12. *Catalysed Hydrogen Peroxide Oxidation of Aromatic Hydrocarbons. Part I.*

By J. W. Cook and (Miss) R. Schoental.

A study has been made of the products formed by oxidation of a series of aromatic hydrocarbons (benzene, naphthalene, phenanthrene, anthracene, 1 : 2-benzanthracene, 1 : 2-5 : 6-dibenzanthracene, chrysene, pyrene, and 3 : 4-benzpyrene) with Milas's reagent (hydrogen peroxide in *tert.*-butanol, catalysed by osmium tetroxide). The reagent was diluted with acetone to promote solubility of the polycyclic hydrocarbons, which are sparingly soluble in *tert.*-butanol; the presence of acetone appeared to have no significant influence on the course of the reaction. The products isolated included a *cis*-diol (in the case of phenanthrene and 1 : 2-benzanthracene), quinones, and acids formed by ring-fission.

1 : 2-Benzanthracene and 1 : 2-5 : 6-dibenzanthracene gave not only the 9 : 10-quinones but also the 3 : 4-quinones (XII and XIV) and in spite of the relatively low yields the method furnishes a convenient method of preparation of these *ortho*-quinones. Benzene gave, together with other products, *allo*mucic acid, an interesting example of a simple and direct route from an aromatic hydrocarbon to a sugar acid.

The mechanism of these transformations is discussed, and attention is drawn to their bearing on the mode of biochemical oxidation of the hydrocarbons, for which an exact parallel by purely chemical methods has yet to be found.

The authors have shown (Cook and Schoental, *J.*, 1948, 170; *Nature*, 1948, **161**, 237) that oxidation of polycyclic aromatic hydrocarbons with Criegee's osmium tetroxide reagent (osmium tetroxide in benzene–pyridine, followed by hydrolysis of the resulting osmic ester) leads to smooth perhydroxylation with formation of homogeneous α-glycols. From the work of Criegee (*Annalen*, 1936, **522**, 75; 1942, **550**, 99) these were clearly *cis*-α-glycols. In nearly every case the positions attacked were different from those at which oxidation is effected by other reagents. The products of osmium tetroxide oxidation were similar in type to some of the compounds which have been isolated from the excreta of animals to which the hydrocarbons were administered (Boyland and Levi, *Biochem. J.*, 1935, **29**, 2679; Boyland and Shoppee, *J.*, 1947, 801; Boyland and Wolf, *Biochem. J.*, 1948, **42**, xxxii; Booth and Boyland, *ibid.*, 1947, **41**, xxix; 1949, **44**, 361). Such metabolic glycols are probably intermediates in the formation of the phenols which have been isolated in other cases (see, *e.g.*, Weigert and Mottram, *Cancer Res.*, 1946, **6**, 109; Berenblum and Schoental, *ibid.*, p. 699; *Biochem. J.*, 1949, **44**, 604). There are, however, important differences between the osmium tetroxide oxidations and the biochemical oxidations. Usually the positions in the molecules at which oxidation occurs are different in the two cases. Moreover, evidence is accumulating that the metabolic diols which have been isolated are *trans*-α-glycols. The experiments now recorded were initiated in an endeavour to find a reagent

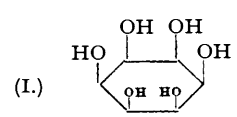
which would provide a closer parallel to the biochemical perhydroxylations of aromatic hydrocarbons.

As long ago as 1912, Dakin (" Oxidations and Reductions in the Animal Body," New York) pointed out that hydrogen peroxide, alone of all the known chemical oxidising agents, could bring about the same types of oxidation of a variety of compounds as do enzyme systems, and the widespread occurrence of catalase and peroxidases is a pointer to the importance of hydrogen peroxide in some types of biochemical oxidation. The oxidation of aromatic hydrocarbons by hydrogen peroxide was first reported by Leeds (*Ber.*, 1881, **14**, 975, 1382), who obtained phenol and oxalic acid from benzene, β-naphthol from naphthalene, and anthraquinone from anthracene. In recent years knowledge of the variety of conditions under which oxidations by hydrogen peroxide, and by peroxides and per-acids, can be effected has been widely extended (see, *e.g.*, Waters, *Ann. Reports*, 1945, **42**, 145) and the reaction mechanisms have been extensively studied. It was decided, therefore, to re-examine the oxidation of these hydrocarbons by hydrogen peroxide under varying conditions, and also to examine some more complex polycyclic hydrocarbons which are of interest on account of their carcinogenic activity. The present communication deals mainly with oxidations by hydrogen peroxide in *tert.*-butanol in presence of osmium tetroxide as catalyst. For comparison, some preliminary attention has also been devoted to oxidation by hydrogen peroxide alone, and by hydrogen peroxide catalysed by light, by chloride ions, and by haematin.

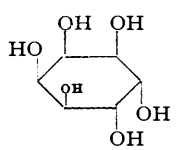
The osmium tetroxide reagent was introduced by Milas and Sussman (*J. Amer. Chem. Soc.*, 1936, **58**, 1302), who showed that olefins are not attacked by a solution of anhydrous hydrogen peroxide in *tert.*-butanol at room temperature until osmium tetroxide is added, whereupon they become oxidised to α-glycols. This reagent was found unsatisfactory in many of the cases which we studied on account of the low solubility of the polycyclic hydrocarbons in *tert.*-butanol. Acetone was therefore used as a solvent in conjunction with the Milas reagent. Treibs (*Ber.*, 1939, **72**, 7) had successfully used acetone as a solvent in catalysed hydrogen peroxide oxidations, but under the conditions which he used he did not apparently observe the formation of the cyclic peroxide of acetone (Wolffenstein, *Ber.*, 1895, **28**, 2265) which we regularly encountered. This appeared to have no significant influence on the course of the reaction. Parallel experiments on the oxidation of benzene by the Milas reagent led to the isolation of the same products, both in presence and in absence of acetone.

