REFERENCES

Anderson, T. G. & Rettger, L. F. (1937). *J. dent. Res.* **16**, 489.
Arnold, F. A. & McClure, F. J. (1941). *Publ. Hlth Rep., Wash.*, **56**, 1495.
Barker, S. B. & Summerson, W. H. (1941). *J. biol. Chem.* **138**, 535.
Bibby, B. G., Volker, J. F. & Van Kesteren, M. (1942). *J. dent. Res.* **21**, 61.
Bunting, R. W. (1934). *J. dent. Res.* **14**, 97.
Deakins, M., Cheyne, V. D., Bibby, B. G. & Van Kesteren, M. (1941). *J. dent. Res.* **20**, 161.
Dreizen, S., Mann, A. W., Cline, J. K. & Spies, T. D. (1946). *J. dent. Res.* **25**, 213.
Florestano, H. J. (1942). *J. dent. Res.* **21**, 263.
Fosdick, L. S., Campaigne, E. E. & Fancher, O. E. (1941). *Illinois dent. J.* **10**, 85.
Fosdick, L. S., Hansen, H. L. & Epple, C. (1937). *J. Amer. dent. Ass.* **24**, 1275.
Fosdick, L. S., Hansen, H. L. & Wessenger, G. D. (1937). *J. Amer. dent. Ass.* **24**, 1445.
Frisbie, H. E. & Nuckolls, J. (1947). *J. dent. Res.* **26**, 181.
Gottlieb, B. (1947). *Dental Caries.* Philadelphia: Lea and Febiger.
Jay, P., Hadley, F. P., Bunting, R. W. & Koehne, M. (1936). *J. Amer. dent. Ass.* **23**, 846.
Miller, W. D. (1890). *The Micro-organisms of the Human Mouth.* Philadelphia: S. S. White Co.
Nuckolls, J. & Frisbie, H. E. (1946). *J. Amer. Coll. Dent.* **13**, 84.
Osborn, T. W. B., Noriskin, J. N. & Staz, J. (1937). *J. dent. Res.* **16**, 165.
Snyder, M. L. & Teachout, J. J. (1942). *J. dent. Res.* **21**, 461.
Stephan, R. M. (1944). *J. dent. Res.* **23**, 257.
Stralfors, A. (1948). *J. dent. Res.* **27**, 587.
Thoma, K. H. (1944). *Oral Pathology.* London: Henry Kimpton.
Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1945). *Manometric Techniques.* Minneapolis: Burgess Publishing Co.
Volker, J. F. & Pinkerton, D. M. (1947). *J. dent. Res.* **26**, 229.

Metabolism of Polycyclic Compounds

6. CONVERSION OF PHENANTHRENE INTO DIHYDROXYDIHYDROPHENANTHRENES

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Bergel & Pschorr (1903) showed that rabbits dosed with phenanthrene excrete a phenolic derivative, phenanthrylglucuronide. Investigation of the metabolism of anthracene in rats and rabbits (Boyland & Levi, 1935) and of naphthalene (Young, 1947; Booth & Boyland, 1947, 1949) has shown that these hydrocarbons are transformed into dihydroxydihydro derivatives. Such diols are readily converted to phenols by dehydration in the presence of acid, and it is possible that some of the phenolic metabolic products which have been isolated are decomposition products of dihydroxydihydro derivatives. In the present paper this reaction of diol formation, which has been called perhydroxylation because it involves addition of the elements of hydrogen peroxide, is shown to take place with phenanthrene.

