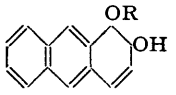
154. *The Metabolism of Polycyclic Compounds: the Configuration of the Dihydroxydihydroanthracenes produced from Anthracene.*

By E. Boyland and C. W. Shoppee.

The metabolism of carcinogenic and non-carcinogenic polycyclic hydrocarbons appears to conform to a general pattern. The configurations of the isomeric 1 : 2-dihydroanthracene-1 : 2-diols excreted by rats and rabbits respectively (Boyland and Levi, *Biochem. J.*, 1935, **29**, 2679) have therefore been investigated. The evidence now adduced shows that the isomeride from rats is either the *d*- or the *l-cis*-enantiomorph, but that the isomeride from rabbits is the optically inactive *dl-cis*-compound.

Some years ago it was shown that anthracene fed to rats and rabbits is partly excreted in the urine as isomeric 1 : 2-dihydroanthracene-1 : 2-diols (I*a*) (Boyland and Levi, *Biochem. J.*, 1935, **29**, 2679) or as the *l*-glycuronic acid conjugates (I*b*) of these compounds (*idem*, *ibid.*, 1936, **30**, 728), and that all four substances are converted by dilute mineral acid into 1-hydroxyanthracene.



(I*a*; R = H)
(I*b*; R = $C_6H_9O_6$)

The structures of the diols (I*a*) were established by suitable oxidation and reduction experiments, but their configurations were not investigated.

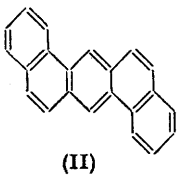
Subsequently, after having fed the carcinogen 1 : 2 : 5 : 6-dibenzanthracene (II) to rabbits,

* The mean values of k_a and $k_a/Re^{0.5}$ from Tables I—III are given below, together with the percentage root-mean-square deviations. The values of the same quantities calculated by application of equation (7) to the experimental data of Chambers and Sherwood are also given in the table. The deviation of

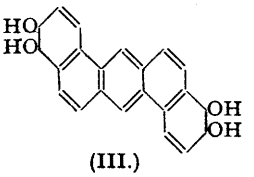
	$k_a \times 10^2$.	Devn., %.	$k^3/Re^{0.5} \times 10^3$.	Devn., %.
Present work: all acid concns. at 40°	1·9	24	1·1	10
,, ,, ,, 25°	1·6	16	0·8	13
Chambers and Sherwood: 8 results on acids up to 44% at 25°	3·3	25	0·6	19
,, ,, 4 ,, 50·7% acid at 25°	1·6	42	0·3	25
,, ,, 1 ,, 54·5% ,, ,,	1·0	—	0·2	—

$k_a/Re^{0.5}$ is seen to be rather smaller than the deviation of k_a; $k_a/Re^{0.8}$ is less satisfactory for the results as a whole. It may also be noted that Chambers and Sherwood's data show evidence of an effect of acid concentration on k_a or $k_a/Re^{0.5}$.

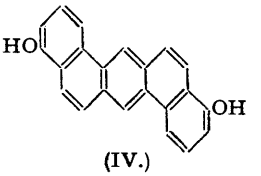
Boyland and Levi (*Chem. and Ind.*, 1938, **15**, 446) isolated a dihydroxy-derivative, also obtained by Dobriner, Rhoads, and Lavin (*Cancer Research*, 1942, **2**, 95; cf. Jones, *ibid.*, p. 245). Dobriner, Rhoads, and Lavin also isolated an apparently different dihydroxy-derivative from the urine and fæces of rats; this was later identified by Cason and Fieser (*J. Amer. Chem. Soc.*, 1940, **62**, 2681) as 4′ : 8′-dihydroxy-1 : 2 : 5 : 6-dibenzanthracene (IV). Boyland and Levi suggested that the production of (IV) might occur by way of an intermediate metabolite (III), analogous to (I*a*) or (I*b*), although no positive evidence for this was obtained. Fieser (Univ. of Pennsylvania Bicentennial Conference, 1940) also assumed that " perhydroxylation " of (II) precedes the metabolic formation of (IV) in rats and rabbits.



(II)

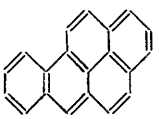


(III.)

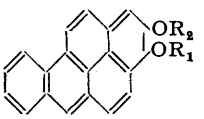


(IV.)

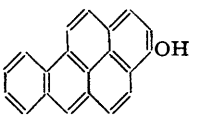
In the case of benzpyrene (V) fed to rats, it has been shown that the phenolic metabolite discovered by Chalmers and Crowfoot (*Biochem. J.*, 1941, **35**, 1270) is 8-hydroxybenzpyrene (VII) (Berenblum *et al.*, *Cancer Research*, 1943, **3**, 145, 151), whilst recently Weigert and Mottram (*ibid.*, 1946, **6**, 97, 109) have adduced evidence that here also metabolism proceeds by way of intermediates (VI) produced by " perhydroxylation ".