Results.

Benzene.—The hydroxylation of benzene by hydrogen peroxide in *tert.*-butanol catalysed by osmium tetroxide has already been reported by Milas and Sussman (*J. Amer. Chem. Soc.*, 1937, **59**, 2345) who obtained phenol in 23% yield. A somewhat higher yield (30%) of phenol was obtained by Milas (*ibid.*, p. 2342) when vanadium pentoxide was used as the catalyst. We also observed the formation of phenol when benzene was treated with the osmium tetroxide catalysed reagent, but if the experiment was continued for several months with excess of hydrogen peroxide a deposit separated, and this was identified as *allo*mucic acid; from the mother-liquors there were isolated also *meso*tartaric and oxalic acids. The transformation of benzene into a sugar acid is noteworthy. It is uncertain whether phenol is an intermediate or whether benzene becomes fully hydroxylated to a hexahydroxy*cyclo*hexane (I or II) which then undergoes ring-



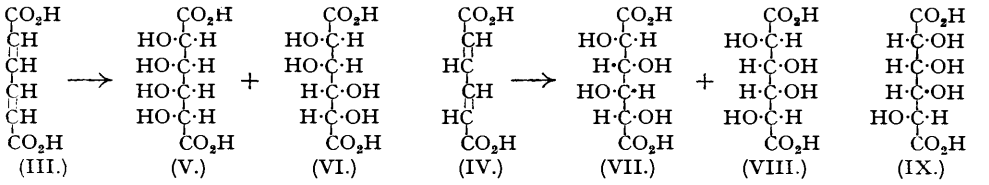
(I.)



(II.)

fission. This is a point which we are examining. Böeseken and Engelberts (*Proc. Akad. Wetensch. Amsterdam*, 1931, **34**, 1292; 1932, **35**, 750; compare Böeseken and Metz, *Rec. Trav. chim.*, 1935, **54**, 345) have shown that oxidation of phenol by peracetic acid gives, with other products, a 35% yield of *cis-cis*-muconic acid (III). *cis*-Hydroxylation of the double bonds of this acid should give a mixture of *allo*mucic (V) and mannosaccharic (VI) acids [compare Behrend and Heyer, *Annalen*, 1919, **418**, 294, who obtained idosaccharic (VII) and mucic (VIII) acids by oxidation of *trans-trans*-muconic acid (IV) with sodium chlorate in presence of osmium tetroxide]. If a hexahydroxy*cyclo*hexane is formed in our conversion of benzene into *allo*mucic acid then this probably has the completely *cis*-configuration (I). The alternative configuration (II) which could arise from *cis*-addition in pairs of hydroxyl groups to the benzenoid double bonds (see

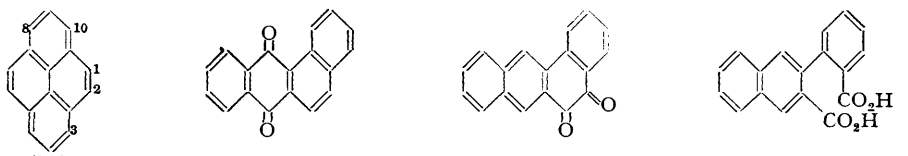
later) could give, on ring-fission, not only *allo*mucic acid (V) but also mucic (VIII), DL-manno-saccharic (VI and enantiomorph), and DL-talomucic (IX and enantiomorph) acids, and there was no indication of the formation of such a complex mixture of hexahydroxyadipic acids.



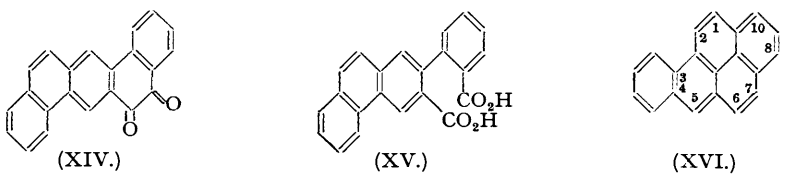
This degradation of benzene by oxidation with the Milas reagent is of interest in connection with the metabolic conversion of benzene into *trans-trans*-muconic acid (IV), which is possibly an artefact formed by isomerisation of the *cis-cis*-acid (III) which should be the primary product of ring fission (Jaffé, *Z. physiol. Chem.*, 1909, **62**, 58; Drummond and Finar, *Biochem. J.*, 1938, **32**, 79; Bernhard and Greesley, *Helv. Chim. Acta*, 1941, **24**, 83). Such isomerisation is known to be readily effected (Grundmann and Trischmann, *Ber.*, 1936, **69**, 1755).

Naphthalene, Phenanthrene, Chrysene, Anthracene, and Pyrene.—The products formed from these hydrocarbons by oxidation with the Milas reagent call for little comment. In the case of naphthalene the only product isolated was phthalic acid, also obtained by Charrier and Moggi (*Gazzetta*, 1927, **57**, 736) by oxidation with hydrogen peroxide in an acid medium. Less extensive degradation occurs when naphthalene, α- or β-naphthol, or β-naphthaquinone is oxidised with peracetic acid, *o*-carboxy*allo*cinnamic acid being formed in each case (Böeseken and Slooff, *Rec. Trav. chim.*, 1930, **49**, 100; Böeseken and von Königsfeldt, *ibid.*, 1935, **54**, 313).

