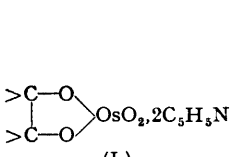
104. *The Relative Reactivity of Aromatic Double Bonds.*

By G. M. Badger.

In the presence of pyridine, osmium tetroxide is added to certain reactive aromatic double bonds with the formation of brown complexes. A method for studying the rate of addition has been developed, and the reaction shown to be of the second order. The rate of addition to 18 carcinogenic and related non-carcinogenic hydrocarbons has been studied. The substitution of alkyl groups, especially in " favourable " positions, increases the rate of addition, while the cyano-group decreases it. These results are discussed in relation to theories of chemical constitution and carcinogenic activity.

Criegee *et al.* (*Annalen*, 1936, **522**, 75; 1942, **550**, 99) observed that osmium tetroxide, in the presence of pyridine, reacts with compounds containing an ethylenic double bond with the formation of coloured complexes having the structure of osmic esters (I). This reaction has recently been extended to polycyclic aromatic hydrocarbons by Cook and Schoental (*J.*, 1948, 170; *Nature*, 1948, **161**, 237), who observed that, in every case, the addition was to adjacent carbon atoms. With anthracene, for example, addition to the 1 : 2- and 3 : 4-bonds took place,

and with 1 : 2-benzanthracene (II), addition to the 3 : 4-bond. This should be compared with the fact that most reagents which have been investigated give rise to *meso*-substitution or *meso*-addition products of anthracene and its derivatives. Again, with pyrene (III), the addition was to the 1 : 2-bond although most reagents oxidise, or substitute, this hydrocarbon



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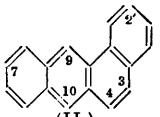

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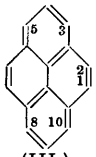

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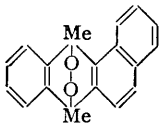

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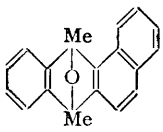

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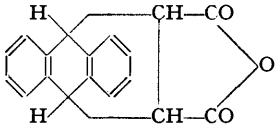

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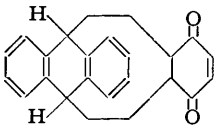

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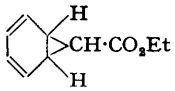


(VIII.)

at positions 3, 5, 8, and 10, to give 3 : 8- and 3 : 10-disubstituted, or 3 : 5 : 8 : 10-tetrasubstituted pyrenes. These facts favour the view that osmium tetroxide can only react as a " double bond " reagent, for there does not seem to be any theoretical objection to a transannular addition with anthracene and its derivatives. Anthracene is known to form a photo-oxide by the transannular addition of oxygen (Dufraise and Gérard, *Bull. Soc. chim.*, 1937, **4**, 2052). 9 : 10-Dimethyl-1 : 2-benzanthracene, and many other substituted anthracenes, are also known to form stable photo-oxides which have been shown to have the structure of *endo*-peroxides (IV) (Cook and Martin, *J.*, 1940, 1125). It is of some interest that a stable *endo*mono-oxide (V) has also been reported (Badger, Goulden, and Warren, *J.*, 1941, 18), having been prepared by transannular dehydration of 9 : 10-dimethyl-9 : 10-dihydroxy-9 : 10-dihydro-1 : 2-benzanthracene. Anthracene and its derivatives also form transannular adducts (VI and VII) with maleic anhydride and with *p*-benzoquinone (see Clar, " Aromatische Kohlenwasserstoffe ", 1941; also Bartlett, Ryan, and Cohen, *J. Amer. Chem. Soc.*, 1942, **64**, 2649).

