228. *Polycyclic Aromatic Hydrocarbons. Part XXXVI. Synthesis of the Metabolic Oxidation Products of* 3 : 4-*Benzpyrene.*

By J. W. Cook, (Miss) R. S. Ludwiczak, and (Miss) R. Schoental.

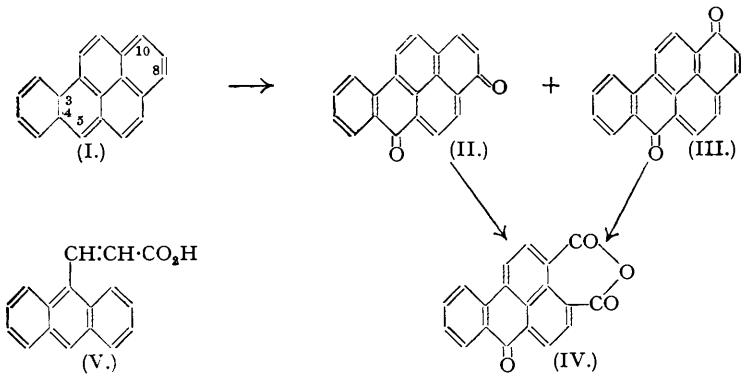
8-*Hydroxy*-3 : 4-*benzpyrene* (XVII) has been synthesised from anthracene by reactions which establish the structure of the product. These reactions represent a new type of synthesis of the 3 : 4-benzpyrene ring system. The *methyl ether* of the synthetic compound has been shown to be identical with one of two methoxybenzpyrenes formed by methylation of metabolic oxidation products of 3 : 4-benzpyrene. The second of these methoxybenzpyrenes is 10-*methoxy*-3 : 4-*benzpyrene* which has been prepared from the hydrocarbon through 5-chloro- and 5-*chloro*-10-*methoxybenzpyrene*. Oxidation of 8-hydroxy-3 : 4-benzpyrene and 10-methoxy-3 : 4-benzpyrene has given 3 : 4-*benzpyrene*-5 : 8-*quinone* (II) and 3 : 4-benzpyrene-5 : 10-quinone (III) respectively; both these quinones are formed by direct oxidation of the hydrocarbon. These reactions establish the structures of the quinones and confirm those already assigned to benzpyrene derivatives with substituents at position 10. The two quinones have been further characterised by conversion into 5 : 8- and 5 10-*dimethoxy*-3 : 4-*benzpyrenes*, which have been used to show that the corresponding quinones are present in extracts of excreta after administration of 3 : 4-benzpyrene to rodents.

When 3 : 4-benzpyrene (I), a carcinogenic hydrocarbon present in coal tar, is administered to rodents, it undergoes transformations which can be followed by changes in fluorescence (Peacock, *Brit. J. Exp. Path*, 1936, **17**, 164; *Amer. J. Cancer*, 1940, **40**, 251; Chalmers, *Biochem. J.*, 1938, **32**, 271; Weigert and Mottram, *ibid.*, 1943, **37**, 497; *Cancer Res.*, 1946, **6**, 97, 109; Weigert, *Nature*, 1945, **155**, 479). The metabolic products have not been isolated in sufficient amount for analysis and complete characterisation. Nevertheless, Chalmers and Crowfoot (*Biochem. J.*, 1941, **35**, 1270) were able to isolate a minute quantity of crystalline fluorescent material, insufficient for full purification but found to be alkali-soluble and to have micro-m. p. 190—196°. On the assumption that this was a hydroxybenzpyrene, possible positions for the hydroxyl group were suggested by *X*-ray crystallographic measurements. Further studies by Berenblum and Schoental, assisted by spectroscopic and crystallographic methods (Berenblum and Schoental, *Cancer Res.*, 1943, **3**, 145; Berenblum, Crowfoot, Holiday, and Schoental, *ibid.*, p. 151; Berenblum, Schoental, Holiday, and Jope, *ibid.*, 1946, **6**, 699), provided evidence of the presence, in the excreta of animals into which benzpyrene had been injected, of the 8- and 10-hydroxy-derivatives of 3 : 4-benzpyrene and of the 5 : 8- and 5 : 10-quinones. These quinones may arise by oxidation during working-up of the two hydroxybenzpyrenes, which have been found very susceptible to oxidation. In the case of the metabolite believed to be 8-hydroxy-3 : 4-benzpyrene the methylation product was isolated in crystalline form but its melting point was not recorded as its degree of purity was uncertain. This applied also to the product of reductive methylation of the 5 : 8-quinone. In order that these metabolic hydroxy-derivatives of benzpyrene might be adequately characterised and their identification completed their synthesis was undertaken, and the present communication describes this work. The statement made by Williams ("Detoxication Mechanisms," London, 1947, p. 54) that 8-hydroxy-3 : 4-benzpyrene was prepared synthetically by Berenblum and Schoental is a misinterpretation of the work of these authors.

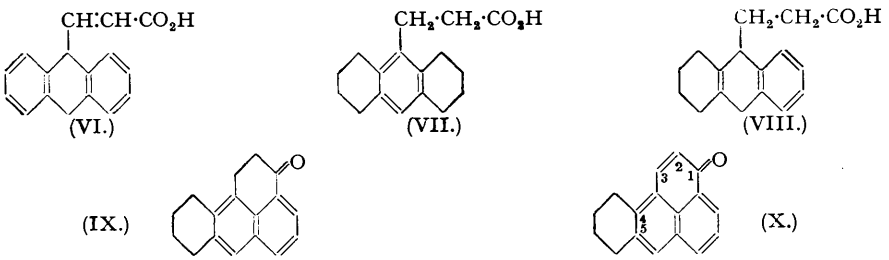
The evidence adduced by Berenblum, Schoental, *et al.* (*loc. cit.*) in support of their conclusion that the alkali-soluble metabolites of 3 : 4-benzpyrene were the 8- and 10-hydroxy-derivatives consisted, in part, of the demonstration that they differed spectroscopically from the known 5-hydroxy-3 : 4-benzpyrene but could be oxidised to the 5 : 8- and 5 : 10-quinones, respectively. These two quinones were obtained by Vollmann, Becker, Corell, and Streeck (*Annalen*, 1937, **531**, 51) by chromic acid oxidation of 3 : 4-benzpyrene (I) and the structures assigned to the red 5 : 8-quinone (II) and the yellow 5 : 10-quinone (III) were based on analogy with the 3 : 8- and 3 : 10-pyrenequinones, which are red and yellow, respectively. Moreover, the two benzpyrenequinones were each oxidised to the same *meso*benzanthrone-3 : 4-dicarboxylic anhydride (IV). This evidence from oxidation would seem to show that the two quinones have the 5 : 8- and the 5 : 10-structure but does not distinguish between them, and the evidence based on colour is inconclusive. However, the evidence from oxidation is greatly weakened by the fact that the red quinone of Vollmann *et al.* must have been heavily contaminated, for these authors give m. p. 245° for their 5 : 8-quinone whereas we have found that the pure quinone has m. p. 284°.

None of the existing methods of synthesis of 3 : 4-benzpyrene seemed adaptable to the preparation of the 8-hydroxy-derivative, and a new route was therefore devised.