Phenanthrene gave *cis*-9 : 10-dihydroxy-9 : 10-dihydrophenanthrene, phenanthraquinone, and diphenic acid. Charrier and Moggi (*loc. cit.*) had obtained diphenic acid by oxidising phenanthrene with hydrogen peroxide in boiling acetic acid, a result which we confirmed. Chrysene was oxidised to 1 : 2-chrysaquinone, and anthracene to 9 : 10-anthraquinone, which are the normal products of oxidation with chromic acid. Pyrene (X) was rapidly attacked and after about a week the products were separated into a mixture of quinones which appeared to be the 3 : 8- and 3 : 10-quinones which are the normal oxidation products. When the action of the Milas reagent was prolonged for a year the product was naphthalene-1 : 4 : 5 : 8-tetracarboxylic acid, characterised as its *methyl* ester. There was no evidence of attack at the 1 : 2-positions of pyrene, which are the positions exclusively attacked when pyrene is treated with osmium tetroxide in benzene–pyridine (Cook and Schoental, *loc. cit.*).



1 : 2-*Benzanthracene and* 1 : 2-5 : 6-*Dibenzanthracene.*—After a few days 1 : 2-benzanthracene gave the 3 : 4-quinone (XII) (isolated in 15% yield) and the 9 : 10-quinone (XI) (isolated in 20% yield). The *o*-quinone (XII), which is conveniently prepared in this way, is evidently formed through the intermediary of *cis*-3 : 4-dihydroxy-3 : 4-dihydro-1 : 2-benzanthracene (Cook and Schoental, *loc. cit.*), a small amount of which was isolated by chromatography. When 1 : 2-benzanthracene was heated on the water-bath with the reagent acidic products were formed more rapidly than in the cold. Phthalic acid and *2-phenylnaphthalene-3 : 2'-dicarboxylic acid* (XIII) were isolated, and there was also evidence of the formation of keto-acids.



With 1 : 2-5 : 6-dibenzanthracene the reaction took a similar course. On account of the low solubility of the hydrocarbon a more dilute solution was used, and the reaction was very

sluggish at room temperature. When the reactants were heated on the water-bath the dark red 1 : 2-5 : 6-dibenzanthra-3 : 4-quinone (XIV) separated after a few days and was isolated in 35% yield. From the liquors the 9 : 10-quinone was also isolated and, in addition, 2-phenylphenanthrene-3 : 2'-dicarboxylic acid (XV), which was also obtained directly from the *o*-quinone (XIV) by oxidation with alkaline hydrogen peroxide.

3 : 4-*Benzpyrene* (XVI).—This was readily oxidised and the solution became deep-red. A mixture of quinones was then separated from other products of oxidation by chromatography on alumina. These quinones did not account wholly for the intensity of colour (noted also in the case of some of the other hydrocarbons). Possibly the high colour intensity was due to formation of hydroxylated quinones. Warren (*Biochem. J.*, 1943, **37**, 338), who studied the atmospheric oxidation of aqueous acetone solutions of 3 : 4-benzypyrene in presence of ascorbic acid, also observed the formation of deeply coloured products in addition to known quinones.

Chromatography of the quinone fraction from benzpyrene resulted in only partial separation and the individual constituents were not isolated. Evidence was obtained, however, of the presence of the known 5 : 8- and 5 : 10-quinones (Vollmann *et al.*, *Annalen*, 1937, **531**, 1) and of a new *o*-quinone. The latter is probably 3 : 4-*benzpyrene*-6 : 7-*quinone*, which was prepared for comparative purposes by oxidation of the dihydroxydihydro-3 : 4-benzpyrene of Cook and Schoental (*loc. cit.*), and characterised by formation of a *quinoxaline* by reaction with *o*-phenylenediamine. The diol is believed to be the 6 : 7-diol, although the alternative 1 : 2-structure is not excluded (see numbering on formula XVI).

Oxidation of 1 : 2-*Benzanthracene with Hydrogen Peroxide in Absence of Osmium Tetroxide.*—These experiments were carried out for comparison with the action of the Milas reagent. A solution of the hydrocarbon in acetone and *tert.*-butanol containing hydrogen peroxide remained unchanged for several weeks when kept in the dark. If methylamine hydrochloride was also present (used as a source of chloride ions) then oxidation took place and after two months a considerable proportion of the hydrocarbon had been oxidised to 1 : 2-benzanthra-9 : 10-quinone (XI) and phthalic acid. There was no evidence of the formation of the 3 : 4-quinone (XII). A similar result was obtained when haematin was used in place of methylamine hydrochloride.

Discussion.