Young (1947) reported the isolation of a crystalline product from the urine of rats dosed with phenanthrene. This product with m.p. 186–187° and analysis corresponding to a formula $C_{14}H_{12}O_2$ would appear to be identical with the 9:10-dihydroxy-9:10-dihydrophenanthrene characterized below. Criegee, Marchand & Wannowius (1942) prepared *cis*-9:10-dihydroxy-9:10-dihydrophenanthrene by oxidation of phenanthrene with osmic acid. Attempts to synthesize *trans*-9:10-dihydroxy-9:10-dihydrophenanthrene by published methods (e.g. Skita, 1925) or by oxidation of phenanthrene have so far been unsuccessful, but reduction of phenanthraquinone with lithium aluminium hydride by the method described by Finholt, Bond & Schlesinger (1947) has given the *trans* compound identical with this metabolite (Booth, Boyland & Turner, 1950).

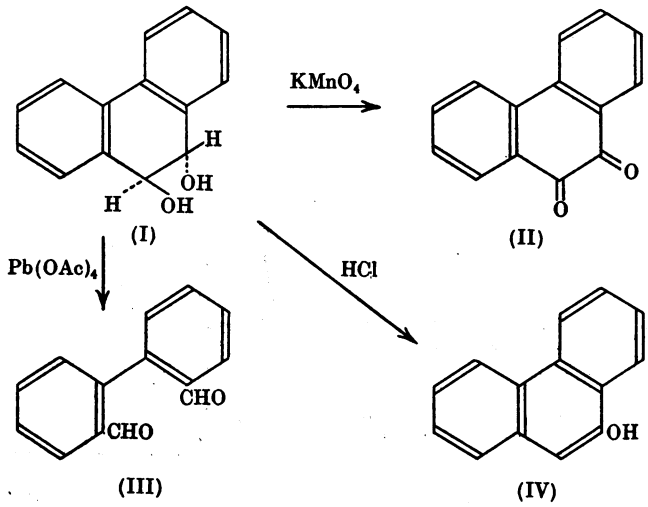
EXPERIMENTAL

A solution of phenanthrene (20%, w/v) in arachis oil was injected intraperitoneally into rats and rabbits. Rats were dosed with 10 ml./kg. body weight and rabbits with 5 ml./kg. body weight twice weekly and showed no ill effects from the treatment. The fresh urine which was collected in metabolism cages was extracted with ether as soon as possible. The ethereal extract was evaporated to dryness. In some experiments, the residue could be crystallized from benzene without further treatment. When the material was too impure for direct crystallization, it was dissolved in methanol containing 1% (v/v) water

and this solution extracted in a continuous extractor with light petroleum, b.p. 40–60°. Crystalline material separated from the light petroleum extract and was further purified by recrystallization from benzene after decolorization with charcoal.

Product from rat urine

9:10-*Dihydroxy*-9:10-*dihydrophenanthrene*. The compound from rat urine was dimorphic and crystallized from benzene as long, silky colourless needles, m.p. 185–187° (Found: C, 79·1; H, 5·6. $C_{14}H_{12}O_2$ requires C, 79·2; H, 5·6%), and as prisms, m. p. 194° (Found: C, 79·2; H, 5·7%). It was soluble in water and hexane and easily soluble in ethanol. This product is probably identical with that described by Young (1947). It had the same melting points as the two forms of the compound obtained synthetically by Booth *et al.* (1950). The products isolated from urine in this way were usually optically inactive, but material with *laevo* rotation was sometimes obtained. The problem of the optical activity is still under investigation.



Acetylation. Specimens (0·05 g.) of the diol were dissolved in pyridine (0·05 ml.) and acetic anhydride (0·4 ml.), and kept at room temperature for 16 hr. After neutralization of the pyridine with N-HCl the reaction products were extracted with ether and the extract shaken with M-Na_2CO_3 to remove excess acetic anhydride. The ether extracts were then shaken with water, dried with anhydrous Na_2SO_4 and evaporated to dryness. The *diacetate* crystallized from light petroleum (b.p. 40–60°) in colourless elongated prisms and was recrystallized from ethanol, m.p. 173°, alone or mixed with a synthetic specimen (Found: C, 73·0; H, 5·4. $C_{18}H_{16}O_4$ requires C, 72·9, H, 5·4%). The compound was sparingly soluble in water or cold ethanol but soluble in ether and benzene.