9-Anthraldehyde (Fieser and Hartwell, *J. Amer. Chem. Soc.*, 1938, **60**, 2555) was condensed with malonic acid, and the product decarboxylated to β-9-anthranylacrylic acid (V)

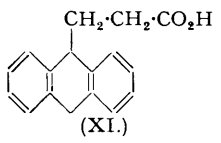


essentially as described by Davis and Carmack (*J. Org. Chem.*, 1947, **12**, 76). Reduction of the unsaturated acid (V) was studied under various conditions which mostly gave mixtures from which homogeneous acids were obtained only with difficulty. Sodium amalgam in alkaline solution gave a colourless acid, m. p. 152—154°, which appeared to be β-(9 : 10-*dihydro*-9-*anthranyl*)*acrylic acid* (VI). When its oily methyl ester was heated with palladium-black it was isomerised, and subsequent hydrolysis gave β-9-*anthranylpropionic acid*, which crystallised in yellowish prisms, m. p. 191—192°. Attempted cyclisation of this acid with anhydrous hydrogen fluoride gave a deep-red product, soluble in alkali to give a violet solution, and this was also the main product of the action of stannic chloride on the acid chloride of (VI). Hydrogenation of the *methyl* ester of anthranylacrylic acid with Raney nickel gave the *methyl* ester of the β-(9-*s*-octahydroanthranyl)propionic acid (VII) (Badger, Carruthers, Cook, and Schoental, *J.*, 1949, 169). When the acrylic acid (V) was reduced in alkaline solution with nickel–aluminium alloy (Papa *et al.*, *J. Org. Chem.*, 1942, **7**, 587) it gave an acid, m. p. 143—144·5°, which appeared to be β-(*hexahydro*-9-*anthranyl*)*propionic acid* (probably VIII). This acid, on cyclisation with hydrogen fluoride, gave a mixture of ketones from which was isolated by chromatography 4 : 5-*tetramethyleneperinaphthan*-1-*one* (IX), the orange 4 : 5-*tetramethyleneperinaphthen*-1-*one* (X) soluble in cold concentrated hydrochloric acid (cf. Cook and Hewett, *J.*, 1934, 365), and 1′-keto-9 : 10-*cyclo*penteno-*s*-octahydrophenanthrene which Badger *et al.* (*loc. cit.*) have shown to be formed under these conditions from octahydroanthranylpropionic acid (VII). This acid is probably formed as an intermediate by disproportionation of (VIII), together with the tetrahydroanthranylpropionic acid which would lead to (IX) and then, by dehydrogenation, to (X).

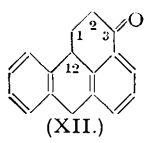


The method of reduction which eventually proved to be admirably suited to our purpose was treatment of the acrylic acid (V) with sodium and boiling amyl alcohol. The principal product, readily obtained pure through its sparingly soluble sodium salt, was β-(9 : 10-*dihydro*-9-*anthranyl*)*propionic acid* (XI). This was cyclised by hydrogen fluoride to 3-*keto*-1 : 2 : 3 : 12-*tetrahydro*meso*benzanthrene* (XII), the structure of which was confirmed by its oxidation by chromic acid to anthraquinone-1-carboxylic acid. For the introduction of the three-carbon chain necessary for the construction of the fifth ring of the molecule the ketone (XII) was condensed with ethyl succinate in presence of potassium *tert.*-butoxide. This Stobbe reaction led to the *half-ester* (XIII), from which the corresponding *dicarboxylic acid* was

obtained by hydrolysis. Riegel and Burr (*J. Amer. Chem. Soc.*, 1948, **70**, 1070) showed that analogous half-esters are partly decarboxylated and also lactonised by hydrobromic acid in boiling acetic acid. However, when our half-ester (XIII) was submitted to this treatment decarbethoxylation was accompanied by disproportionation, so that the product was a mixture of meso*benzanthrene*-3-β-*propionic acid* (XIV) and its tetrahydro-derivative-A (XV). The benzanthrene acid (XIV), which had an ultra-violet absorption spectrum very similar to that of *meso*benzanthrene (Fig. 1), was oxidised to meso*benzanthrone*-3-β-*propionic acid* and then to anthraquinone-1-carboxylic acid. The benzanthrone acid with anhydrous hydrogen fluoride gave ill-defined products, and the benzanthrone acid was mostly unattacked. Reduction of the benzanthrene acid (XIV) with sodium and boiling amyl alcohol gave a mixture of 1 : 2 : 3 : 12-tetrahydro*meso*benzanthrene-3-β-propionic acid-A (XV), m. p. 185°, and the stereoisomeric *acid-B*, m. p. 180—181°. These were separated by taking advantage of the sparing solubility of the sodium salt of acid-B, and the two acids clearly differ from one another in the configurations of carbon atoms 3 and 12.



(XI.)



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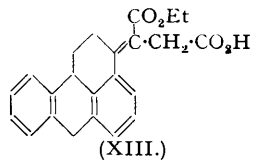

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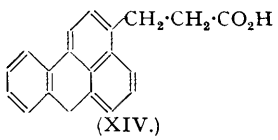
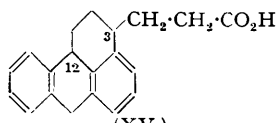
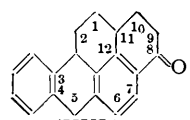


(XIV.)



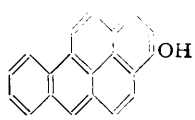
(XV.)

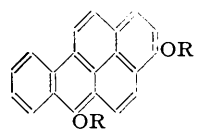


(XVI.)

Cyclisation of the stereoisomeric acids (A and B) (XV) by hydrogen fluoride gave 8-*keto*-1 : 2 : 5 : 8 : 9 : 10 : 11 : 12-*octahydro*-3 : 4-*benzpyrene-A* and -*B* (XVI), respectively. These ketones had almost identical ultra-violet absorption spectra (Fig. 3), and the presence of the ring-system of 3 : 4-benzpyrene was established by reduction of the ketone-A to a liquid hydrocarbon which was dehydrogenated to 3 : 4-benzpyrene by heating it with palladium-black. By means of palladium-black in boiling 1-methylnaphthalene both ketones, A and B (XVI), were dehydrogenated to 8-*hydroxy*-3 : 4-*benzpyrene* (XVII), characterised as its *methyl ether* and *acetate*.

(XVII.)





(XVIII.)

Oxidation of 8-hydroxy-3 : 4-benzpyrene with sodium dichromate in acetic acid gave pure 3 : 4-benzpyrene-5 : 8-quinone (II), identical with the red quinone formed as one of the products of direct oxidation of the hydrocarbon (see above). (II) was characterised by reductive methylation to 5 : 8-*dimethoxy*- (XVIII; R = Me) and by reductive acetylation to 5 : 8-*diacetoxy*-3 : 4-*benzpyrene* (XVIII; R = Ac).

The synthesis of 8-hydroxybenzpyrene by a series of reactions which establishes the structure of the product, and its oxidation to Vollmann's 5 : 8-quinone, confirm not only the structures assigned to the two benzpyrenequinones, but also the orientation of a series of benzpyrene derivatives which have been related to the 5 : 10-quinone (cf. Fieser and Hershberg, *J. Amer. Chem. Soc.*, 1939, **61**, 1565).