Considerable interest attaches to the mechanism of the addition of osmium tetroxide to double bonds. The data available at present are few, but it seems to be important that osmium tetroxide reacts with aromatic hydrocarbons, and with compounds containing ethylenic double bonds, in a manner analogous to that of reagents such as ozone and diazoacetic ester. As has already been mentioned, the addition of osmium tetroxide to pyrene takes place at the 1 : 2-bond, and this is also the first bond to be attacked by ozone (Vollmann, Becker, Corell, and Streeck, *Annalen*, 1937, **531**, 1; Fieser and Novello, *J. Amer. Chem. Soc.*, 1940, **62**, 1855). This seems to indicate that the 1 : 2-bond is the most reactive *bond* in pyrene, although the 3, 5, 8, and 10 positions are the most reactive *centres* (compare Robertson and White, *J.*, 1947, 358; Moffitt and Coulson, *Proc. Physical Soc.*, 1948, **60**, 309). Ozone attacks anthracene, but the products do not seem to have been determined. With naphthalene and its derivatives, either a diozonide or a pentozonide is formed (Long, *Chem. Reviews*, 1940, **27**, 437; Harries, *Annalen*, 1905, **343**, 311; Wibaut and van Dijk, *Rec. Trav. chim.*, 1946, **65**, 413; Kooyman, *ibid.*, 1947, **66**, 201). As mentioned above, osmium tetroxide gives rise to anthracene-1 : 2-3 : 4-diosmate, and a similar result may be expected with naphthalene, although this has not yet been confirmed. Diazoacetic ester also attacks aromatic and ethylenic doubles bonds (Smith, *Chem. Reviews*, 1938, **23**, 193). The products are pyrazolines, which decompose with evolution of nitrogen to give norcaradiene derivatives (VIII). Some other diazo-compounds behave similarly (Fieser and Peters, *J. Amer. Chem. Soc.*, 1931, **53**, 4080).

The additions of these reagents to double bonds is therefore very similar. For the present purpose, however, the detailed mechanism is not important. The relative reactivities of two substances will be governed by two factors : (i) the relative polarisabilities of the double bonds, and (ii) the percentages of double-bond character. It seems unlikely that relative polarisability will exert an important effect, but, in any case, with closely related substances the polarisabilities may be assumed to be identical, and the relative rates of reaction with osmium tetroxide should be comparable with the density of π electrons, or with the double-bond character.

For preparative purposes (cf. Criegee *et al.*, *loc. cit.*; Cook and Schoental, *loc. cit.*), the reaction is usually carried out in a solvent such as benzene, in which the complexes are sparingly soluble.

The complexes are, however, soluble in methylene chloride, chloroform, etc., and these solvents were therefore indicated for kinetic work. A preliminary account of a comparison between the rate of addition of osmium tetroxide to 1 : 2-benzanthracene and to phenanthrene has already been published (Badger and Reed, *Nature*, 1948, **161**, 238). In this case the reaction between the hydrocarbon and osmium tetroxide was carried out at 30°, in pure chloroform.

Pure chloroform, from which the alcohol has been removed, is notoriously unstable, giving rise to hydrogen chloride and carbonyl chloride, and in further experimental work difficulties were often encountered owing to the development of these decomposition products, and the apparent instability of the complex. An investigation into the stability of osmium tetroxide and of the complexes, in chloroform, was accordingly undertaken. Osmium tetroxide was found to be stable in solution in chloroform, for at least a week, so that standard solutions may safely be made up and used within that time. On the other hand, such solutions give rise to varying amounts of chloroform decomposition products within a few days. In chloroform B.P. (*i.e.*, pure anæsthesia chloroform containing alcohol) osmium tetroxide is unstable, and rapidly gives a black precipitate. The 1 : 2-benzanthracene-3 : 4-osmate–pyridine complex was found to be stable in pure chloroform for at least 24 hours, but unstable in chloroform containing decomposition products. On the other hand, the complex was found to be stable in chloroform containing decomposition products to which a little pyridine had been added. Hydrogen ions are evidently involved in the decomposition : this has been demonstrated by passing hydrogen chloride through a solution of the complex in pure chloroform. Criegee (*loc. cit.*) has previously observed that osmic esters are converted into " diesters " by treatment with acids.

In view of these results, it was decided to carry out the reaction in pure chloroform containing 4% of pyridine. A temperature of 20° was used. Colour intensities were measured at suitable intervals with a Spekker absorptiometer, using green filters, the instrument being calibrated with known concentrations of the appropriate complex. Green filters gave the most satisfactory results, reducing the effect of the yellow osmium tetroxide–pyridine solution to a minimum. A correction for the absorption of this solution was, however, applied. The reactions were followed for 24 hours.

According to Criegee (*loc. cit.*), pyridine and osmium tetroxide quickly interact to form a complex, OsO_4,C_5H_5N. The author has found, however, that the prolonged action of osmium tetroxide on pyridine gives rise to a highly crystalline, sparingly soluble, light brown *complex*, $OsO_4,2C_5H_5N$. This means that the use of pyridine in the hydrocarbon–osmium tetroxide reaction mixture must introduce an error which, although small for fast reactions, may be quite large with very slow reactions. For this reason the method is unsuitable for the accurate determination of slow reactions, and in the present work no rate constants have been calculated for those substances found to react substantially slower than benzanthracene (*i.e.*, those reactions less than 40% complete in 24 hours). The absolute accuracy of the present determinations may not be high, but for determinations of the reaction rates of two similar compounds the relative accuracy is probably about $\pm 5\%$.

