying at 12 mm. and 100° for 2 hr.: C, 52.1; H, 2.7; S, 10.2; 9.6. Calc. for $\text{C}_{14}\text{H}_9\text{KO}_4\text{S}$, $\frac{1}{2}\text{H}_2\text{O}$: C, 52.3; H, 3.1; S, 10.0%). The product, which was sparingly soluble in cold water but soluble in cold 2N-KOH, yielded 9-hydroxyphenanthrene, m.p. and mixed m.p. 152° , on hydrolysis with 5N-HCl at 100° for 15 min. Its properties on paper chromatograms and its u.v. and i.r. spectra differed from those of 9-phenanthryl sulphate. Since it did not appear to be a product of phenanthrene metabolism it was not further investigated.

In the preparation of ammonium 9-hydroxy-10-phenanthryl sulphate, 9,10-dihydroxyphenanthrene (2 g.) and sulphamic acid (2 g.), in pyridine (100 ml.), were heated under reflux for 24 hr. The mixture was poured into aq. 2N-KOH (50 ml.), and the solution was diluted with water (250 ml.) and evaporated to 50 ml. under reduced pressure. The solution was acidified with HCl and washed twice with ether (100 ml.). Activated charcoal (10 g.; British Drug Houses Ltd.) was added to the aqueous layer, and the mixture was filtered and the charcoal washed with water (500 ml.). The sulphuric ester was eluted from the charcoal

with methanol containing 5% (w/v) of aq. NH_3 (sp.gr. 0.88), and the solution was evaporated under reduced pressure to leave a solid (1.5 g.) which was recrystallized from aq. ethanol to yield *ammonium 9-hydroxy-10-phenanthryl sulphate* in light-brown plates (Found: C, 54.5; H, 4.1; S, 10.1. $\text{C}_{14}\text{H}_{13}\text{NO}_4\text{S}$ requires C, 54.7; H, 4.3; S, 10.4%). When the ester was heated to 100° with 5N-HCl for 15 min., 9,10-dihydroxyphenanthrene, m.p. and mixed m.p. 147° , and inorganic sulphate were obtained. Hydrolysis of the ester with Taka-diastrase yielded only 9,10-phenanthraquinone, in orange needles, m.p. and mixed m.p. 205° .

Ammonium 2-hydroxy-1-phenanthryl sulphate was prepared by the Elbs persulphate oxidation of 2-hydroxyphenanthrene. The phenol (2 g.), in a solution of KOH (3.5 g.) in water (200 ml.), was stirred at room temperature while a solution of $\text{K}_2\text{S}_2\text{O}_8$ (3 g.) in water was added dropwise over 4 hr. The mixture was kept overnight and the dark-brown solid filtered off and discarded. The filtrate was acidified with HCl and the orange solid was separated and discarded. The filtrate was washed twice with ether (100 ml.) and the sulphuric ester isolated from the aqueous layer by means of charcoal as described above to yield *ammonium 2-hydroxy-1-phenanthryl sulphate* (150 mg.), separating from aq. ethanol in light-brown plates (Found: C, 54.3; H, 4.4; S, 10.2%). When the ester was heated with 5N-HCl at 100° for 15 min., 1,2-dihydroxyphenanthrene separated in needles, m.p. 172° . The diacetate formed prisms from aq. ethanol, m.p. $143\text{--}145^\circ$ (Found: C, 73.5; H, 4.9. Calc. for $\text{C}_{18}\text{H}_{14}\text{O}_4$: C, 73.6; H, 4.8%), and the dimethyl ether formed needles from aq. ethanol, m.p. 100° . 1,2-Dihydroxyphenanthrene has m.p. 178° (Fieser & Peters, 1931), the diacetate has m.p. $146\text{--}147^\circ$ (Fieser, 1929c) and the dimethyl ether, m.p. 102° (Fieser, 1929b). The hydrolysis with Taka-diastrase failed to yield any recognizable products.