Dehydration of 9:10-*dihydroxy*-9:10-*dihydrophenanthrene*. When the diol was heated in 2N-HCl at 100° for 2 hr., 9-phenanthrol (IV), m.p. 153° (alone and when mixed with an authentic specimen) was formed. This compound, obtained from the diol, was dissolved in NaOH and shaken with dimethyl sulphate, when it gave 9-methoxyphenanthrene, m.p. 96° (alone and when mixed with an authentic specimen). When treated with pyridine and acetic anhydride, the phenanthrol derived from the optically active diol yielded 9-acetoxyphenanthrene, m.p. 77°, alone and when mixed with an authentic specimen (Found: C, 80·8; H, 5·1. Calc. for $C_{16}H_{12}O_2$: C, 81·2; H, 5·0%).

Oxidation of 9:10-*dihydroxy*-9:10-*dihydrophenanthrene*. (*a*) By $KMnO_4$: 15 ml. of 0·25M-$KMnO_4$ was added to the diol (0·05 g.) in water (10 ml.) and the mixture heated at 100° for 1 hr. The solution was acidified with 10N-HCl and extracted with a large volume of ether. The extract was washed with N-NaOH and water. After drying and evaporation of the solvent, phenanthraquinone (II) separated and was recrystallized from ethanol, m.p. 208°, alone and when mixed with an authentic specimen (Found: C, 80·2; H, 4·0. Calc. for $C_{14}H_8O_2$: C, 80·6; H, 3·9%).

(*b*) By Pb tetraacetate: the diol (0·025 g.) was added to a solution of Pb tetraacetate (0·1 g.) in anhydrous benzene (10 ml.) and kept at room temperature for 8 hr. The solution was then washed, first with N-HCl to remove the excess Pb tetraacetate, then with M-Na_2CO_3, and filtered to remove the insoluble Pb salt. After evaporation of the solvent, diphenyl-2:2′-dialdehyde (III) separated and was crystallized from light petroleum (b.p. 40–60°), m.p. 64° (alone and when mixed with an authentic specimen, prepared by oxidation of synthetic *cis*-9:10-diol with Pb tetraacetate according to Criegee *et al.* 1942). The dioxime was prepared and crystallized from aqueous methanol as colourless needles, m.p. 183–184°.

Spatial configuration. The hydroxyl groups appear to have the *trans* configuration as indicated by the following pieces of evidence.

(*a*) Identity with the synthetic *trans* compound.

(*b*) Difference from synthetic *cis* derivative in physical properties (Table 1).

(*c*) Differences in rate of oxidation of *cis* and *trans* diols. Lead tetraacetate oxidation was carried out in glacial acetic acid as described for naphthalene derivatives (Booth & Boyland, 1949).

Table 1. *Properties of* 9:10-*dihydroxy*-9:10-*dihydrophenanthrenes*

	Cis form (synthetic)	*Trans* form (synthetic)	Natural product
M.p. of diol	178°	185–187° and 194°	185° and 194°*
M.p. of diacetate	110°	173°	173°†
Oxidation with lead tetraacetate:			
Time for half reaction at 20° (min.)	4·2	0·3	0·3
$k_{20°}$ (t = min.)	13·8	130	130
Dehydration with N-HCl:			
Time for half reaction at 100° (min.)	0·85	11·5	12·0
$k_{100°}$ (t = min.)	8·1	0·60	0·58

* Mixed m.p. of natural product and synthetic *trans* form of m.p. 185–187° was 184–185°.
† Mixed m.p. of natural product and synthetic *trans* compound 173°.

The rates of reaction of the natural 9:10-dihydroxy-9:10-dihydrophenanthrene and synthetic *cis* and *trans* forms of 9:10-dihydroxy-9:10-dihydrophenanthrene with Pb tetra-acetate are shown in Fig. 1 and Table 1. Under these conditions phenanthrene itself was not oxidized. The natural product was oxidized much more rapidly than the synthetic *cis* compound.