The results of this investigation are mainly of interest on account of the additional evidence which they furnish as to the mechanism of oxidation by the Milas reagent, and they also perhaps provide one further span towards bridging the gap between biochemical and purely chemical oxidation of the aromatic hydrocarbons. Our earlier results with Criegee's osmium tetroxide reagent showed that it is possible to bring about selective chemical attack at positions in the molecules of polycyclic aromatic hydrocarbons which are not normally the most reactive. They also showed how it is possible to simulate the " perhydroxylation " reactions which are a characteristic feature of several of the biochemical oxidations. The positions attacked by osmium tetroxide are not those at which biochemical hydroxylation takes place, but in this connection it has been postulated that combination between the hydrocarbon and the oxidising enzyme may immobilise some of the more reactive centres in the hydrocarbon molecule.*

The diols and the related phenols which have been isolated in metabolic experiments represent only a small proportion of the hydrocarbon administered, and it is clear that most of the material undergoes more extensive degradation. Except in the case of benzene (see above) the products of this degradation have not been identified. Using 1 : 2-5 : 6-dibenzanthracene containing isotopic carbon, Heidelberger and his collaborators (*Cancer*, 1948, **1**, 252, 261) have shown that acids are formed by ring-fission of the hydrocarbon and also that some of the material is completely oxidised to carbon dioxide. Metabolic studies by one of us (R. S.) with chrysene, 1 : 2-benzanthracene, 1 : 2-5 : 6-dibenzanthracene, and 3 : 4-benzpyrene have likewise indicated that in all these cases acidic degradation products of the hydrocarbons are present in the excreta of the animals. To this extent oxidation by the Milas reagent imitates the metabolic oxidation, for prolonged treatment with the reagent always led to extensive conversion of the hydrocarbons into acidic degradation products, only some of which have been identified.

There remains the question as to how far the chemical perhydroxylations really follow the

* Berenblum and Schoental (*Cancer Res.*, 1943, **3**, 686) pointed out that in the cases of 1 : 2-benzanthracene and 1 : 2-5 : 6-dibenzanthracene the positions of the molecules where metabolic oxidation occurs are those at which sulphonation takes place *in the corresponding quinones*. This has been misinterpreted by Neish (*Biochem. J.*, 1948, **43**, 534) who states, incorrectly, that Berenblum and Schoental observed that benzanthracene and dibenzanthracene are metabolically attacked at points where sulphonation *of these hydrocarbons* occurs.

pattern of the corresponding biochemical processes. Osmium tetroxide oxidation of olefins and of phenanthrene has been shown by Criegee (*loc. cit.*) to give *cis*-diols through the intermediary of cyclic osmic esters. It is quite conceivable that metallic catalysts could function in a similar way in biochemical " perhydroxylation." But if it can be shown that one method of oxidation consistently gives *cis*-diols and the other *trans*-diols, then it is a reasonable inference that the reaction mechanisms are different. It is for this reason that great importance attaches to the determination of the configurations of the metabolic diols, and as already stated, evidence is accumulating that these are *trans*-isomers. An alternative possibility is that biochemical perhydroxylation is effected by free hydroxyl radicals, and there is much current opinion in favour of the view that free radicals play an important role in biological oxidations (see Waters, " Chemistry of Free Radicals," Oxford, 1946, p. 259; LuValle and Goddard, *Quart. Review Biol.*, 1948, **23**, 197).

Milas (*J. Amer. Chem. Soc.*, 1937, **59**, 2342) was inclined to regard oxidation by osmium tetroxide-catalysed hydrogen peroxide as involving attack by free hydroxyl radicals, formed by the action of perosmic acid on hydrogen peroxide, as in its dissociation by light (compare Spring, *Ann. Reports*, 1943, **40**, 108). That free hydroxyl radicals can oxidise benzene to phenol was shown by Stein and Weiss (*Nature*, 1948, **161**, 650; cf. Loebl, Stein, and Weiss, *J.*, 1949, 2074), who found phenol among the products of the action of *X*-rays on water and benzene. To this extent the Milas reagent produces the same result as free hydroxyl radicals. Moreover, Milas, Kurz, and Anslow (*J. Amer. Chem. Soc.*, 1937, **59**, 543) observed the oxidation of maleic acid to *meso*tartaric acid by hydrogen peroxide in presence of light and attributed this to addition of free hydroxyl radicals. This *cis*-addition rather than the *trans*-addition which might have been expected by such a mechanism excited no comment. Recently, Merz and Waters (*J.*, 1949, S 15) have shown that free hydroxyl radicals, produced by the action of ferrous salts on aqueous hydrogen peroxide, do not add to the double bond of maleic and fumaric acids. Hence the photochemical oxidation of maleic acid is not to be ascribed to the action of free hydroxyl radicals.

The same type of argument may be adduced to show that the perhydroxylation of double bonds by hydrogen peroxide in presence of osmium tetroxide is not due to the action of free hydroxyl radicals. It is true that in the Milas reagent part of the hydrogen peroxide is present as *tert.*-butyl hydroperoxide (compare Milas and Surgenor, *J. Amer. Chem. Soc.*, 1946, **68**, 205; Criegee and Dietrich, *Annalen*, 1948, **560**, 135), and that an alkyl hydroperoxide has been shown to break down to give free radicals (Robertson and Waters, *Trans. Faraday Soc.*, 1946, **42**, 201; *J.*, 1948, 1578). But the Milas reagent does not normally bring about the extensive degradations which Merz and Waters (*loc. cit.*) observed with free hydroxyl radicals; it does effect perhydroxylation of the double bonds in compounds of the maleic acid type, and moreover the method leads to *cis*-addition to the double bond (Milas and Sussman, *J. Amer. Chem. Soc.*, 1937, **59**, 2345; Milas, Sussman, and Mason, *ibid.*, 1939, **61**, 1844). It is clear, in fact, that the oxidation involves the intermediate formation of cyclic osmic esters, as in the Criegee method in which such esters are isolated.