The ammonium salt of 3-hydroxy-4-phenanthryl sulphate was similarly prepared from 3-hydroxyphenanthrene (2 g.). In this case, however, the product obtained was a mixture of two sulphuric esters which were separated on four sheets of Whatman no. 1 chromatography paper developed with solvent 1 described above. The positions of the two esters on the chromatograms were found by examination in u.v. light, and these regions were cut out and the esters eluted from the paper with methanol containing 5% of aq. NH_3 (sp.gr. 0.88). The solution of the faster-moving compound was evaporated to dryness under reduced

pressure to yield ammonium 3-hydroxy-4-phenanthryl sulphate (75 mg.), which formed grey plates from aq. ethanol (Found: C, 53.9; H, 4.4; S, 10.0%). The ester (30 mg.) was heated with 5*N*-HCl (1 ml.) at 100° for 15 min. and the cooled solution extracted with ether (5 ml.). The gum obtained by evaporation of the ether was treated with acetic anhydride in pyridine to yield the diacetate of 3,4-dihydroxyphenanthrene, which, after three recrystallizations from aq. ethanol, formed needles (10 mg.), m.p. 157–158°. Fieser (1929*a*) reports m.p. 159° for the diacetate. The second ester (110 mg.), which formed light-brown plates from aq. ethanol, was not investigated further.

When ammonium 2-hydroxy-1-phenanthryl sulphate (50 mg.), in methanol, was treated with diazomethane in ether, a methoxy derivative was obtained which was heated to 100° for 15 min. with 5*N*-HCl. On cooling the solution, 1-hydroxy-2-methoxyphenanthrene (20 mg.) was obtained which separated from aq. ethanol in grey plates, m.p. 113° (Found: C, 80.2; H, 5.5. $C_{15}H_{12}O_2$ requires C, 80.3; H, 5.4%). Acetylation with acetic anhydride in 2*N*-NaOH yielded 1-acetoxy-2-methoxyphenanthrene in plates from aq. ethanol, m.p. 185° (Found: C, 76.9; H, 5.5. $C_{17}H_{14}O_3$ requires C, 76.7; H, 5.3%). When 1,2-dihydroxyphenanthrene was heated to 100° with sulphamic acid in pyridine for 2 hr. and the products examined on paper chromatograms, two products were detected, one of which was indistinguishable from 2-hydroxy-1-phenanthryl sulphate. The second product, which gave a blue colour with diazotized *p*-nitroaniline and aq. Na_2CO_3 (see Table 1), is believed to be the isomeric ester, 1-hydroxy-2-phenanthryl sulphate. When the ester mixture, in methanol, was treated with diazomethane in ether and the product hydrolysed with 5*N*-HCl as before, two products were detected on thin-layer chromatograms, one of which was indistinguishable from 1-hydroxy-2-methoxyphenanthrene, and the second product, which gave a purple-red colour with reagent (i) above, is believed to be 2-hydroxy-1-methoxyphenanthrene.

When ammonium 3-hydroxy-4-phenanthryl sulphate was similarly treated with diazomethane and the product hydrolysed with acid, 4-hydroxy-3-methoxyphenanthrene, in plates from aq. ethanol, m.p. 60–62°, was obtained, which yielded 4-acetoxy-3-methoxyphenanthrene, m.p. 131°, on acetylation with acetic anhydride in 2*N*-NaOH. Pschorr & Dickhäuser (1910) report m.p. 62–63° for the hydroxy and Fischer & Gerichten (1886) m.p. 131° for the acetoxy derivative.

The only product isolated when ammonium 9-hydroxy-10-phenanthryl sulphate was treated with diazomethane and the product hydrolysed with acid as before was 9,10-phenanthraquinone, m.p. and mixed m.p. 204–205°. 9-Hydroxy-10-methoxyphenanthrene was therefore prepared by the method of Goldschmidt & Schmidt (1922).

Hydrolysis of sulphuric esters. Acid hydrolyses were carried out by heating the material under examination with 5*N*-HCl at 100° for 15 min. The products were isolated from the cooled reaction mixtures by extraction with ether. Enzymic hydrolyses were carried out in acetate buffer at pH 5.7, with the sulphatases of Taka-diaxase (Parke, Davis Ltd.). The solutions were incubated at 37° overnight and the products were isolated with ether as before.

Animal experiments. A solution of phenanthrene (20%, w/v) in arachis oil was injected intraperitoneally into rabbits and rats. Rabbits were dosed twice weekly with 5 ml./kg.

body wt. and rats with 10 ml./kg. body wt. of this solution. Urines were collected daily and were stored at 0° until required. In the main experiments, the combined urines obtained from two rabbits which had received a total of 30 g. of phenanthrene and the combined urines from 22 rats which had received a total of 32 g. of phenanthrene were examined. Other smaller-scale experiments are described below. The health of the animals appeared to be unaffected by the large doses of phenanthrene administered.