(V.)



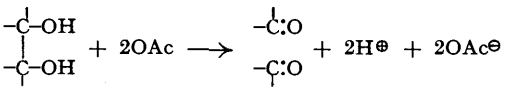
(VI.)



(VII.)

Thus it appears that an essential similarity exists between the metabolism of carcinogenic and non-carcinogenic polycyclic hydrocarbons. It is important to seek for some relation between the activity of a carcinogen and its metabolism because it is not known whether the change from a normal to an abnormal cell is induced by the hydrocarbon itself or by some locally produced derivative. Rats and rabbits metabolise ingested anthracene differently * (Boyland and Levi, *loc. cit.*); and it is known that rats and rabbits react differently to 1 : 2 : 5 : 6-dibenzanthracene—rats, but not rabbits, readily afford tumours at injection sites (Burrows and Boyland, *Amer. J. Cancer*, 1938, **32**, 367), which may conceivably be due to some difference in metabolism. It seemed therefore of possible value to attempt to determine the stereochemical configuration of the isomeric diols (I*a*), and an examination of these has now been made by one of us (C. W. S.) with material supplied by the other (E. B.).

Four isomeric 1 : 2-dihydroanthracene-1 : 2-diols can exist, comprising a pair of *cis*-enantiomorphs and a pair of *trans*-enantiomorphs. Since the isomerides described by Boyland and Levi were both optically active with specific rotations of +16° and −100°, it appeared that the method of Criegee (*Ber.*, 1931, **64**, 260) would be diagnostic since it has been shown (Criegee, Kraft, and Rank, *Annalen*, 1933, **507**, 159) that lead tetra-acetate invariably oxidises cyclic *cis*-1 : 2-diols much more rapidly than the *trans*-isomerides by a reaction which may be symbolised :



so that 1 mol. of diol requires 1 mol. of lead tetra-acetate. In applying this method to 1 : 2-dihydroanthracene-1 : 2-diols, it is necessary to take into consideration the reactivity of the *meso*-positions of the anthracene nucleus. K. H. Meyer (*Annalen*, 1911, **379**, 75) found that with 1 mol. of lead dioxide in acetic acid at 50° anthracene gives anthranyl acetate, whilst use of 2 mols. of lead dioxide at 70° affords *meso*-hydroxyanthranyl acetate which is stable to lead dioxide in acetic acid at 20°. Fieser and Hershberg (*J. Amer. Chem. Soc.*, 1938, **60**, 1893) have shown that lead tetra-acetate in acetic acid is equivalent to the lead dioxide–acetic acid mixture used by Meyer and is a more convenient reagent; with N/10-lead tetra-acetate in acetic acid at 20°,

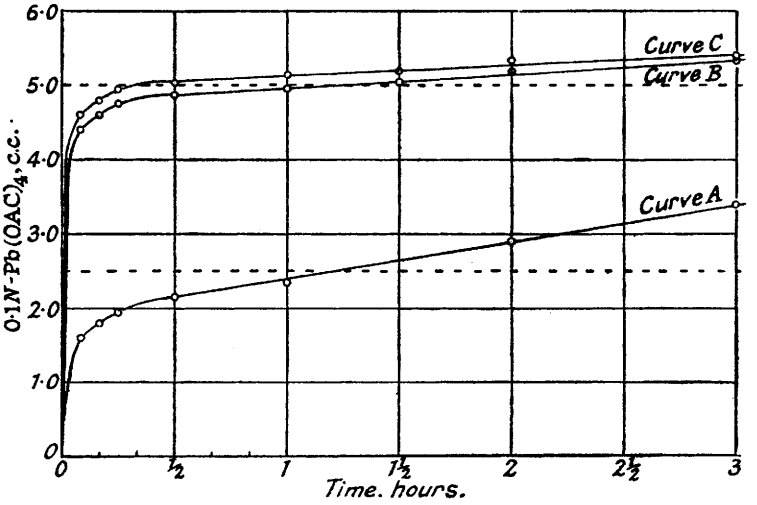
* Compare also Weigert and Mottram (*loc. cit.*) who state that it is certain that the metabolism of benzpyrene in rabbits is different from that in mice.

the main reaction would be expected to be the production of anthranyl acetate, which may be symbolised :

$$\geqslant CH + 2OAc \longrightarrow \geqslant C{\cdot}OAc + H^{\oplus} + OAc^{\ominus}$$

so that 1 mol. of anthracene should require 1 mol. of lead tetra-acetate. Curve *A* of the figure shows that this is in fact the case; under the conditions specified, anthracene undergoes fairly rapid oxidation by lead tetra-acetate to anthranyl acetate. The reaction is substantially complete in about 1 hour, and is followed by a further, slower oxidation, which probably represents the conversion of anthranyl acetate to *meso*-hydroxyanthranyl acetate.*