Our own results with polycyclic hydrocarbons show that in part oxidation at a " potential " double bond takes place by the same mechanism. The isolation of *cis*-α-diols from phenanthrene and 1 : 2-benzanthracene, with no evidence of the presence of stereoisomeric *trans*-diols, points clearly to the intermediate formation of cyclic osmic esters. These diols undoubtedly represent the first stage also in the reactions which lead to 1 : 2-benzanthra-3 : 4-quinone (XII) and the acid (XIII) from 1 : 2-benzanthracene and the allied compounds (XIV) and (XV) from 1 : 2-5 : 6-dibenzanthracene. Moreover, the quite appreciable amounts of these products isolated indicate that the reactions by this route represent a considerable proportion of the whole. However, quite a different mechanism must operate in the oxidation of anthracene, 1 : 2-benzanthracene, and 1 : 2-5 : 6-dibenzanthracene to the 9 : 10-quinones (*e.g.*, XI), and in the oxidation of pyrene and 3 : 4-benzpyrene to their heteronuclear quinones. These oxidations may be due to the action of free hydroxyl radicals formed by dissociation of hydrogen peroxide, but there is no evidence bearing on this point, and judgment must be reserved. We are extending this work to study the effect on polycyclic hydrocarbons of free hydroxyl radicals and also of hydrogen peroxide in the presence of other catalysts. In this connection, it may be noted that Sequin (*Compt. rend.*, 1943, **216**, 667) obtained only the *trans*-α-glycol by oxidising *cyclo*hexene with hydrogen peroxide in acetone, in presence of selenium dioxide. A similar result was reported by Treibs (*Angew. Chem.*, 1939, **52**, 698) with pervanadic acid as catalyst.

Experimental.

General.—A solution of the hydrocarbon (0·001 g.-mol.) in acetone was treated with a stock solution of hydrogen peroxide (2—10 c.c.), prepared by diluting 90% hydrogen peroxide to 20—40% with *tert.*-butanol, and with a 1—2% solution of osmium tetroxide in *tert.*-butanol, so that the final concentration of the tetroxide varied between 0·01% and 0·5%. The solution was kept in a stoppered flask at room temperature in diffused light. Addition of the *tert.*-butanol solutions usually resulted in partial separation of the crystalline hydrocarbons, but the crystals redissolved in the course of the reaction. The colourless solutions slowly developed yellow or red colours of increasing intensity. There was usually an induction period when highly purified material was used. After the hydrogen peroxide initially added had been consumed the osmium tetroxide became reduced so that the solution became almost black. This colour was discharged by addition of more hydrogen peroxide solution. The rapidity with which this darkening of colour occurred furnished an index of the rate of oxidation of the hydrocarbons except in the cases of pyrene and 3 : 4-benzpyrene when very dark oxidation products were formed and the dark colour then persisted for some months. In view of the complex mixtures of oxidation products formed and the difficulty of isolating individual constituents a quantitative study was not attempted. Yields are recorded only when reaction products actually crystallised from solution.

For isolation of the products, any solid which had separated was collected, and the filtrate concentrated under reduced pressure at 40—50°. By this means solvents, osmium tetroxide, and volatile products were removed. Colourless crystals which collected in the still-head and condenser were identified as the trimeric cyclic peroxide of acetone, m. p. 94—95° (Wolffenstein, *loc. cit.*). Attempts to isolate the products without this preliminary concentration resulted in formation of dark colloidal suspensions of lower oxides of osmium, and as far as could be judged the distillation process did not bring about any alteration in the oxidation products.

The residue after distillation was a yellow or brownish viscous oil, which was treated with dilute sodium hydrogen carbonate solution and extracted with benzene. Dark resinous material remained insoluble in both phases, but dissolved when sodium hydroxide solution was added, after removal of the sodium hydrogen carbonate layer. Acidification of the dark sodium hydroxide extracts gave brownish material which did not crystallise and gave no crystalline derivatives by reductive methylation or acetylation.

The benzene extracts were submitted to chromatography on alumina and were thus separated into unchanged hydrocarbon, diol (in two cases), and quinones. In all cases highly coloured zones of strongly adsorbed material were visible on the upper part of the column, but pure products could not be obtained after these were eluted with acidified alcohol.

The sodium hydrogen carbonate extract was acidified, and the resulting acids separated by fractional crystallisation from various solvents or by vacuum distillation of their methyl esters, formed by treatment with ethereal diazomethane.

Benzene.—Two parallel experiments were made. In one, the reaction was carried out in acetone solution, as in the other cases recorded in this paper. In the other, *tert.*-butanol alone was used as solvent. Oxidation took place more rapidly in acetone solution, but in both cases the same products were isolated. The reaction mixtures, prepared in each case by using 10 c.c. of benzene, became yellow in a few days and phenol was detected by its smell, by the preparation of tribromophenol, m. p. 93°, from a sample of distillate, and by the blue colour given with 2 : 6-dichloroquinonechloroimide in neutral or alkaline solution (Porteous and Williams, *Biochem. J.*, 1949, **44**, 56).