Except in the Friedel–Crafts reaction, substitution in 3 : 4-benzpyrene has been shown to take place at the 5-position. A second substituent appears to enter mainly or wholly at the 10-position (Fieser and Hershberg, *loc. cit.*). It seemed, therefore, that the second, and less abundant, hydroxybenzpyrene formed by biochemical oxidation of the hydrocarbon might be obtained chemically from 3 : 4-benzpyrene after the 5-position had been blocked by a removable substituent. This has proved to be the case. Windaus and Raichle (*Annalen*, 1939, **537**, 157) showed that benzpyrene is chlorinated by sulphuryl chloride to give 5-chloro-3 : 4-benzpyrene. By increasing the proportion of sulphuryl chloride we have obtained not only this monochloro-compound, which is the main product, but also two isomeric *dichloro*-compounds and a

trichloro-compound. Oxidation of the monochlorobenzpyrene with lead tetra-acetate led to partial conversion into 5-chloro-10-acetoxy-3 : 4-benzpyrene. This was not isolated, the crude product being hydrolysed with alkali and the phenol then methylated with methyl sulphate. Subsequent chromatography led to the isolation, in small yield, of 5-*chloro*-10-*methoxy*-3 : 4-*benzpyrene*, which was dechlorinated by catalytic hydrogenation to 10-*methoxy*-3 : 4-*benzpyrene*. This was compared with the methylated second metabolite of benzpyrene and was indistinguishable from it (Berenblum, Schoental, Holiday, and Jope, *Cancer Res.*, 1946, **6**, 699). Oxidation gave 3 : 4-benzpyrene-5 : 10-quinone (III), identical with that separated from the mixture obtained by oxidation of the hydrocarbon, and this quinone was further characterised by reductive methylation to 5 : 10-*dimethoxy*-3 : 4-*benzpyrene*.

Spectroscopic Data.

Ultra-violet-absorption measurements, kindly made by Dr. E. Clar, were used to supplement the chemical evidence of structure of some of the intermediates obtained in the synthesis of 8-methoxy-3 : 4-benzpyrene and to assist in establishing the identity of this compound with the product of methylation of one of the metabolic hydroxybenzpyrenes.

Fig. 1. Fig. 2.

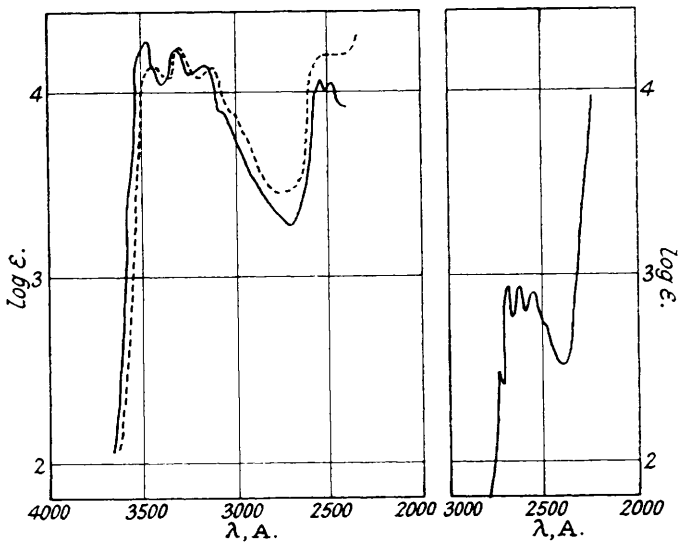


Fig. 1.—*Absorption spectra of* meso*benzanthrene*-3-β-*propionic acid* (XIV) ———, *and* meso*benzanthrene* .....

Fig. 2.—*Absorption spectrum of the sodium salt of* 1 : 2 : 3 : 12-*tetrahydro*meso*benzanthrene*-3-β-*propionic acid*-A (XV).

The structure of a *meso*benzanthrene derivative (XIV) assigned to one of the acids formed by the action of hydrobromic acid on (XIII) is confirmed by the comparison given in Fig. 1 of the absorption spectra (in ethanol) of *meso*benzanthrene and the acid in question, maxima for (XIV) being :

λ, A.	3470	3310	3160	2565	2480
log ε	4·26	4·22	4·15	4·06	4·03

Fig. 2 gives the spectrum of the sodium salt (in water) of the second acid (XV) formed by the action of hydrobromic acid on (XIII). The curve is benzenoid in character and is consistent with the presence of two isolated benzene rings. Maxima are :

λ, A.	2720	2670	2610	2550
log ε	2·50	2·97	2·96	2·94

The absorption spectra of the two stereoisomeric ketones (XVI-A and -B) (in ethanol) are shown in Fig. 3, with the maxima at 2980 (log ε 3·46), 2850 (log ε 3·65), and 2670 A. (log ε 4·25).

Fig. 4 gives the curve for synthetic 8-methoxy-3 : 4-benzpyrene (in ethanol). There is agreement in the positions of the maxima of the main bands with those recorded by Holiday and Jope (*Cancer Res.*, 1946, **6**, 704) for the crystalline methylated metabolite of benzpyrene (in hexane) (p. 1121) :

λ, A.	4225	3985	3825	3630	3460	3070	2920	2680	2590
log ε	4·37	4·28	4·58	4·24	3·88	4·78	4·63	4·74	4·72

We have also measured the wave-lengths of the maxima of the fluorescence bands of the four compounds named in the following table and have found them respectively identical with re-determined values of the fluorescence maxima of (*a*) the methylated metabolite obtained by Berenblum and Schoental (*ibid.*, p. 699) from rabbit fæces, (*b*) the methylated metabolite

FIG. 3. FIG. 4.

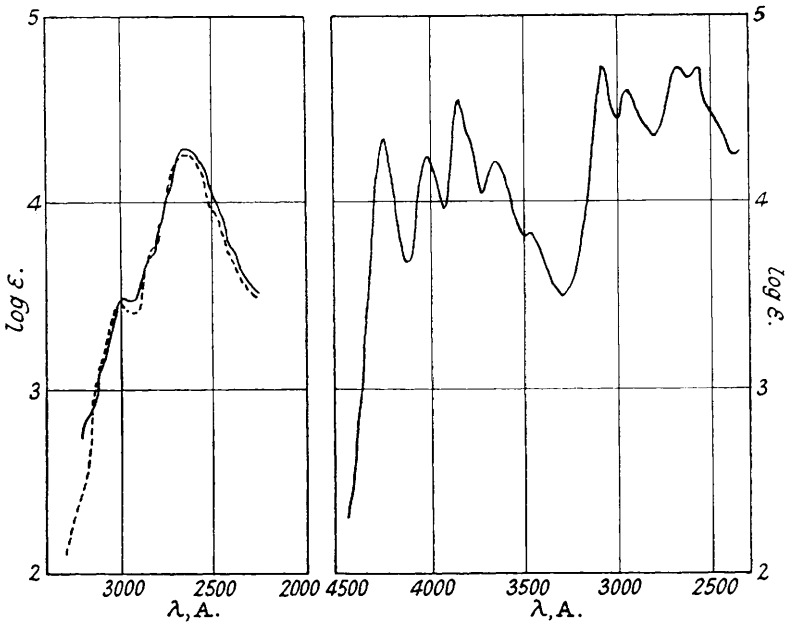


FIG. 3.—*Absorption spectra of stereoisomeric* 8-*keto*-1 : 2 : 5 : 8 : 9 : 10 : 11 : 12-*octahydro*-3 : 4-*benzpyrenes* (XVI): *isomer A* ———; *isomer B* .....