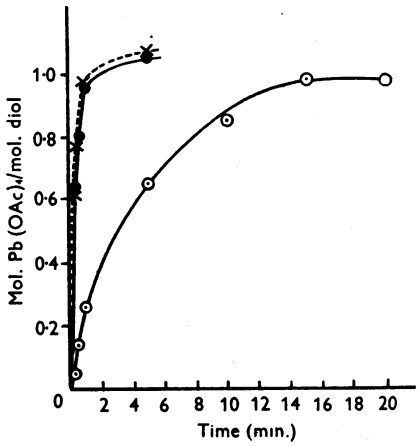


Fig. 1. Oxidation of phenanthrene diols with lead tetra-acetate. Natural, ×- - ×; synthetic *trans*, ●—●—; *cis*, ⊙—⊙—.

(*d*) Rates of dehydration with acid. Solutions of the natural 9:10-dihydroxy-9:10-dihydrophenanthrene and the synthetic *cis* and *trans* compounds were heated in N-HCl at 100°. The amount of 9-phenanthrol formed was estimated colorimetrically by coupling with diazotized sulphanilic acid in alkaline solution. The rates of phenol formation of the two isomers are shown in Fig. 2 and Table 1.

(*e*) Colour reaction with potassium triacetyl osmate. With this reagent (Criegee *et al.* 1942) synthetic *cis*-9:10-dihydroxy-9:10-dihydrophenanthrene gave a positive reaction, while 9:10-dihydroxy-9:10-dihydrophenanthrene from rat urine, or the synthetic *trans* compound, did not react.

(*f*) Ultraviolet absorption spectrum. Ultraviolet absorption measurements made by Mr R. N. Beale and Dr E. M. F. Roe and to be published separately, show the natural 9:10-dihydroxy-9:10-dihydrophenanthrene to have a similar absorption to that of synthetic *trans*-9:10-dihydrophenanthrene.

Product from rabbit urine

The crystals obtained from the extract had an indefinite melting point and were shown to be a mixture of diols. The mixture was acetylated with cold acetic anhydride and pyridine. The mixed

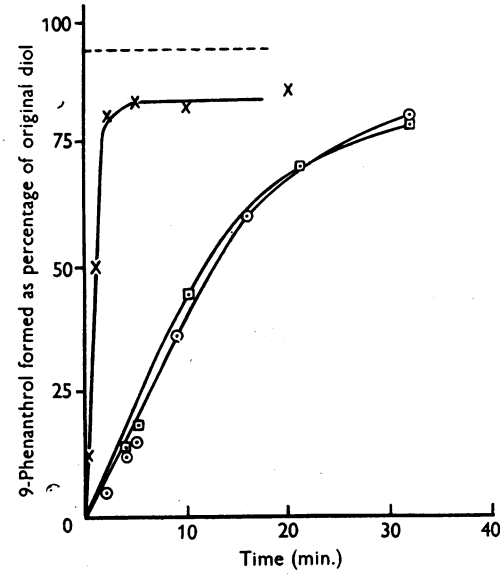


Fig. 2. Elimination of water from isomeric 9:10-dihydroxy-9:10-dihydrophenanthrenes in N-HCl at 100°. Theoretical yield, - - - - -; synthetic *cis* compound, ×—×—; natural product, ⊙—⊙—; synthetic *trans* compound, ⊡—⊡—.

acetates were dissolved in light petroleum (b.p. 40–60°) with a little ether, and fractionally crystallized. The first crop consisted of the diacetate of *trans*-9:10-dihydroxy-9:10-dihydrophenanthrene, m.p. 173° (alone and when mixed with a specimen of the compound obtained from rat urine). By treatment with N-KOH in methanol at the boiling point for 2 min., 9:10-dihydroxy-9:10-dihydrophenanthrene (I) was obtained, m.p. 185–187° (alone and when mixed with a specimen of the compound ob-

tained from rat urine). This was identical with the compound obtained from rat urine in every respect.