Preliminary separation of the phenanthrene metabolites. The pooled urines from rabbits and rats were treated separately but in a similar manner. The urines were filtered and the pH of the filtrates was adjusted to 4 with acetic acid. Activated charcoal (100 g.) was added to each filtrate and the mixtures were stirred for a short time. The charcoal was filtered off and washed with water (2 l.) and the metabolites were eluted by washing the charcoal first with methanol (10 l.) containing 5% (v/v) of aq. NH_3 (sp.gr. 0.88) and then with methanol-benzene (9:1, v/v) (5 l.). The eluates were evaporated under reduced pressure to yield brown gums, which were chromatographed on cellulose-powder columns, each prepared from Whatman standard-grade cellulose powder (750 g.), with butan-1-ol-cyclohexane-aq. 2*N*- NH_3 (9:2:1, by vol.) as developing solvent. Fractions of 250 ml. were collected and evaporated under reduced pressure and the residues examined on paper chromatograms with the solvents and reagents described above. From the results of these examinations, the fractions were divided into five groups. Thus, in a typical separation, fractions 1 and 2 contained phenols, fractions 3–5 dihydrodihydroxyphenanthrenes, fractions 7–10 sulphuric esters, fractions 12–15 mercapturic acids and related compounds, and fractions 17–28 glucosiduronic acids. Fractions lying between the main groups contained mixtures and were either discarded or chromatographed on smaller cellulose-powder columns according to the amounts of the materials involved.

The present paper describes the investigations carried out on the groups of fractions containing phenols and sulphuric esters. The natures of the other groups of fractions are described by Boyland & Sims (1962*a, b, c*).

RESULTS

Phenolic fractions from the urines of rabbits and rats. The fractions from both rabbits and rats each formed dark-brown gums (about 0.5 g.), in which crystals formed after some weeks. Examination of the gums on paper and thin-layer chromatograms showed that compounds indistinguishable from 1-, 2-, 3-, and 4-hydroxyphenanthrene and 9,10-phenanthraquinone were present in those of both species. Since some of the products may have arisen from precursors during the working-up processes, samples of fresh urine from animals treated with phenanthrene were extracted with ether and the ether extracts examined on thin-layer chromatograms. The chromatograms showed the presence of small amounts of 1-, 2-, 3-, 4- and 9-hydroxyphenanthrene, the 1- and 2-derivatives being present in larger amount than the 3-, 4- and 9-derivatives. 9,10-Phenanthraquinone was not detected in the extracts of these urines.

Sulphuric ester fractions from the urine of rabbits. The combined fractions formed a dark-brown gum (about 2.5 g.). Preliminary examination on paper chromatograms

showed the presence of compounds indistinguishable from 1-, 2- and 3-phenanthryl sulphate and 2-hydroxy-1- and 9-hydroxy-10-phenanthryl sulphate. Another compound which gave a blue colour with *p*-nitroaniline and aq. Na_2CO_3 was indistinguishable from the product believed to be 1-hydroxy-2-phenanthryl sulphate. When a little of the gum was hydrolysed with acid as described above, compounds indistinguishable from 1-, 2-, 3-, 4- and 9-hydroxyphenanthrene and 9,10-phenanthraquinone were detected on thin-layer chromatograms. The quinone was probably an artifact arising from the oxidation of 9,10-dihydroxyphenanthrene, since only the quinone could be detected when the dihydroxy compound was run on thin-layer chromatograms. 1,2- and 3,4-Dihydroxyphenanthrene were also oxidized on these chromatograms, giving unidentified dark products. When the ester mixture was treated with diazomethane in ether before being heated with acid, then, as well as the products previously detected, 1-hydroxy-2-methoxyphenanthrene and a smaller amount of the compound believed to be 2-hydroxy-1-methoxyphenanthrene were also found on thin-layer chromatograms. The methoxy derivatives presumably arise from the methylation of the two isomeric sulphuric esters of 1,2-dihydroxyphenanthrene. 4-Hydroxy-3-methoxyphenanthrene, which would be expected if 3-hydroxy-4-phenanthryl sulphate were present in the mixture of sulphuric esters, was not found.