FIG. 4.—*Absorption spectrum of* 8-*methoxy*-3 : 4-*benzpyrene.*

obtained by Berenblum *et al.* (*ibid.*, 1943, **3**, 145) from fæces of rats and mice, (*c*) the product obtained by reductive methylation of the yellow metabolic quinone, and (*d*) the product similarly obtained from the red metabolic quinone (cf. p. 1121). The values given in the table were obtained with solutions in light petroleum (b. p. 60—80°) and differ somewhat from those recorded by Berenblum and Schoental (*loc. cit.*) for solutions in liquid paraffin. This latter solvent causes a shift towards the red.

3 : 4-Benzpyrene derivative.	Maxima of main bands.			Maxima of subsidiary bands.	
10-Methoxy-	4125	4370	4620 *	4175	4430
8-Methoxy-	4230	4470	4750 *	4280	4530
5 : 10-Dimethoxy-	4220	4460	4740 *	4270	4520
5 : 8-Dimethoxy-	4330	4580 *	4900 *	4370	—

* These were diffuse bands so that the values are less accurate.

EXPERIMENTAL.

β-9-*Anthranylacrylic Acid* (V).—9-Anthraldehyde (Fieser and Hartwell, *J. Amer. Chem. Soc.*, 1938, **60**, 2555) was condensed with malonic acid under conditions essentially the same as those which have since been reported by Davis and Carmack (*loc. cit.*) which, however, resulted in recovery of 40% of unchanged aldehyde. The substituted malonic acid was decarboxylated by boiling its solution in acetic anhydride (10 parts) for 2 hours. With quantities of 1 g. this gave the acrylic acid (V) in yields up to 70%, but on a larger scale the yields were smaller (*e.g.*, 40 g. of crude substituted malonic acid

gave 20 g. of the acrylic acid). The acrylic acid had m. p. 247°, in agreement with Davis and Carmack. Its *methyl* ester, prepared with ethereal diazomethane, formed canary-yellow needles (from light petroleum), m. p. 112—113° (Found: C, 82·4; H, 5·3; OMe, 12·1. $C_{18}H_{14}O_2$ requires C, 82·5; H, 5·3; OMe, 11·8%). The mother-liquors from the acrylic acid contained other acids which were partly separated by crystallisation of their methyl esters, but products of undoubted homogeneity were not isolated.

β-(9 : 10-Dihydro-9-anthranyl)acrylic Acid (VI).—A solution of the acrylic acid (V) (2·5 g.) in 1·6% aqueous sodium hydroxide (200 c.c.) was shaken with sodium amalgam (from 1·8 g. of sodium and 160 g. of mercury) until it was decolourised. Repeated crystallisation of the resulting acid from aqueous methanol gave the *dihydroanthranylacrylic acid* (VI) as colourless rhombic prisms, m. p. 152—154° (Found: C, 81·6; H, 5·6. $C_{17}H_{14}O_2$ requires C, 81·6; H, 5·6%). A sample of the methyl ester, which did not crystallise, was heated under nitrogen with palladium-black at 260° for an hour, and the product was hydrolysed with methanolic potassium hydroxide. Acidification of an aqueous solution of the resulting potassium salt gave *β-9-anthranylpropionic acid*, which crystallised from benzene in pale yellow prisms, m. p. 191—192° (Found: C, 81·4; H, 5·9. $C_{17}H_{16}O_2$ requires C, 81·6; H, 5·6%), and gave a *methyl* ester which formed yellowish needles (from methanol), m. p. 75—76° (Found: C, 81·5; H, 6·05; OMe, 12·2. $C_{18}H_{16}O_2$ requires C, 81·8; H, 6·1; OMe, 11·7%). Hydrolysis of the ester gave an acid of m. p. 191—192°. A small amount of anthraquinone was also formed in the rearrangement with palladium-black.

Attempted cyclisation of anthranylpropionic acid with anhydrous hydrogen fluoride (procedure of Fieser and Hershberg, *J. Amer. Chem. Soc.*, 1939, **61**, 1272) gave a reddish-purple product which was strongly adsorbed on alumina from benzene and only partly eluted from the column by ethanol. It gave a violet solution in aqueous alkali and a deep-red solution in concentrated sulphuric acid, which characteristics are recorded for 1-hydroxy*meso*benzanthrone (Maki and Nagai, *J. Soc. Chem. Ind. Japan* [Suppl.], 1934, **37**, 213B). A similar product was formed by the action of stannic chloride on the chloride prepared by the action of phosphorus pentachloride on a benzene solution of dihydroanthranylacrylic acid (VI). By repeated chromatography on alumina a small amount of a *by-product* was isolated in this case. It formed yellow needles (from acetone), m. p. 135—136° (Found: C, 87·3; H, 6·0. $C_{17}H_{14}O$ requires C, 87·2; H, 6·0%).

Hydrogenation of Methyl β-9-Anthranylacrylate.—(*a*) A suspension of this methyl ester (2 g.) in ethanol (120 c.c.) was shaken with hydrogen and platinum oxide (150 mg.) containing 10% of palladium at room temperature. After 20 hours 230 c.c. of hydrogen had been absorbed and the ester had dissolved but the solution remained yellow. The oil remaining after evaporation of the solvent was hydrolysed. The resulting mixture of acids was fractionally crystallised into anthranylacrylic acid (V) and anthranylpropionic acid, m. p. 191°.

(*b*) A solution of methyl anthranylacrylate (2·5 g.) in ethanol (150 c.c.) was hydrogenated at 100 atmospheres below 100°, in presence of Raney nickel. Concentration of the colourless solution gave colourless needles, m. p. 54—55°, of *methyl β-(s-octahydro-9-anthranyl)propionate* (Found: C, 79·5; H, 8·6; OMe, 10·85. $C_{18}H_{24}O_2$ requires C, 79·4; H, 8·8; OMe, 11·4%). The acid formed by hydrolysis of this ester had m. p. 167—168°, alone or mixed with the octahydroanthranylpropionic acid (VII) of Badger *et al.* (*loc. cit.*).

β-(Hexahydro-9-anthranyl)propionic Acid (VIII).—A solution of anthranylacrylic acid (25 g.) in 10% aqueous potassium hydroxide (500 c.c.) was heated on the water-bath and stirred mechanically while, during 2—3 hours, nickel–aluminium alloy (100 g.) was added. Reduction decolorised the solution, which was filtered into 20% hydrochloric acid. The precipitate was collected, dried, and recrystallised several times from benzene–light petroleum. The resulting *hexahydroanthranylpropionic acid* (probably VIII), m. p. 142—144°, was further purified through its *methyl* ester, colourless rhombic plates (from methanol), m. p. 45—46° (Found: C, 80·2; H, 8·2. $C_{18}H_{22}O_2$ requires C, 80·0; H, 8·1%). The acid obtained by hydrolysis of the ester formed colourless prisms, m. p. 143—144·5° (Found: C, 79·9; H, 7·7. $C_{17}H_{20}O_2$ requires C, 79·7; H, 7·8%).