The second crop of crystals consisted of the *diacetate* of 1:2-*dihydroxy*-1:2-*dihydrophenanthrene* crystallizing from ethanol in colourless, short, thick needles, m.p. 106° (Found: C, 72·8; H, 5·4. $C_{18}H_{16}O_4$ requires C, 72·9; H, 5·4%). Optical rotation $[\alpha]_D^{20°} = -380°$ (*c*, 0·4% in ethanol).

laevo-1:2-*Dihydroxy*-1:2-*dihydrophenanthrene* (V). The above diacetate was dissolved in N-KOH in methanol and the solution boiled for 2 min. and poured into water. The diol was then extracted with ether and crystallized from benzene in colourless thick prisms, m.p. 155–159° (Found: C, 79·1; H, 5·6. $C_{14}H_{12}O_2$ requires C, 79·2; H, 5·6%). The compound is soluble in water, benzene and light petroleum, and easily soluble in ethanol. Optical rotation $[\alpha]_D^{20°} = -240°$ (*c*, 0·5% in ethanol).

Dehydration (*Method a*). *laevo*-1:2-Dihydroxy-1:2-dihydrophenanthrene (0·02 g.) was dissolved in 0·25 N-HCl in methanol and the solution boiled for 30 min. Water was then added, the resulting mixture of phenols extracted with ether, the ether extract washed with M-$NaHCO_3$ and dried over Na_2SO_4. After evaporation of the solvent, the product was dissolved in pyridine and acetic anhydride and heated at 100° for 3 hr. After removal of the pyridine with N-HCl, the mixture was dissolved in ether and, after washing with M-$NaHCO_3$ and drying over Na_2SO_4, evaporated to dryness. The resulting solid was dissolved in light petroleum (b.p. 60–80°), and by slow crystallization, a product m.p. 168–169° was obtained. This is now being further investigated. The residue from the mother liquors was dissolved in hot N-NaOH and treated with successive portions of dimethyl sulphate until the fluorescence of the solution had changed from green to blue. The solids obtained were extracted with ether, the extracts washed with water, dried over Na_2SO_4 and the solvent evaporated. The residual solid was dissolved in light petroleum (b.p. 40–60°) and chromatographed on a column of Al_2O_3 (Savory & Moore Ltd.) made with the same solvent. Two fluorescing bands were seen in ultraviolet light; the less strongly adsorbed band, on elution, was found to be 1-methoxyphenanthrene (VI), m.p. 105°, alone and when mixed with an authentic specimen (Found: C, 85·9; H, 5·8. Calc. for $C_{15}H_{12}O$: C, 86·4; H, 5·8%).

Dehydration (*Method b*). *laevo*-1:2-Dihydroxy-1:2-dihydrophenanthrene (0·02 g.), when suspended in 2 N-HCl and heated to 100° for 1 hr., gave 1-hydroxyphenanthrene, m.p. 156° (alone and when mixed with an authentic specimen). This on treatment with dimethyl sulphate in NaOH solution as described above, gave 1-methoxyphenanthrene (VI), m.p. 105° (alone and when mixed with an authentic specimen).

Hydrogenation. *laevo*-1:2-Dihydroxy-1:2-dihydrophenanthrene takes up Br_2 from a solution in $CHCl_3$ and can be hydrogenated. The diacetate of the diol (0·15 g.) in ethanol (10 ml.) was treated with Pt (Adams's catalyst) and hydrogenated at atmospheric pressure and room temperature. The volume of H_2 taken up was 7·3 ml. When absorption of H_2 ceased, the catalyst was removed by filtration and the solvent evaporated. The *diacetate* of 1:2-*dihydroxy*-1:2:3:4-*tetrahydrophenanthrene* (VII) crystallized in colourless needles from ethanol, m.p. 112° (Found: C, 72·0; H, 6·5. $C_{18}H_{18}O_4$ requires C, 72·4; H, 6·1%). The compound is optically active, $[\alpha]_D^{20°} = -105°$ (*c*, 0·6% in ethanol).