The hydrolysis of the ester mixture with Taka-diastase as described above yielded compounds indistinguishable on thin-layer chromatograms from 1-, 2-, 3- and 4-hydroxyphenanthrene and 9,10-phenanthraquinone. 9-Hydroxyphenanthrene was not present in detectable amounts.

The mixture of sulphuric esters (1 g.) was heated to 100° for 15 min. with 5*N*-HCl and the products were extracted from the cooled solution with ether. Removal of the ether gave a gum which was dissolved in benzene and chromatographed in this solvent on a column prepared from 100 g. of a mixture of silica gel G and Hyflo Super-Cel (Johns-Manville Co. Ltd. London, S.W. 1) (2:1, w/w). Fractions of 30 ml. were collected and examined on thin-layer chromatograms, and those which contained similar products were combined and evaporated and the residues methylated with methyl sulphate in 2*N*-NaOH. Fractions 4 and 5 yielded 4-methoxyphenanthrene (22 mg.), needles from aq. ethanol, m.p. and mixed m.p. 68°; fractions 7-9 yielded 1-methoxyphenanthrene (77 mg.), needles from aq. ethanol, m.p. and mixed m.p. 105°; fractions 11-14 yielded 3-methoxyphenanthrene (24 mg.), plates from methanol, m.p. and mixed m.p. 60-61°; fractions 15-18 yielded 2-methoxyphenanthrene (58 mg.), plates from ethanol, m.p. and mixed m.p. 99-100°. Although 9-hydroxyphenanthrene could be detected in fractions 6 and 7, the methoxy derivative could not be crystallized. After fraction 19 had been collected, the eluting solvent was changed to 5% (v/v) of ethanol in benzene. Fractions 27-30 were combined and evaporated to yield 9,10-phenanthraquinone (23 mg.) in orange needles, m.p. and mixed m.p. 204-205°. Dark substances remaining on the column are presumed to be oxidation products of the hydroxyphenanthrenes.

The remaining sulphuric ester mixture was chromatographed on a cellulose-powder column (prepared from 250 g. of cellulose powder) with butan-1-ol-cyclohexane-aq. 2*N*- NH_3 (18:5:1, by vol.) as the developing solvent. Fractions of 50 ml. were collected and examined on paper

chromatograms, and the five which contained sulphuric esters were separately evaporated under reduced pressure. The residual dark-brown gums were each dissolved in aq. 2*N*-KOH (5 ml.), with heating if necessary, and the solutions were kept at 0° for several days. Fraction 1 failed to crystallize, but fraction 2 deposited a solid (75 mg.) which was recrystallized three times from water to yield plates which were mainly potassium 1-phenanthryl sulphate (Found: S, 10.10. Calc. for $\text{C}_{14}\text{H}_9\text{KO}_4\text{S}$: S, 10.3%), although paper chromatography showed the presence of small amounts of the 2-isomer. When the ester (50 g.) was hydrolysed by heating with 5*N*-HCl (0.5 ml.) for 15 min., needles (20 mg.) separated, m.p. 154°, undepressed in admixture with 1-hydroxyphenanthrene. The mother liquors were shown with aq. BaCl_2 to contain inorganic sulphate. Fractions 3 and 4 deposited a powder which was recrystallized from water to yield potassium 2-phenanthryl sulphate (100 mg.) (Found: S, 9.95%). Acid hydrolysis as described above yielded 2-hydroxyphenanthrene (m.p. and mixed m.p. 168-169°) and inorganic sulphate. The mother liquors of fractions 1-4 all contained 1-, 2-, 3- and 4-phenanthryl sulphate (detected on chromatograms), but no solid material could be isolated. Paper chromatography showed that fraction 5 contained 2-hydroxy-1-phenanthryl sulphate (and its isomer) and 9-hydroxy-10-phenanthryl sulphate, but no solid material could be isolated. The solution was therefore heated to 100° for 15 min. with HCl (2 ml.) and the products were extracted from the reaction mixture with ether. The residue obtained on evaporating the ethereal solutions was treated with acetic anhydride in pyridine at room temperature for 2 hr. The reaction mixture was poured into water (50 ml.), when 9,10-phenanthraquinone (15 mg.), m.p. and mixed m.p. 203-205°, (evidently arising from the oxidation of 9,10-dihydroxyphenanthrene) was slowly deposited. The mother liquors were extracted twice with ether (50 ml.) and the combined extracts were washed with 2*N*- H_2SO_4 (100 ml.) and then with saturated aq. NaHCO_3 (100 ml.). Removal of the ether gave a gummy solid (35 mg.) which was recrystallized three times from aq. ethanol to yield the diacetate of 1,2-dihydroxyphenanthrene in prismatic needles, m.p. and mixed m.p. 141-144° (Found: C, 73.3; H, 5.0. Calc. for $\text{C}_{18}\text{H}_{14}\text{O}_4$: C, 73.6; H, 4.8%).