The analytical figures for both the acid and the ester are in excellent agreement with those required for hexahydroanthracene derivatives, and the products formed by cyclisation probably arise by prior disproportionation by means of hydrogen fluoride to tetrahydro- and octahydro-anthranylpropionic acids (cf. Johnson, Johnson, and Petersen, *J. Amer. Chem. Soc.*, 1945, **67**, 1360). Even so, it is surprising that reduction should be arrested at the hexahydro-stage especially in view of the recent finding of Papa *et al.* (*J. Org. Chem.*, 1949, **14** 366) that substituted naphthalenes are reduced by this method to tetrahydronaphthalenes. We cannot completely exclude the possibility that our hexahydroanthranyl propionic acid is largely tetrahydro-acid contaminated with some of the octahydro-acid.

For cyclisation, the foregoing acid, m. p. 142—144° (8 g.), was added to anhydrous hydrogen fluoride (150 c.c.), the solution was kept overnight, and the hydrogen fluoride then allowed to evaporate. The residue was freed from acidic material by means of sodium carbonate solution, and the neutral products separated by repeated chromatography on alumina, using benzene–light petroleum mixtures as solvents. The following represents approximately increasing degrees of adsorption of the materials: (i) The first eluates gave 1′-keto-9 : 10-*cyclo*penteno-*s*-octahydrophenanthrene, m. p. 196—197°, which Badger *et al.* (*loc. cit.*) have shown is formed by the action of hydrogen fluoride on *s*-octahydroanthranylpropionic acid. (ii) A ketone which formed almost colourless thick rhombic crystals (from benzene), m. p. 131—132° (Found: C, 86·3; H, 6·7. $C_{17}H_{16}O$ requires C, 86·4; H, 6·8%); this is evidently 4 : 5-*tetramethyleneperinaphthan*-1-*one* (IX); its crimson 2 : 4-*dinitrophenylhydrazone* had m. p. 247° (decomp.) (Found: C, 66·2; H, 4·9. $C_{23}H_{20}O_4N_4$ requires C, 66·4; H, 4·8%). (iii) Pinkish fluffy needles, m. p. 116°, probably not homogeneous. (iv) Yellow crystals, m. p. *ca.* 120—122°. (v) A compound which crystallised from benzene in stout orange needles, m. p. 147—148°; this was readily obtained pure by making use of its solubility in cold concentrated hydrochloric acid, from which the compound was recovered by dilution with water; it is accordingly considered to be 4 : 5-*tetramethyleneperinaphthen*-1-*one* (X) (Found C, 87·4; H, 6·1. $C_{17}H_{14}O$ requires C, 87·2; H, 6·0%). (vi) Strongly adsorbed purple material.

β-(9 : 10-*Dihydro*-9-*anthranyl*)*propionic Acid* (XI).—Sodium (17 g.) was added during 4½ hours to a gently boiling solution of anthranylacrylic acid (V) (12·4 g.) in amyl alcohol (300 c.c.). The alcohol was then removed in steam and the alkaline solution boiled with charcoal, filtered, and cooled. Colourless glistening scales of the *sodium* salt of (XI) crystallised (13 g.) and were recrystallised from boiling water (Found : loss of weight, at 105°, 19·3%. $C_{17}H_{15}ONa,4H_2O$ requires H_2O, 20·8%). Acidification of a solution of this salt precipitated *β*-(9 : 10-*dihydro*-9-*anthranyl*)*propionic acid* (XI) which formed colourless rhombs (from benzene), m. p. 139—140° (Found : C, 81·3; H, 6·3. $C_{17}H_{16}O_2$ requires C, 81·0; H, 6·35%). The *methyl* ester, prepared by the Fischer–Speier method, formed colourless transparent rhombs (from methanol), m. p. 64·5—65·5° (Found : C, 80·9; H, 6·7. $C_{18}H_{18}O_2$ requires C, 81·2; H, 6·8%).

Unidentified reduction products were also formed to a minor extent and remained in solution when the above sodium salt crystallised.

3-*Keto*-1 : 2 : 3 : 12-*tetrahydro*meso*benzanthrene* (XII).—Cyclisation of the acid (XI) (1 g.) with hydrogen fluoride was carried out in the usual way (18 hours' storage) and gave a homogeneous product which was freed from traces of coloured impurity by passing its solution in benzene through a column of alumina. The *ketone* (XII) (0·7 g.) formed thick prisms with a cream-coloured tinge (from benzene–light petroleum), m. p. 131—132° (Found : C, 86·95; H, 6·1. $C_{17}H_{14}O$ requires C, 87·2; H, 6·0%). The m. p. was strongly depressed when mixed with the ketone, m. p. 131—132°, to which the structure (IX) has been assigned. The 2 : 4-*dinitrophenylhydrazone* of (XII) formed deep-red needles (from xylene), m. p. 240° (decomp.) (Found : C, 66·95; H, 4·4; N, 13·5. $C_{23}H_{18}O_4N_4$ requires C, 66·7; H, 4·4; N, 13·5%).

A solution of the ketone (XII) (0·5 g.) in acetic acid (3 c.c) was treated with sodium dichromate (2·3 g.) in acetic acid (25 c.c.) and heated, first at 100° for 2 hours and then at the boil for ½ hour. The resulting anthraquinone-1-carboxylic acid crystallised from concentrated nitric acid in yellow needles, m. p. 293—294° (decomp.), and gave a methyl ester, m. p. 189°, in agreement with recorded values.

*Stobbe Reaction with Ketotetrahydro*meso*benzanthrene* (XII).—The ketone (4·3 g.) was condensed with ethyl succinate (7 g.) by means of potassium (0·95 g.) in *tert.*-butyl alcohol (12·9 c.c.) as described by Riegel and Burr (*J. Amer. Chem. Soc.*, 1948, **70**, 1070) for an analogous case, the reaction mixture being heated under reflux in a nitrogen atmosphere for 4 hours. While still warm, the mixture was treated with dilute hydrochloric acid (4 c.c. of concentrated acid, 20 c.c. of water) and kept overnight. The solvent was removed by distillation under reduced pressure. The residual brown oil was dissolved in ether, and the acidic product was extracted with dilute ammonia solution. Acidification of the ammoniacal solution, followed by extraction with ether and then evaporation, gave a reddish-brown oil (7·1 g.) which crystallised after addition of a little benzene. Recrystallisation from benzene–light petroleum gave the *half-ester* (XIII) as colourless crystals, m. p. 115—116° (Found : C, 76·4; H, 5·9; OEt, 11·9. $C_{23}H_{22}O_4$ requires C, 76·2; H, 6·1; OEt, 12·4%). Hydrolysis with aqueous sodium hydroxide gave the corresponding *dicarboxylic acid*, m. p. 208—209° (from ethyl acetate–light petroleum) (Found : C, 75·7; H, 5·3. $C_{21}H_{18}O_4$ requires C, 75·4; H, 5·4%).

meso*Benzanthrene*-3-*β*-*propionic Acid* (XIV) *and* 1 : 2 : 3 : 12-*Tetrahydro*meso*benzanthrene*-3-*β*-*propionic Acid-A* (XV).—A suspension of the half-ester (XIII) (4 g.) in acetic acid (12 c.c.), 48% hydrobromic acid (8 c.c.), and water (4 c.c.) was boiled gently for 6½ hours. The molten half-ester was gradually replaced by a solid. After cooling, this (3·05 g.) was collected, more (0·45 g.) being obtained by diluting the filtrate with water and then extracting with ether. The crude products of decarbethoxylation and rearrangement (3·5 g.) were extracted with boiling benzene (150 c.c.). The insoluble portion, m. p. 202—203° (1·65 g.), was twice recrystallised from ethanol and gave colourless prisms (0·9 g.) of meso*benzanthrene*-3-*β*-*propionic acid* (XIV), m. p. 218—219° (Found : C, 83·2, 83·3; H, 5·2, 5·6. $C_{20}H_{16}O_2$ requires C, 83·3; H, 5·6%). The *methyl* ester formed long colourless needles (from benzene–light petroleum), m. p. 104·5—105·5° (Found : C, 83·5; H, 6·1. $C_{21}H_{18}O_2$ requires C, 83·4; H, 6·0%). This benzanthrene acid gave the same reddish-violet colour with concentrated sulphuric acid as does *meso*benzanthrene.