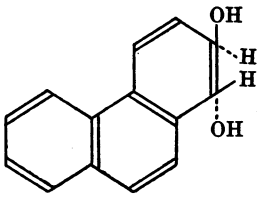
Steric configuration. The result of the potassium triacetyl osmate colour reaction of Criegee *et al.* (1942) on the 1:2-dihydroxy-1:2-dihydrophenanthrene was negative, which would indicate that the hydroxyl groups in the compound have the *trans* configuration.

DISCUSSION

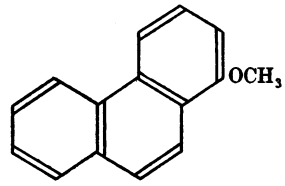
The experiments show that phenanthrene is metabolized to dihydroxydihydro derivatives by rats and rabbits. The presence of two hydroxyl groups is shown by the formation of diacetates. That the hydroxyl groups are on adjacent carbon atoms is shown by the ease of oxidation with lead tetraacetate to dialdehydes.

That the hydroxyl groups in the derivative isolated from rat urine must be in the 9:10 positions is shown by (*a*) the oxidation to 9:10:phenanthraquinone (II) with potassium permanganate, (*b*) the oxidation to diphenyl-2:2′-dialdehyde (III) with lead tetraacetate, and (*c*) the conversion to 9-phenanthrol on treatment with hot acid. That the hydroxyl groups have the *trans* configuration is indicated by the fact that the derivative differs from the synthetic *cis* compound in several respects, viz.: (1) melting point, (2) not giving an acetone derivative, (3) reacting with lead tetraacetate at a faster rate, (4) not giving the colour reaction with potassium triacetyl osmate, (5) being dehydrated more slowly to 9-phenanthrol. This evidence for the structure and the *trans* configuration was obtained before the synthetic compound was available. The identity with the synthetic compound proves the structure previously assigned to the compound.

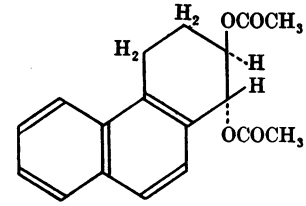
The experiments are of interest in that in this compound *trans*-9:10-dihydroxy-9:10-dihydrophenanthrene is more rapidly oxidized by lead tetra-



(V)



(VI)



(VII)

acetate than is the *cis* derivative. This would appear to be an exception to Criegee's rule (Criegee, Kraft & Rank, 1933) that *cis* glycols are more rapidly oxidized than the corresponding *trans* compounds. The evidence seems quite clear in the case of these 9:10-diols derived from phenanthrene. Another similar anomaly is the oxidation of the *trans*-9:10-dimethyl-9:10-dihydroxyphenanthrene which Criegee (personal communication) has found to react with lead tetraacetate more rapidly than the corresponding *cis* derivative.

As the natural optically inactive 9:10-dihydroxy-9:10-dihydrophenanthrene has been shown to be the *trans* compound it must be a racemic form. The racemate is possibly formed by racemization of the optically active material after excretion.

One of the products isolated from rabbit urine is identical with the *trans*-9:10-dihydroxy-9:10-dihydrophenanthrene isolated from rat urine. The other diol from rabbit urine is shown to be *trans*-1:2-dihydroxy-1:2-dihydrophenanthrene (V). The evidence for this structure depends upon the following observations: (*a*) the conversion to 1-phenanthrol on treatment with hot, aqueous acid; (*b*) the formation of 1-phenanthrol on treatment with acid in methanol; (*c*) the formation of 1:2-dihydroxy-1:2:3:4-tetrahydrophenanthrene (VIII) by catalytic hydrogenation. The only evidence for the configuration of the hydroxyl groups is the fact that the compound does not discharge the blue colour of potassium triacetyl osmate. In the absence of contrary evidence this would indicate that the compound is *laevo-trans*-1:2-dihydroxy-1:2-dihydrophenanthrene.