The colour of the solution of reactants deepened on storage, but after some months, during which several additions of hydrogen peroxide solution were made, the colour became pale yellow and a white precipitate was formed. This (35 mg.) was recrystallised from hot water and formed a microcrystalline powder which decomposed at 197°. Analysis showed the compound to be a tetrahydroxyadipic acid (Found : C, 34·0; H, 5·0. Calc. for $C_6H_{10}O_8$: C, 34·3; H, 4·8%). Its ethyl ester, prepared by heating it under reflux with ethanol containing a few drops of concentrated hydrochloric acid, had m. p. *ca.* 150°. These data are in approximate agreement with those given for *allo*mucic acid by Posternak (*Helv. Chim. Acta*, 1935, **18**, 1283) (acid, m. p. 197—198°; ethyl ester, m. p. 153—154°) and by Lapsley, Robertson, and Patterson (*J.*, 1940, 862) (acid, m. p. 199—200°; ethyl ester, m. p. 155°). Through the kindness of Mr. J. Robertson, who generously gave us a sample of *O*-tetra-acetyl*allo*mucic acid prepared by the method described by himself and his collaborators, we have been able to establish that our oxidation product is in fact *allo*mucic acid. The methyl ester of our acid had m. p. 170° and gave an *O*-tetra-acetyl derivative, m. p. 204—206°. The m. p.s of the acid and its three derivatives were not depressed by mixing with the acid (prepared by acid hydrolysis of the authentic *O*-tetra-acetyl*allo*mucic acid) and its corresponding derivatives. On the other hand, a mixture of the methyl ester of the tetra-acetate of our acid and methyl *O*-tetra-acetylmucate, m.p. 196—197° (Simon and Guillaumin, *Compt. rend.*, 1924, **179**, 1324), melted at 180—196°. Pure *methyl* allo*mucate* formed transparent prisms (from methanol), m. p. 175° after softening (Found : C, 40·6; H, 5·9. $C_8H_{14}O_8$ requires C, 40·3; H, 5·9%). *Methyl O-tetra-acetyl*allo*mucate*, prepared from methyl *allo*mucate by acetylation with acetic anhydride, formed transparent rhombic prisms, m. p. 205—206° (Found : C, 47·3; H, 5·6. $C_{16}H_{22}O_{12}$ requires C, 47·3; H, 5·5%).

In another experiment, a mixture of benzene (10 c.c.) and acetone (10 c.c.) was treated with 2% osmium tetroxide (2 c.c.) and 40% hydrogen peroxide (60 c.c.), both in *tert.*-butanol. After nine months the filtered solution was concentrated under reduced pressure at 40—50°. A solution in water of the residual oil was partly neutralised with sodium carbonate and the solution heated under reflux for 2½ hours with *p*-bromophenacyl bromide (0·5 g.) in ethanol (10 c.c.). After concentration and cooling the resulting *p*-bromophenacyl ester was recrystallised from ethanol and formed glistening leaflets, m. p. 198—200°. This was shown to be p-*bromophenacyl* meso*tartrate*, a sample of which was prepared from authentic *meso*tartaric acid and had m. p. 198—200°, alone or mixed with the above product (Found : C, 44·25; H, 3·2. $C_{20}H_{16}O_8Br_2$ requires C, 44·1; H, 3·0%).

In yet another experiment the filtrate from the crude *allo*mucic acid was concentrated under reduced pressure, the residue dissolved in water and almost neutralised with sodium hydroxide, and the boiling solution then treated with saturated calcium acetate solution. Without cooling, the white precipitate was collected, washed, and dried. It was shown by titration with warm acid permanganate solution to consist of calcium oxalate. The filtrate was concentrated and gave large crystals which were redissolved in hot water; the solution was acidified and then extracted with ether. After removal of the ether the residue was treated with ethereal diazomethane and gave colourless prisms, m. p. 111—112°, shown by mixed m. p. to consist of methyl *meso*tartrate.

In yet another experiment, the crude mixture of acids, freed from volatile substances, was esterified with diazomethane and the esters were distilled. The fraction, b. p. 110°/0·3 mm., gave again methyl *meso*tartrate. The higher-boiling esters could not be separated; much of the material underwent decomposition during distillation. Although the hydroxyl groups of *meso*tartaric acid were not affected by diazomethane it may be noted that Schmidt and Zeiser (*Ber.*, 1934, **67**, 2120) found that tartaric and other hydroxy-acids undergo etherification as well as esterification with diazomethane.

Naphthalene.—A solution of the hydrocarbon (5 g.) in acetone (25 c.c.) with 1% osmium tetroxide in *tert.*-butanol (2·5 c.c.) and hydrogen peroxide solution (15 c.c.) immediately became yellow and oxidation proceeded rapidly; further quantities of hydrogen peroxide solution were added as required. After 18 days the solution was worked up in the usual way. The acidic fraction was mainly soluble in water. It was extracted with ether, recovered from the extract, and recrystallised several times from ethyl acetate and aqueous methanol. The resulting acid had m. p. *ca.* 200°, and on sublimation gave long needles, m. p. 137°, shown by mixed m. p. to be phthalic anhydride.

When the solution of the reactants was worked up after shorter periods there were shown to be present phenolic material and also coloured neutral products, probably naphthaquinones (compare Milas, U.S.P., 2,395,638).

Phenanthrene.—The hydrocarbon (3 g.) in acetone (15 c.c.) was treated with the Milas reagent (2 c.c. of osmium tetroxide solution and 10 c.c. of hydrogen peroxide solution) at room temperature. Further additions of hydrogen peroxide solution were made from time to time, about 60 c.c. of a 20% solution being used in all. After 26 days the red solution was concentrated *in vacuo* and then deposited crystals (55 mg.), m. p. 204—206°, which were shown to consist of phenanthraquinone. The filtrate was worked up in the usual way, and on passing the benzene solution through a column of alumina there were obtained from the first benzene filtrate some unchanged phenanthrene and then a fraction which gave fine colourless needles (from light petroleum) of *cis*-9 : 10-dihydroxy-9 : 10-dihydrophenanthrene (25 mg.), m. p. 178° alone or mixed with a specimen prepared as described by Criegee, Marchand, and Wannowius (*Annalen*, 1942, **550**, 99). A yellow zone was cut from the alumina column and gave on elution with benzene more phenanthraquinone, m. p. 206—207°.