Oxidation of the acid (XIV) (0·2 g.) with sodium dichromate (0·34 g.) in acetic acid (20 c.c.) at 100° (5 minutes) gave deep-yellow crystals (0·2 g.) of meso*benzanthrone*-3-*β*-*propionic acid*, m. p. 232—233° (Found : C, 78·85; H, 5·0. $C_{20}H_{14}O_3$ requires C, 79·45; H, 4·7%). The *methyl* ester formed yellow needles (from ethanol), m. p. 127—128° (Found : C, 79·9; H, 5·2. $C_{21}H_{16}O_3$ requires C, 79·7; H, 5·1%). Further oxidation of the benzanthrone acid with excess of alkaline permanganate at 80—90° gave anthraquinone-1-carboxylic acid, identified by m. p.s and mixed m. p.s of the acid and its methyl ester.

The benzene extract from which the benzanthrenepropionic acid had been separated was concentrated under reduced pressure and gave crystals which after several recrystallisations from ethanol formed colourless prisms (0·85 g.), m. p. 185°, of 1 : 2 : 3 : 12-*tetrahydro*meso*benzanthrene*-3-*β*-*propionic acid-A* (XV) (Found : C, 82·1; H, 6·9. $C_{20}H_{20}O_2$ requires C, 82·2; H, 6·9%). This acid gave a yellow solution in concentrated sulphuric acid and formed a *methyl* ester which crystallised from ethanol in clumps of colourless needles, m. p. 67—68° (Found : C, 82·35; H, 7·3. $C_{21}H_{22}O_2$ requires C, 82·3; H, 7·2%).

Other acids appeared to be present in small amount in the liquors from which (XIV) and (XV) were isolated, but these were not identified.

Reduction of meso*Benzanthrene*-3-*β*-*propionic Acid* (XIV).—A boiling solution of this acid (1·15 g.) in amyl alcohol (70 c.c.) was reduced by addition, during 2 hours, of sodium (2·3 g.). The alcohol was removed in steam, and the alkaline solution was boiled with charcoal and filtered. On storage, a sparingly soluble sodium salt crystallised; the acid obtained from this was crystallised from benzene–light petroleum. The resulting 1 : 2 : 3 : 12-*tetrahydro*meso*benzanthrene*-3-*β*-*propionic acid-B* (XV) formed colourless hexagonal plates (0·25 g.), m. p. 180—181°, depressed to 156—160° on admixture with its stereoisomer-A (Found : C, 82·1; H, 7·0%). The methyl ester did not crystallise.

The alkaline filtrate from the sparingly soluble sodium salt was acidified and the precipitated acid was recrystallised several times from benzene–light petroleum. It then had m. p. 183° (yield, 0·24 g.) and was shown by mixed m. p. to be 1 : 2 : 3 : 12-tetrahydro*meso*benzanthrene-3-*β*-propionic acid-A.

8-*Keto*-1 : 2 : 5 : 8 : 9 : 10 : 11 : 12-*octahydro*-3 : 4-*benzpyrene-A*.—Tetrahydro*meso*benzanthrenepropionic

acid-A (1·8 g.) was cyclised with hydrogen fluoride in the usual way. A solution of the product in benzene was passed through a column of alumina. Coloured impurities were adsorbed in narrow bands at the top of the column; these showed brown, yellowish-green, and red fluorescence colours in ultra-violet light. The main slightly yellow zone had a blue fluorescence. This was eluted with benzene, and the solution concentrated and treated with light petroleum. The resulting yellowish crystals (1·1 g.) were recrystallised from ethanol and 8-*keto*-1 : 2 : 5 : 8 : 9 : 10 : 11 : 12-*octahydro*-3 : 4-*benzpyrene-A* (XVI) was obtained as colourless rods, m. p. 156° (Found : C, 87·7; H, 6·55. $C_{20}H_{18}O$ requires C, 87·6; H, 6·6%). Its 2 : 4-*dinitrophenylhydrazone* formed bright red needles (from xylene), m. p. 274° (decomp.) (Found : C, 68·9; H, 5·0; N, 12·5. $C_{26}H_{22}O_4N_4$ requires C, 68·7; H, 4·9; N, 12·3%).

8-*Keto*-1 : 2 : 5 : 8 : 9 : 10 : 11 : 12-*octahydro*-3 : 4-*benzpyrene*-B.—This was similarly obtained by the action of hydrogen fluoride on tetrahydro*meso*benzanthrenepropionic acid-B (0·2 g.). The *ketone-B* (XVI) (0·15 g.) formed colourless prismatic needles (from benzene–light petroleum), m. p. 185·5° (Found : C, 87·8; H, 6·6%), and gave a 2 : 4-*dinitrophenylhydrazone* which crystallised from xylene in bright red needles, m. p. 281—282° (decomp.) (Found : C, 69·2; H, 4·8; N, 12·4%).

3 : 4-*Benzpyrene* (I).—A mixture of ketone-A (XVI) (0·2 g.), potassium hydroxide (0·15 g.), 90% hydrazine hydrate (0·1 c.c.), and diethylene glycol (1 c.c.) was boiled under reflux for 1½ hours. The water was then drained from the condenser and the temperature allowed to rise to 195—200°, heating under reflux being then continued for 3 hours. The reaction mixture was cooled, diluted with water, and extracted with benzene. The benzene solution was passed through a column of alumina, and the solvent evaporated. The residual oil, which did not crystallise, was heated in a nitrogen atmosphere at 300—320° for an hour with a fifth of its weight of palladium-black. The product was dissolved in benzene, and the filtered solution concentrated and treated with light petroleum. 3 : 4-Benzpyrene (0·1 g.) crystallised and after recrystallisation from benzene–light petroleum formed yellow needles, m. p. 175°, alone or mixed with an authentic specimen. Its *s*-trinitrobenzene complex formed red needles, m. p. 225—226°, not depressed on admixture with an authentic specimen of m. p. 226—227° (Fieser and Hershberg, *J. Amer. Chem. Soc.*, 1938, **60**, 1664).