The results are of interest in relation to those obtained with other hydrocarbons. Phenanthrene differs from naphthalene and anthracene in having one bond with high reactivity. In naphthalene and anthracene there are four equivalent bonds all of equal reactivity. If in the metabolism of anthracene or naphthalene the hydrocarbons are attached to an enzyme through a 3:4 bond by an addition reaction, the adjacent double bond (1:2) would then become more reactive so that oxidation might readily take place in this 1:2 position. Thus a 1:2-dihydroxy-1:2-dihydro-3:4-'tissue'-complex may be formed which breaks down to give the 1:2-dihydroxy-1:2-dihydro derivative of the hydrocarbon.

The formation of the 1:2-dihydroxy-1:2-dihydrophenanthrene is analogous to the metabolism of the more complex hydrocarbons such as 1:2-benzanthracene and 1:2:5:6-dibenzanthracene in that the addition occurs in positions of secondary chemical activity. The region corresponding to the phenanthrene 9:10 double bond, which is the region with the highest charge of electrons, has been called the '*K* region' by French workers (cf. Pullman & Pullman, 1946). If the bond of the *K* region is saturated, because the hydrocarbon is held to a tissue constituent through this bond by an addition reaction, then a second bond may be activated. If phenanthrene is bound through the 9:10 bond then the 1:2 bond would be available and possibly activated. Thus the formation of the 1:2-dihydroxy-1:2-dihydrophenanthrene is similar to the metabolism of other hydrocarbons which have been investigated.

The formation of the 9:10-dihydroxy-9:10-dihydrophenanthrene is anomalous as this process involves the addition of hydroxyl groups to the bond of high activity. In this reaction phenanthrene is metabolized in a manner unlike that occurring with the carcinogenic hydrocarbons.

Although *trans*-9:10-dihydroxy-9:10-dihydrophenanthrene can be prepared by reduction of the corresponding quinone this is unlikely to be the mechanism of the biological production of the diol because no diol could be isolated from the urine when phenanthraquinone was injected into rats or rabbits.

The fact that the hydroxyl groups in the metabolic diols obtained from naphthalene, anthracene (Booth & Boyland, 1949) and phenanthrene have the *trans* configuration suggests that they may arise by hydration of an epoxide. The bond to be oxidized may become activated by attachment of another part of the molecule to the enzyme system by an addition reaction. The activated bond might then react with an oxidizing system to yield an epoxide which might then react with water (by enzyme action in the cases where optically active diols are formed), with a tissue to form a complex or with an acid to yield an ester. The reaction of phenanthrene-9:10-epoxide with water might lead to formation of a racemic product, because the molecule is symmetrical about the reacting group. This and other possible mechanisms of the reaction of perhydroxylation are being investigated.

SUMMARY

1. When phenanthrene is injected into rats, *trans*-9:10-dihydroxy-9:10-dihydrophenanthrene is excreted in the urine; when injected into rabbits a mixture of the same diol and *laevo-trans*-1:2-dihydroxy-1:2-dihydrophenanthrene is excreted.

2. The oxidation of the 9:10-dihydroxy-9:10-dihydrophenanthrene with lead tetraacetate is unusual in that the *trans* compound reacts more rapidly than the *cis* form.