With most of the compounds studied, a concentration of about 0·015 or 0·020M was found to give a satisfactory rate of reaction. Indeed, it has not been possible to study these compounds over as large a range of concentration as might be desired. The sparing solubility of most of the polycyclic aromatic compounds, and the intense yellow colour of the osmium tetroxide–pyridine solutions, prevents the study of high concentrations, while the slowness of the reaction and the side reaction with pyridine already mentioned make it difficult to follow the reaction at great dilutions. With these limitations the reaction with benzanthracene has been studied at five concentrations. Within experimental error, the same rate constant (in g.-mol.$^{-1}$ sec.$^{-1}$ l.) was obtained (see Table I). This indicates that the reaction is of the second order.

TABLE I.

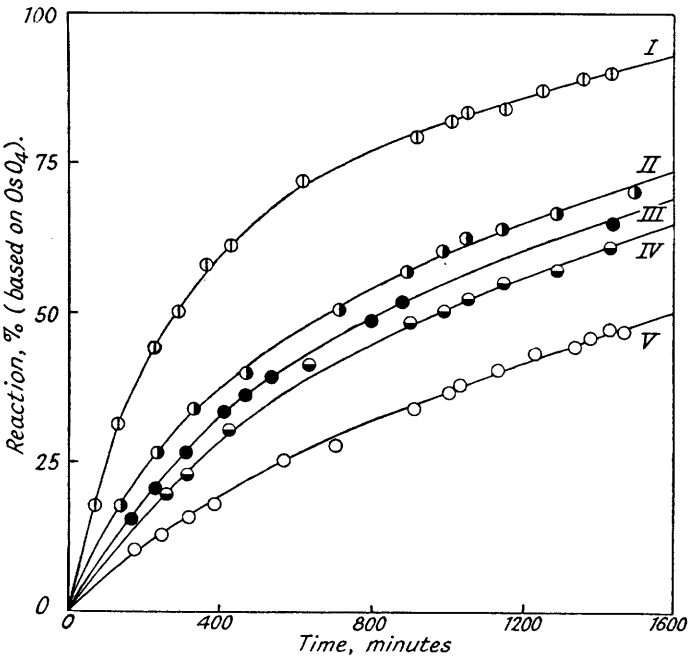
Reaction between 1 : 2-*benzanthracene and osmium tetroxide in* 4% *pyridine in chloroform, at* 20°.

1 : 2-Benzanthracene, M.	OsO_4, M.	10^4k_2.	1 : 2-Benzanthracene, M.	OsO_4, M.	10^4k_2.
0·02000	0·01542	4·75±0·24	0·03000	0·03064	4·57±0·36
0·02000	0·02145	4·86±0·30	0·03000	0·02208	4·74±0·24
0·02500	0·01766	4·86±0·29		Mean	4·76

It appeared to be desirable to confirm that the reaction follows the same course in solution in chloroform as has been demonstrated (Cook and Schoental, *loc. cit.*) for benzene solution. This was carried out by using the reaction mixture remaining unused after the above determinations with 1 : 2-benzanthracene. The mixture was hydrolysed to 3 : 4-dihydroxy-3 : 4-dihydro-

1 : 2-benzanthracene, and this was converted into the diacetyl derivative, shown to be identical with a specimen prepared by Dr. R. Schoental.

The rate of addition of osmium tetroxide to 18 carcinogenic and related non-carcinogenic polycyclic aromatic compounds has been studied under standard conditions : hydrocarbon, 0·020M; osmium tetroxide, 0·0154M. Attention was concentrated on derivatives of 1 : 2-benz-



Curves illustrating the effect of methyl and methylene group on the rate of addition of osmium tetroxide to 1 : 2-*benzanthracenes.* I, 9 : 10-*Dimethyl*-1 : 2-*benzanthracene.* II, *Methylcholanthrene.* III, 10-*Methyl*-1 : 2-*benzanthracene.* IV, 2′ : 7-*Dimethyl*-1 : 2-*benzanthracene.* V, 1 : 2-*Benzanthracene.*

anthracene, which may reasonably be assumed to have comparable polarisabilities, although examples of other types of carcinogen have been examined. The results clearly show (see figure) that alkyl groups act as electron donors to the ring, and increase the density of π electrons at the point of addition. This is reflected in the increased reactivity towards osmium tetroxide. The effect varies in magnitude with the position of the alkyl group, and is at a maximum when

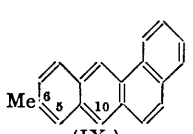
TABLE II.