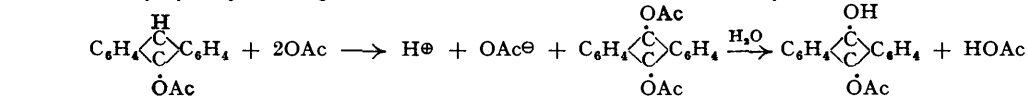
If the behaviour of anthracene towards lead tetra-acetate may be expected to be approximately reproduced in the corresponding oxidation under the same conditions of a 1 : 2-dihydroanthracene-1 : 2-diol, then 2 mols. of the reagent should be required. This is found to be so; curve *B* of the figure represents the oxidation of the " rat "-diol with N/10-lead tetra-acetate



in acetic acid at 20°, and curve *C* that of the " rabbit "-diol. The two curves are nearly coincident and show that an extremely rapid oxidation is succeeded by a slower, but still fairly fast, oxidation (analogous to that exhibited by anthracene, curve *A*), the process becoming substantially complete in about ½ hour and requiring 2 mols. of lead tetra-acetate; finally there occurs a further, much slower oxidation which is again analogous to that shown by anthracene.

It appears certain from the close similarity of the curves *B* and *C* that the two diols possess the same stereochemical configuration, and reasonably certain that this configuration must be *cis*- on account of the high speed with which oxidation occurs. Only two *cis*-1 : 2-diols, related as *d*- and *l*-forms, can exist, and these would be expected to possess approximately equal and opposite rotatory powers. Since this was not so according to the original observations of Boyland and Levi, specimens of the two diols were rigorously purified by sublimation in a high vacuum and subsequent crystallisation and their optical rotatory powers redetermined. The optical activity reported previously by Boyland and Levi for the " rat "-diol has been confirmed ($[\alpha]_D^{22°} -80° \pm 2°$), but the pure " rabbit "-diol has been found to be optically inactive. Boyland and Levi had reported only a small specific rotation ($[\alpha]_D^{20°} +16°$) for the " rabbit "-diol (they stated that it was not definitely proved that the substances they isolated were optically pure), and it may be noted that the specific rotation which they recorded for its dihydro-derivative

* The production of this compound is difficult to formulate; it seems likely that acetoxylation is followed by hydrolysis to regenerate the anthranol–anthrone tautomeric system :



This may be compared with the alcoholysis of tetraethyl 1-methyl-1 : 3-dicarboxyglutaconate to afford ethyl carbonate (Ingold and Thorpe, *J.*, 1919, 143) with regeneration of the three-carbon prototropic system :

$$(CO_2Et)_2C{=}CH{-}CMe(CO_2Et)_2 \xrightarrow{EtOH} (CO_2Et)_2C{=}CH{-}CHMe{\cdot}CO_2Et + CO(OEt)_2$$

($[\alpha]_D^{20°}$ −1°) and the diacetate of this ($[\alpha]_D^{20°}$ −3°) are within the limits of experimental error. The only earlier observations not satisfactorily accounted for are the physical constants of the " rabbit "-diol diacetate (reported to have m. p. 184°, $[\alpha]_D^{20°}$ 309°); whether prepared according to the directions of Boyland and Levi, or by use of acetic anhydride in pyridine at 20°, the pure diacetate has m. p. 120—122° (with indications of a polymorphic form, m. p. 131—132°), $[\alpha]_D^{22°}$ 0° ± 2°, and by alkaline hydrolysis regenerates the parent diol, m. p. 184°. A specimen of the crude crystalline diol, acetylated following the procedure of Boyland and Levi, furnished a crude diacetate, m. p. 120—121°, $[\alpha]_D^{22°}$ +23° ± 3°. It was noticed that, whereas the " rat "-diol sublimed in a high vacuum leaving practically no residue, the " rabbit "-diol left a small carbonaceous deposit; it seems probable therefore that the small dextro-rotations shown by the crude crystalline " rabbit "-diol and diacetate are due to the presence of a small proportion of some substance of high dextro-rotatory power, such as the " rabbit "-diol-*l*-glyconuric acid ($[\alpha]_D^{20°}$ +195°) or some similar conjugate. It is to be concluded that the " rat "-diol is either *d*- or *l*-1 : 2-dihydroanthracene-*cis*-1 : 2-diol whilst the " rabbit "-diol is the *dl*-1 : 2-dihydroanthracene *cis*-1 : 2-diol.

An attempt was made to support this conclusion by the preparation of acetone compounds, which are furnished by *cis*- but not *trans*-1 : 2-diols; use of anhydrous copper sulphate as condensing agent (Reichstein and von Euw, *Helv. Chim. Acta*, 1941, **24**, 401) was unsuccessful, whilst employment of acidic condensing agents was precluded by the tendency of the 1 : 2-dihydroanthracene-1 : 2-diols to undergo dehydration under the influence of dilute mineral acid.