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REFERENCES

Bergel, P. & Pschorr, R. (1903). *Hoppe-Seyl. Z.* **38**, 16.
Booth, J. & Boyland, E. (1947). *Biochem. J.* **41**, xxix.
Booth, J. & Boyland, E. (1949). *Biochem. J.* **44**, 361.
Booth, J., Boyland, E. & Turner, E. E. (1950). *J. chem. Soc.* p. 1188.
Boyland, E. & Levi, A. A. (1935). *Biochem. J.* **29**, 2679.
Criegee, R., Kraft, L. & Rank, B. (1933). *Liebigs Ann.* **507**, 161.
Criegee, R., Marchand, B. & Wannowius, H. (1942). *Liebigs Ann.* **550**, 99.
Finholt, A. E., Bond, A. C. jun. & Schlesinger, H. J. (1947). *J. Amer. chem. Soc.* **69**, 1199.
Pullman, A. & Pullman, B. (1946). *Rev. Sci.* **84**, 145.
Skita, A. (1925). *Ber. dtsch. chem. Ges.* **58**, 2685.
Young, L. (1947). *Biochem. J.* **41**, 417.

A Study of the Pigments of the Sea-urchins, *Echinus esculentus* L. and *Paracentrotus lividus* Lamarck

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Although the purple pigments of the test (shell) and spines of the common sea-urchin *Echinus esculentus* L. have attracted some attention they have not been unequivocally identified. In his pioneer investigations MacMunn (1885) noticed a red quinonoid pigment which he named echinochrome in the perivisceral fluid of a number of sea-urchins; it was first isolated in crystalline form by McClendon (1912) from the ovaries of *Arbacia pustulosa* Leske, but it was not until much later that its empirical formula was established as $C_{12}H_{10}O_7$ (Ball, 1936; Glaser & Lederer, 1939; Tyler, 1939). Kuhn & Wallenfels (1939, 1940) isolated the same pigment, together with small amounts of two similar pigments, from the gonads of *Paracentrotus lividus* Lamarck and decided that it was 2-ethyl-3:5:6:7:8-pentahydroxy-1:4-naphthaquinone (I). Confirmation of this structure was obtained when Wallenfels & Gauhe (1943) achieved a synthesis of the pigment. Meanwhile, Lederer & Glaser (1938) isolated a new quinonoid pigment (spinochrome, spinochrome A, spinochrome P) from the spines and tests of the purple variety of *P. lividus*; this was confirmed by Musajo & Minchilli (1940), who later isolated a further green pigment spinochrome P_1 from the spines of the green variety of *P. lividus*.

Apart from MacMunn's (1885) work, the only other reports on the pigments of *Echinus esculentus* indicated that reduced echinochrome exists in the elaeocytes and that echinochrome itself is the pigment of the shell, dermis and spines (Lederer, 1940).

It was because of the paucity of information regarding the pigments of *E. esculentus* that the present investigation was undertaken. As the investigation proceeded it became apparent that two pigments were present in the tests and spines and that they were in fact spinochrome A (P) and spinochrome B (P_1); no evidence for the presence of echinochrome was ever obtained. Authentic samples of these two pigments were obtained from *Paracentrotus lividus* and the opportunity was taken not only of recording their properties more fully than had been done previously, but also of determining their distribution in the olive-green and violet varieties of this urchin.

Considerable confusion exists in the literature of sea-urchin pigments for two reasons: (i) various names have been given at different times to the same pigment, and (ii) authors have often been unaware of the synonomy existing in the systematic naming of sea-urchins. In order to clarify the position, Table 1, recording all the published information together with indications of synonomy in both chemical and systematic nomenclatures, has been compiled; names to be used in this paper are also indicated. The logical procedure has been adopted of identifying the pigments by adding the suffixes A, B, C, etc. after the general terms echinochrome and spinochrome, according to the order in which the pigments were isolated; this follows the procedure initiated by Kuhn & Wallenfels (1939). Much less difficulty arises in this way than in adding a suffix indicating the species from which the pigment was first isolated. In this way difficulties soon arise, e.g. spinochrome 'P' (*Paracentrotus*), has now been found in *Echinus esculentus*. The two group terms echinochrome and spinochrome have been accepted, for there appears to be good reason to believe that echinochromes occur only in the gonads and body fluid, whilst the spinochromes are characteristic of the spines and tests. The pigments obtained from Japanese sea-urchins (Kuroda & Oshima, 1940) are