8-*Hydroxy*-3 : 4-*benzpyrene* (XVII).—A solution of keto-octahydrobenzpyrene-A or -B (XVI) (0·2 g.) in 1-methylnaphthalene (2 c.c.) was boiled under reflux with palladium-black (0·03 g.) for 7 hours, in an atmosphere of nitrogen. After being cooled, the solution was kept overnight in a nitrogen atmosphere. The yellow needles which crystallised were collected and washed with benzene–light petroleum. The yield was almost theoretical. After recrystallisation from benzene–light petroleum, 8-*hydroxy*-3 : 4-*benzpyrene* formed small yellow needles, m. p. 226—227° (decomp.), in an evacuated sealed capillary (Found : C, 89·7; H, 4·7. $C_{20}H_{12}O$ requires C, 89·5; H, 4·5%). The micro-m. p. on a heated microscope stage was 207°, after previous softening (cf. Chalmers and Crowfoot, *loc. cit.*).

The hydroxy-compound, dissolved in ethanol, was treated with excess of ethereal diazomethane. The product was purified by passing its benzene solution through a column of alumina. The main yellow zone on the column had a strong blue fluorescence. 8-*Methoxy*-3 : 4-*benzpyrene* formed transparent yellow prisms (from benzene), m. p. 182—183° (Found : C, 89·5; H, 4·7. $C_{21}H_{14}O$ requires C, 89·3; H, 5·0%). It formed a *picrate*, which crystallised from benzene in almost black needles, m. p. 207—208° (decomp.) (Found : C, 63·8; H, 3·7; N, 8·2. $C_{21}H_{14}O,C_6H_3O_7N_3$ requires C, 63·4; H, 3·35; N, 8·2%), and a *s-trinitrobenzene* complex, long dark reddish-brown needles (from benzene), m. p. 213—214° (decomp.) (Found : C, 65·5; H, 3·5; N, 8·6. $C_{21}H_{14}O,C_6H_3O_6N_3$ requires C, 65·5; H, 3·5; N, 8·5%).

The hydroxybenzpyrene was dissolved in acetic anhydride and the solution treated with a few drops of pyridine. Yellow crystals soon began to separate. After storage overnight these were collected and recrystallised from benzene. The *acetate* formed golden-yellow prisms, m. p. 169—170° (Found : C, 85·3; H, 4·55. $C_{22}H_{14}O_2$ requires C, 85·1; H, 4·55%).

3 : 4-*Benzpyrene*-5 : 8-*quinone* (II).—A solution of 8-hydroxybenzpyrene (0·12 g.) and sodium dichromate (0·12 g.) in acetic acid was heated at 90° for 5 minutes. Dilution with water gave a bright red precipitate of the *quinone* (II) which, after recrystallisation from acetic acid, formed red crystals (0·1 g.), m. p. 283—284° (decomp.) (Found : C, 85·1; H, 3·6. $C_{20}H_{10}O_2$ requires C, 85·1; H, 3·6%). It gave a green solution in concentrated sulphuric acid. Vollmann, Becker, Corell, and Streeck (*Annalen*, 1937, **531**, 130) gave m. p. 245° for the compound which they regarded as this quinone. Analytical figures were not quoted. 3 : 4-Benzpyrene was therefore oxidised as described by these workers, the crude mixture of quinones (1·2 g.) was dissolved in tetrachloroethane, and the quinones were adsorbed by passing the solution through a column of alumina. Development of the chromatogram with benzene led to partial separation. The upper portion was deep-red and the lower portion deep-yellow, but the bands merged into one another and complete separation could not be effected by development with solvent. The column was therefore cut, and the red and the yellow portion separated and eluted with chloroform. The fractions so obtained were each re-submitted to similar treatment and the process repeated several times. In this way the 5 : 8-quinone was obtained as red needles, micro-m. p. 291° (decomp.), and the 5 : 10-quinone as yellow rosettes of needles, micro-m. p. 288° (decomp.). The red quinone did not depress the m. p. of the quinone prepared by oxidation of 8-hydroxy-3 : 4-benzpyrene. For the compound which they regarded as the 5 : 10-quinone, Vollmann *et al.* gave m. p. 295°. This was also isolated by Winterstein and Vetter (*Z. physiol. Chem.*, 1934, **230**, 169) by chromatography of the crude mixture of quinones formed by oxidation of benzpyrene. These authors described the quinone as orange-yellow needles, m. p. 292—293° (corr.).

5 : 8-*Dimethoxy*-3 : 4-*benzpyrene* (XVIII; R = Me).—The red quinone, formed by oxidation of (XVII) was treated with zinc dust, concentrated sodium hydroxide solution, and methyl sulphate, and the mixture boiled for some time. The solution was diluted and then extracted with benzene, and the benzene extract was passed through a column of alumina. Concentration of the filtrate, followed by addition of light petroleum, gave yellow rhombic crystals of 5 : 8-*dimethoxy*-3 : 4-*benzpyrene*, m. p. 226—227° (Found : C, 84·8; H, 5·2. $C_{22}H_{16}O_2$ requires C, 84·6; H, 5·2%). The *s-trinitrobenzene* complex formed brownish-red needles (from benzene), m. p. 221·5—222° (Found : C, 64·0; H, 3·65; N, 8·5. $C_{22}H_{16}O_2,C_6H_3O_6N_3$ requires C, 64·0; H, 3·65; N, 8·0%).

5 : 8-*Diacetoxy*-3 : 4-*benzpyrene* (XVIII; R = Ac).—The red quinone obtained by oxidation of 8-hydroxybenzpyrene was suspended in acetic anhydride and treated with zinc dust and a drop of concentrated sulphuric acid. The solution was boiled for ½ hour, cooled, and treated with water. After decomposition of the acetic anhydride the *diacetate* was extracted with benzene, from which it separated after concentration in elongated yellow prisms, m. p. 215—216° (Found: C, 78·0; H, 5·0. $C_{24}H_{18}O_4$ requires C, 77·8; H, 4·9%). Vollmann *et al.* (*loc. cit.*) gave m. p. 204° and did not record analytical figures.

Chlorination of 3 : 4-*Benzpyrene*.—Windaus and Raichle (*Annalen*, 1939, **537**, 168) obtained 5-chloro-3 : 4-benzpyrene by the action of sulphuryl chloride on the hydrocarbon, and we have confirmed their results although with the proportions of reactants which they recommend much benzpyrene remains unattacked. When double the amount of sulphuryl chloride was used, more highly chlorinated derivatives were also formed: A solution of benzpyrene (2 g.) in dry freshly distilled carbon tetrachloride (80 c.c.) was treated dropwise with sulphuryl chloride (2 c.c.) and the solution heated on the water-bath for 6 hours. After being cooled and kept overnight the solid which had separated was collected, washed with carbon tetrachloride, and leached with hot benzene. The insoluble residue was recrystallised several times from chlorobenzene and finally from a large volume of benzene. This gave *trichloro*-3 : 4-*benzpyrene* as pale yellow threads, m. p. 305—306° (Found: Cl, 29·9. $C_{20}H_9Cl_3$ requires Cl, 29·9%).

Evaporation of the benzene leachings gave a residue which was extracted with hot ethanol. Concentration of the extract gave long pale yellow threads which, after crystallisation from benzene had m. p. 219—220° and were found to consist of a *dichloro*-3 : 4-*benzpyrene* (Found: C, 74·5; H, 3·4; Cl, 21·85. $C_{20}H_{10}Cl_2$ requires C, 74·8; H, 3·1; Cl, 22·1%). The residue undissolved by ethanol was crystallised several times from chlorobenzene, and then from chloroform. Yellowish rhombic plates, m. p. 275°, were obtained, consisting essentially of an isomeric *dichloro*-3 : 4-*benzpyrene* (Found: Cl 24·95%). 5-Chloro-3 : 4-benzpyrene (1 g.), m. p. 210°, was recovered from the original carbon tetrachloride liquors and also from the ethanolic liquors after crystallisation of the dichlorobenzpyrene, m. p. 220°.