Sulphuric ester fraction from the urine of rats. The combined fractions yielded a dark-brown gum (about 2.8 g.). Compounds indistinguishable from 1- and 2-phenanthryl sulphate, the two isomeric sulphuric esters of 1,2-dihydroxyphenanthrene and 9-hydroxy-10-phenanthryl sulphate were detected. After the acid hydrolysis of the ester mixture as described above, 1-, 2-, 3-, 4- and 9-hydroxyphenanthrene and 9,10-phenanthraquinone were detected on thin-layer chromatograms, whereas if the mixture were first treated with diazomethane in ether compounds indistinguishable from 1-hydroxy-2-methoxy- and 2-hydroxy-1-methoxyphenanthrene were also found. The hydrolysis of the mixture with the sulphatases of Taka-diastase as described above yielded 1-, 2-, 3- and 4-hydroxyphenanthrene and 9,10-phenanthraquinone, but 9-hydroxyphenanthrene could not be detected.

The remainder of the mixture of the sulphuric esters was chromatographed on a cellulose-powder column as described above. Only potassium 2-phenanthryl sulphate (120 mg.) was isolated: it formed a pale-pink powder (Found: S, 10.0. Calc. for $\text{C}_{14}\text{H}_9\text{KO}_4\text{S}$: S, 10.3%) which on

acid hydrolysis as above yielded 2-hydroxyphenanthrene in plates from aq. ethanol, m.p. and mixed m.p. 167–169°, and inorganic sulphate.

DISCUSSION

No qualitative differences were observed between rabbits and rats in the excretion of the hydroxyphenanthrenes and their sulphuric esters. Small amounts of the five possible isomeric monohydroxyphenanthrenes were present but it is probable that only 1-, 2-, 3- and 4-hydroxyphenanthrene are present in conjugation with sulphuric acid for, although 9-hydroxyphenanthrene was detected when the sulphuric ester fractions were hydrolysed with acid, none was detected after hydrolysis with sulphatase although 9-phenanthryl sulphate is known to be hydrolysed by this enzyme. The presence of 9,10-dihydro-9-hydroxy-10-phenanthryl sulphate, which should give 9-hydroxyphenanthrene only after acid hydrolysis, would account for these results, and a compound with the properties of this ester is present in the urine of rats treated with *trans*-9,10-dihydro-9,10-dihydroxyphenanthrene (Boyland & Sims, 1962c).

The conjugates of the dihydroxyphenanthrenes presumably arise from the dihydroxy compounds formed by the dehydrogenation of the corresponding dihydrodihydroxy compounds in the same way that conjugates of 1,2-dihydroxynaphthalene are formed from *trans*-1,2-dihydro-1,2-dihydroxynaphthalene (Corner & Young, 1955; Sims, 1959). *trans*-9,10-Dihydro-9,10-dihydroxyphenanthrene is converted by rats into the sulphuric ester of 9,10-dihydroxyphenanthrene (Boyland & Sims, 1962c). The sulphuric ester of 3,4-dihydroxyphenanthrene could not be detected but there is evidence that a small amount of 3,4-dihydroxyphenanthrene is conjugated with glucuronic acid (Boyland & Sims, 1962b).

SUMMARY

1. When rabbits and rats are dosed with phenanthrene, they excrete small amounts of 1-, 2-, 3-, 4- and 9-hydroxyphenanthrene. 1-, 2-, 3- and 4-phenanthryl sulphate are excreted, but no 9-phenanthryl sulphate could be detected.

2. Animals of both species excrete 1,2- and 9,10-dihydroxyphenanthryl sulphate.

3. The syntheses of 1-, 2-, 3-, 4- and 9-phenanthryl sulphate, and of 1-hydroxy-2-, 3-hydroxy-4- and 9-hydroxy-10-phenanthryl sulphate are described.

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