The acidic fraction was repeatedly crystallised from aqueous methanol and ethyl acetate. This gave colourless prisms, m. p. 228—230°, shown by mixed m. p. to be diphenic acid. Identification was completed by conversion into the anhydride, m. p. and mixed m. p. 217°. The sodium hydroxide extract on acidification gave a product which was purified by passing its benzene solution through a column of alumina. This gave a small amount of 9-phenanthrol, m. p. and mixed m. p. 148°.

Chrysene.—In this case reaction was slow, but the hydrocarbon in suspension (using 330 mg. in 50 c.c. of acetone) dissolved during the course of 3 weeks. The red solution was concentrated *in vacuo* and gave red needles of 1 : 2-chrysaquinone, m. p. 237—238°, not depressed by mixing with an authentic specimen. Small amounts of acidic and other products were also formed.

Anthracene.—The hydrocarbon (100 mg.) in acetone (10 c.c.) was treated with 1% osmium tetroxide solution (1 c.c.) and 20% hydrogen peroxide solution (2 c.c.). After 4 days long needles of anthraquinone began to separate (m. p. and mixed m. p. 275°). In another experiment 250 mg. of anthracene gave, after 4 months, 170 mg. of anthraquinone and traces of unidentified acidic products.

Pyrene.—A solution of this hydrocarbon (350 mg.) in acetone (10 c.c.) became pink immediately on the addition of the Milas reagent. After a week the deep-red solution was worked up. Chromatography of the benzene extract led to recovery of some unchanged hydrocarbon and gave several coloured bands. Chloroform eluted a mixture of quinones, which were not completely separated by repeated chromatography in benzene. The colours given by the various fractions with concentrated sulphuric acid indicated the presence of the 3 : 8- and 3 : 10-quinones (Vollmann *et al.*, *loc. cit.*). There was no definite evidence of the presence of the 1 : 2-quinone.

In another experiment the solution of reactants was kept for about a year. The deep colour slowly faded and a pale yellow solid was deposited. This was recrystallised from much ethanol and then sublimed under reduced pressure, giving yellowish needles of naphthalene-1 : 4 : 5 : 8-tetracarboxylic dianhydride, m. p. above 360°. Treatment of the silver salt with methyl iodide gave the *methyl* ester as colourless leaflets, m. p. 196—198°, not depressed by mixture with a sample similarly prepared from Cook and Hewett's tetracarboxylic acid (*J.*, 1933, 405) (Found : C, 60·2; H, 4·7. $C_{18}H_{16}O_8$ requires C, 60·0; H, 4·5%).

1 : 2-*Benzanthracene.*—(*a*) A solution of the hydrocarbon (0·5 g.) in acetone (20 c.c.) was treated with 40% hydrogen peroxide (2 c.c.) and 1% osmium tetroxide (1 c.c.), both in *tert.*-butanol. The solution soon became red and in the course of 3—4 days red crystals separated (80 mg.). After crystallisation from chloroform these had m. p. 260—262°, not depressed by admixture with a sample of the quinone (XII) prepared by chromic acid oxidation of *cis*-3 : 4-dihydroxy-3 : 4-dihydro-1 : 2-benzanthracene. The product gave a purple solution in concentrated sulphuric acid, in agreement with Fieser and Dietz (*J. Amer. Chem. Soc.*, 1929, **51**, 3141). The quinone reacted with *o*-phenylenediamine in acetic acid solution to give the *quinoxaline* as pale yellow needles, m. p. 256—257° (Found : C, 86·9; H, 4·3. $C_{24}H_{14}N_2$ requires C, 87·2; H, 4·3%).

The mother-liquors from which this quinone had been separated were worked up in the normal manner. Chromatography of the benzene extract showed that very little unchanged hydrocarbon was present. The benzene eluate gave 1 : 2-benzanthra-9 : 10-quinone (XI) (120 mg.) as orange needles, m. p. 167—168°. Chromatographic purification of the material from the mother-liquors of this gave a

small amount of *cis*-3 : 4-dihydroxy-3 : 4-dihydro-1 : 2-benzanthracene, m. p. 201—205°, not depressed by admixture with a sample prepared as described by Cook and Schoental (*loc. cit.*).

The acidic products insoluble in water (*i.e.*, precipitated by acidification of the sodium hydrogen carbonate extract) were recrystallised several times from ethyl acetate and gave a small amount of colourless crystals, m. p. 245°. This acid was shown to be identical with *2-phenylnaphthalene-3 : 2′-dicarboxylic acid* (XIII), colourless prisms, m. p. 249—250° (from ethyl acetate), formed by oxidation of 1 : 2-benzanthra-3 : 4-quinone (XII) with alkaline hydrogen peroxide (compare Weitz, Schobbert, and Seibert, *Ber.*, 1935, **68**, 1163), and the same acid was also formed from the quinone (XII) by treatment with the Milas reagent for several weeks (Found : C, 74·3; H, 4·1. $C_{18}H_{12}O_4$ requires C, 74·0; H, 4·1%). Its *methyl* ester, prepared by heating it with hydrochloric acid in methanol, formed colourless plates, m. p. 86—87·5° (Found : C, 75·05; H, 5·0. $C_{20}H_{16}O_4$ requires C, 75·1; H, 5·0%)

In another experiment on the oxidation of benzanthracene by the Milas reagent the acidic products were esterified with diazomethane and distilled *in vacuo*. The first fraction, b. p. 90—100°/0·3 mm., was mostly methyl phthalate, identified by conversion into the anhydride. The fraction, b. p. 120—180°/0·3 mm., gave on hydrolysis the acid (XIII). Treatment of the crude acid mixture with 2 : 4-dinitrophenylhydrazine gave a small amount of solid, indicating the presence of keto-acids, but a pure substance could not be isolated.