5-*Chloro*-10-*methoxy*-3 : 4-*benzpyrene*.—A solution of 5-chloro-3 : 4-benzpyrene (2 g.) in benzene (100 c.c.) was treated, on the water-bath, with a solution of lead tetra-acetate (15 g.) in acetic acid (50 c.c.), added in portions during 40 minutes. To the cold mixture water was added, and the benzene layer was separated, washed, and concentrated under reduced pressure. The residual solid was extracted with warm sodium methoxide solution. Most of the material remained undissolved and consisted of unaltered chlorobenzpyrene. The cooled extract was treated with methyl sulphate and kept overnight. Water was then added, the methylated material extracted with benzene, and the solution submitted to chromatography on alumina. There were coloured zones of strongly adsorbed material, followed successively by dark yellow, pale yellow, and greenish-yellow zones, and then an almost colourless zone which showed a violet fluorescence. The latter was eluted with benzene and proved to be unchanged chlorobenzpyrene. The greenish-yellow zone, which was also strongly fluorescent, was eluted and the eluate re-chromatographed from benzene-light petroleum (1 : 3). The fluorescent zone was cut and eluted with benzene, and the solid obtained by concentration was recrystallised several times from light petroleum. 5-*Chloro*-10-*methoxy*-3 : 4-*benzpyrene* (30 mg.) formed yellow rods, m. p. 209—210° (Found: C, 79·6; H, 4·35. $C_{21}H_{13}OCl$ requires C, 79·6; H, 4·1%). From the coloured zone of the original chromatogram there were obtained, by further chromatography and crystallisation from benzene, small amounts of (*a*) deep yellow needles, m. p. 287—288°, consisting of 3 : 4-benzpyrene-5 : 10-quinone, (*b*) red needles, m. p. 287—291·5°, shown to consist of 3 : 4-benzpyrene-5 : 8-quinone, and (*c*) a 5-*chloro*-3 : 4-*benzpyrenequinone* which crystallised from benzene in deep purple needles, m. p. 331—333° (Found: C, 75·8; H, 3·4. $C_{20}H_9O_2Cl$ requires C, 75·8; H, 2·8%).

10-*Methoxy*-3 : 4-*benzpyrene*.—A solution of 5-chloro-10-methoxy-3 : 4-benzpyrene (50 mg.) in purified xylene (5 c.c.) was heated under reflux with palladium-black (prepared as described by Willstätter and Waldschmidt-Leitz, *Ber.*, 1921, **54**, 123) (5 mg.) and a slow stream of purified hydrogen was bubbled through the solution. After an hour evolution of hydrogen chloride had ceased, and the filtered solution was concentrated *in vacuo* and chromatographed on alumina from benzene–light petroleum. The central part of the fluorescent zone was cut and eluted with benzene, and the solution concentrated and treated with light petroleum. 10-*Methoxy*-3 : 4-*benzpyrene* (10 mg.) crystallised in pale yellow rods, m. p. 143—144° (Found: C, 89·6; H, 5·0. $C_{21}H_{14}O$ requires C, 89·4; H, 5·0%). Its s-*trinitrobenzene* complex formed reddish-brown needles (from benzene), m. p. 190° (decomp.) (Found: C, 65·85; H, 3·5; N, 8·4. $C_{21}H_{14}O,C_6H_3O_6N_3$ requires C, 65·5; H, 3·5; N, 8·5%).

5 : 10-*Dimethoxy*-3 : 4-*benzpyrene*.—10-Methoxybenzpyrene was oxidised with sodium dichromate in hot acetic acid. The resulting 3 : 4-benzpyrene-5 : 10-quinone, purified by chromatography on alumina, formed yellow needles, m. p. 286°, not depressed by admixture with the yellow quinone isolated from the products of oxidation of benzpyrene. Reductive methylation, with zinc dust, alkali, and methyl sulphate, gave a fluorescent product which, after chromatographic purification, had the same fluorescence spectrum as 5 : 10-*dimethoxy*-3 : 4-*benzpyrene* which was similarly prepared from a specimen of quinone separated from the oxidation products of the hydrocarbon. It formed golden-yellow rods, m. p. 192—193° (Found: C, 84·75; H, 5·1. $C_{22}H_{16}O_2$ requires C, 84·6; H, 5·2%), and gave a s-*trinitrobenzene* complex as brown needles, m. p. 225—227° (decomp.) (Found: C, 64·5; H, 3·85. $C_{22}H_{16}O_2,C_6H_3O_6N_3$ requires C, 64·1; H, 3·65%).

Preparation of Derivatives of Metabolic Oxidation Products of 3 : 4-*Benzpyrene*.—The publications of Berenblum, Schoental, *et al.* (*loc. cit.*) were concerned with the extraction from the excreta of animals of metabolic products of benzpyrene, and their characterisation by spectroscopic and crystallographic means. The following supplementary notes relate to the more complete chemical characterisation of these metabolites.

The accumulated metabolite from fæces of rats and mice, after methylation with methyl sulphate in presence of alkali (Berenblum and Schoental, *Cancer Res.*, 1943, **3**, 151), was submitted to chromatography on alumina, with light petroleum containing 10% of benzene as solvent. The column,

after development with this solvent, was cut and the middle portion of the fluorescent zone was eluted with benzene. Vacuum-concentration of the eluate was followed by crystallisation. The crystals were washed with a few drops of light petroleum (b. p. 60—80°) and then recrystallised from this solvent. The resulting yellow rhombic needles had micro-m. p. 179—181°, determined in Oxford in 1945. A re-determination in Glasgow in 1949 gave micro-m. p. 173—175°, and the mixed micro-m. p. with synthetic 8-methoxy-3 : 4-benzpyrene, of micro-m. p. 176—177°, was 173—176°. The ultra-violet-absorption spectrum of this crystalline material was determined, in hexane, by Holiday and Jope (*ibid.*, 1946, **6**, 704). The positions of the maxima of the main bands agree with those found by Dr. Clar for synthetic 8-methoxy-3 : 4-benzpyrene, in ethanol solution (Fig. 4).

The red quinone isolated from the excreta of rats and mice was purified by chromatography and recrystallised from acetic acid. Reductive methylation in the usual way led to a product which crystallised from benzene–light petroleum in golden-yellow needles, micro-m. p. 220°, alone or mixed with synthetic 5 : 8-dimethoxy-3 : 4-benzpyrene, which had micro-m. p. 224—225°.

The second methylated metabolite, prepared from the fæces of rabbits to which benzpyrene had been administered (Berenblum and Schoental, *ibid.*, p. 699) has already been shown by Holiday and Jope to have an ultra-violet-absorption spectrum closely resembling that of 10-methoxy-3 : 4-benzpyrene, the synthesis of which is now recorded. These workers also showed that the spectrum of the compound obtained by reductive methylation of the yellow quinone from rabbit excreta resembles closely the spectrum of the 5 : 10-dimethoxy-3 : 4-benzpyrene described in this communication.

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