Experimental.

(All m. ps. were thermo-electrically determined on a Kofler block and are therefore corrected; limit of error ±2°.)

d(or *l*)-1 : 2-*Dihydroanthracene*-1 : 2-*diol*.—The product obtained by crystallisation from benzene of the material from rat urine was sublimed at 140—150° (bath temp.)/<0·001 mm. in a micromolecular still, and the sublimate recrystallised from benzene to give colourless prisms which melt partly at 162°, the last crystals disappearing only at 183°, with development of a reddish colouration; $[\alpha]_D^{22°}$ −80° ±3° (*c*, 1·092 in dioxan).

The diacetate, prepared by use of acetic anhydride in pyridine at 20° and crystallised from ether–pentane, had m. p. 122°, with transformation to prisms melting at 139—143°, $[\alpha]_D^{22°}$ −240° ± 3° (*c*, 1·682 in dioxan), (cf. the constants given by Boyland and Levi, *loc. cit*). Hydrolysis with N/4-methyl alcoholic potassium hydroxide regenerated the parent diol.

dl-1 : 2-*Dihydroanthracene*-1 : 2-*diol*.—The crude crystalline product obtained from rabbit urine was sublimed at 150—160° (bath temp.)/<0·001 mm. in a micromolecular still leaving a small carbonaceous residue, and the sublimate recrystallised from benzene to yield colourless prisms, m. p. 184—186° to a reddish melt. The analysis specimen was dried at 60°/0·01 mm. and divided into two portions, one of these being used for the determination of the specific rotation; $[\alpha]_D^{22°}$ 0° ± 3° (*c*, 1·123 in dioxan) (Found: C, 78·9; H, 5·7. Calc. for $C_{14}H_{12}O_2$: C, 79·3; H, 5·65%).

Diacetate. The pure diol (30 mg.) was dissolved in dry pyridine (0·3 c.c.), acetic anhydride (0·25 c.c.) added, and the mixture kept at 20° for 16 hours. Excess of the reagents was removed in a vacuum at 35°, the residue dissolved in ether, and the ethereal solution washed successively with 2N-hydrochloric acid, 2N-sodium carbonate, and water, then dried (Na_2SO_4), and evaporated. The faintly yellow crystalline product was purified by adsorption on a column of aluminium oxide (Merck–Brockmann, activity III—IV) prepared in pentane. Elution with benzene–pentane mixtures (up to 1 : 1) furnished the diacetate, which after recrystallisation from ether–pentane formed colourless thin prisms, m. p. 121—122°. The crystals were dried at 60°/0·01 mm. and a portion used for determination of the specific rotation: $[\alpha]_D^{22°}$ 0° ± 2° (*c*, 0·991 in dioxan) (Found: C, 73·2, H, 5·2. Calc. for $C_{18}H_{16}O_4$: C, 73·0; H, 5·4%); a further portion was hydrolysed with N/4-methyl-alcoholic potassium hydroxide to regenerate the parent diol, m. p. (crude) 184° (decomp.). Indications of a polymorphic form, m. p. 131—132°, of the diacetate were observed.

A specimen of the crude crystalline diol (50 mg.) was acetylated by refluxing with dry pyridine (0·3 c.c.) and acetic anhydride (0·25 c.c.) for ¼ hour. After evaporation of excess of the reagents in a vacuum and evacuation to 0·01 mm. at 50°, the product was crystallised once from ether–pentane to give the diacetate in thin prisms, m. p. 120—122°, mixed m. p. 120—122° with the above preparation, $[\alpha]_D^{22°}$ +23° ± 4° (*c*, 2·32 in dioxan).

Oxidation with lead tetra-acetate. An N/10-solution of lead tetra-acetate in acetic acid (redistilled over chromium trioxide) (22·2 g./l.) was used; the substance to be oxidised (1/4000 mol.) was dissolved in acetic acid (10·0 c.c., redistilled over chromium trioxide) by warming and the solution cooled to 20°. N/10-Lead tetra-acetate (10·0 c.c.) was rapidly added with simultaneous swirling, and the mixture kept at 20°. At intervals from the time of mixing, aliquots of 1·00 c.c. were withdrawn and run into a sodium acetate–sodium iodide buffer solution, and the iodine so liberated was estimated using N/50 sodium thiosulphate (cf. Hockett and McClenahan, *J. Amer. Chem. Soc.*, 1937, **59**, 377).

The authors desire to acknowledge the generous support of the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund, and the Anna Fuller Fund.

The Chester Beatty Research Institute,
The Royal Cancer Hospital (Free), London, S.W. 3. [*Received, August 8th*, 1946.