(*b*) 1 : 2-Benzanthracene (0·5 g.) in acetone (20 c.c.) was treated with 50% hydrogen peroxide in *tert.*-butanol (3 c.c.) and methylamine hydrochloride (140 mg.). The mixture was kept in the dark for 2 months and gave 1 : 2-benzanthra-9 : 10-quinone (170 mg.) and acidic products from which phthalic acid was isolated by distillation of the esterified material.

(*c*) A similar experiment with haematin (prepared from haemin as described by Fischer *et al.*, *Z. physiol. Chem.*, 1930, **193**, 156) in place of methylamine hydrochloride gave 1 : 2-benzanthra-9 : 10-quinone in 50% yield and some phthalic acid.

1 : 2-5 : 6-*Dibenzanthracene*.—The slight solubility of this hydrocarbon necessitated the use of a very dilute solution and the reaction was exceedingly slow at room temperature. It was therefore accelerated by heating. The hydrocarbon (500 mg.) in acetone (50 c.c.) was treated with 50% hydrogen peroxide (10 c.c.) and 1% osmium tetroxide (10 c.c.), both in *tert.*-butanol, and the solution was heated at 70° for 3—4 days. The purple crystals which separated (160 mg.) were recrystallised from acetic acid and formed dark red needles, m. p. 325°. This substance was shown to be identical with 1 : 2-5 : 6-dibenzanthra-3 : 4-quinone (XIV) prepared as described by Stephenson (*J.*, 1949, 2620), who gives m. p. 327—329° (compare Cook, *J.*, 1933, 1594). Concentration of the original filtrate gave a yellow solid which crystallised from benzene in orange-yellow needles, m. p. 246°, consisting of 1 : 2-5 : 6-dibenzanthra-9 : 10-quinone (Clar, *Ber.*, 1929, **62**, 350).

The sodium hydrogen carbonate fraction on acidification gave a solid precipitate which after several recrystallisations formed a cream microcrystalline powder (from acetone), m. p. 307—309°. This was 2-phenylphenanthrene-3 : 2′-dicarboxylic acid (XV), a sample of which was prepared for comparison by oxidation of the quinone (XIV) with hydrogen peroxide in boiling methanolic sodium hydroxide (compare Weitz *et al.*, *loc. cit.*). The resulting acid had m. p. 316°, and did not depress the m. p. of the acid obtained by oxidising the hydrocarbon with the Milas reagent, or that obtained by another route by Stephenson (*loc. cit.*), who gives m. p. 311—313°. For characterisation, the *methyl* ester of (XV) was prepared by the Fischer–Speier method. It formed almost colourless leaflets (from ethanol), m. p. 144—145° (Found : C, 77·3; H, 5·1. $C_{24}H_{18}O_4$ requires C, 77·8; H, 4·9%).

3 : 4-*Benzpyrene* (XVI).—The oxidation of this hydrocarbon with the Milas reagent proceeded as with pyrene. Chromatography on alumina of the benzene extract after concentration (a large volume of benzene was required) gave a red zone. The column was cut and this was eluted with chloroform. Repeated chromatography from benzene of the concentrated eluate resulted in partial separation into a lower (yellow) band and an upper (red) band. These were cut, eluted with chloroform, and concentrated. The residue from the yellow band gave a cherry-red solution in concentrated sulphuric acid, whereas that from the red band gave an olive-brown solution. These colours are consistent with the view that the yellow quinone is the 5 : 10-quinone and the red quinone the 5 : 8-quinone obtained by Vollmann *et al.* (*loc. cit.*) by chromic acid oxidation of 3 : 4-benzpyrene (XVI).

Some of the fractions from the chromatogram gave a deep violet colour with sulphuric acid and reacted with *o*-phenylenediamine in acetic acid to give a yellow precipitate, indicating an *o*-quinone. For comparison, the dihydroxydihydro-3 : 4-benzpyrene of Cook and Schoental (*loc. cit.*) was oxidised with chromic acid and the resulting *quinone*, probably the 6 : 7-quinone, crystallised from glacial acetic acid. It formed orange-red needles, m. p. 240° (decomp.), and gave the expected deep-violet solution in concentrated sulphuric acid (Found : C, 85·0; H, 3·6. $C_{20}H_{10}O_2$ requires C, 85·1; H, 3·55%). The *quinoxaline*, prepared by treatment with *o*-phenylenediamine in acetic acid, formed small yellow needles, m. p. 318° (decomp.), not depressed by admixture with the precipitate similarly formed from fractions of the chromatogram (Found : C, 87·8; H, 4·2; N, 7·8. $C_{26}H_{14}N_2$ requires C, 88·1; H, 4·0; N, 7·9%).

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