Rates of reaction of osmium tetroxide and carcinogenic compounds, in 4% pyridine in chloroform, at 20°. (k_2 *in* g.-mol.$^{-1}$ sec.$^{-1}$ l.)

Compound.	10^3k_2.	Carcinogenic activity.
5 : 6 : 9 : 10-Tetramethyl-1 : 2-benzanthracene	2·9	+++
9 : 10-Dimethyl-1 : 2-benzanthracene	2·7	++++
9 : 10-Diethyl-1 : 2-benzanthracene	2·1	+++
20-Methylcholanthrene	1·1	++++
Acenaphthanthracene	1·1	+
Cholanthrene	1·0	++++
3 : 4-Benzpyrene	0·94	++++
10-Methyl-1 : 2-benzanthracene	0·91	+++
1 : 2-Dimethylchrysene	0·84	++
2′ : 7-Dimethyl-1 : 2-benzanthracene	0·83	0
5 : 6-Dimethyl-1 : 2-benzanthracene	0·64	++
6-Methyl-1 : 2-benzanthracene	0·64	+
1 : 2 : 5 : 6-Dibenzanthracene	0·64	++
1 : 2-Benzanthracene	0·48	0
10-Cyano-9-methyl-1 : 2-benzanthracene	0·40	++++
10-Cyano-1 : 2-benzanthracene	very slow	+
6-Methyl-3 : 4-benzphenanthrene	,,	+
1 : 2 : 5 : 6-Dibenzphenanthrene	,,	+

the group or groups are in the *meso*-positions (*e.g.*, 10-methyl-, 9 : 10-dimethyl-, and 9 : 10-diethyl-1 : 2-benzanthracene; see also Table II). 9 : 10-Diethyl- was found to react slightly less rapidly than 9 : 10-dimethyl-1 : 2-benzanthracene, as might be expected. The effect of methyl groups situated far from the point of addition was found to be small, or insignificant; *e.g.*, 6-methyl- (IX) and 5 : 6-dimethyl-1 : 2-benzanthracene reacted with osmium tetroxide only slightly more rapidly than benzanthracene; 5 : 6 : 9 : 10-tetramethyl- reacted only slightly more rapidly than 9 : 10-dimethyl-1 : 2-benzanthracene; and methylcholanthrene (X), which may conveniently be considered as a benzanthracene derivative substituted in positions 10, 5, and 6, reacted only slightly faster than cholanthrene.

The rate of addition of osmium tetroxide to acenaphthanthracene (4′ : 3-ace-1 : 2-benzanthracene) (XIII) is of particular interest, for this compound has a substituent methylene group at the point of addition. It was not expected that the presence of this group would prevent, by steric hindrance, the addition of osmium tetroxide to that double bond, and some interesting evidence which supports this view has been obtained. The complex, on hydrolysis, gave a *diol* (XIV), which on acetylation, yielded a *monoacetyl* derivative. This can



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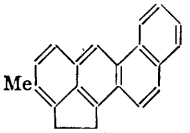

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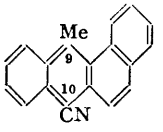

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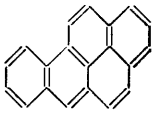
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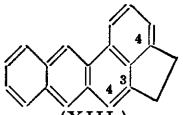

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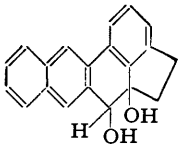

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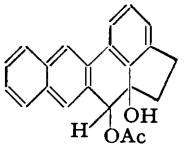

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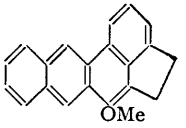


(XVI.)

only be explained if the methylene bridge offers steric hindrance towards one hydroxyl group, and the product was, almost certainly, 3-*hydroxy*-4-*acetoxy*-3 : 4-*dihydro*-4′ : 3-*ace*-1 : 2-*benzanthracene* (XV). Dehydration of the diol proceeded normally, to give the phenol, characterised as 4-*methoxy*-4′ : 3-*ace*-1 : 2-*benzanthracene* (XVI). In this connection, it is also of interest that Cook and Schoental (private communication) have found that osmium tetroxide adds normally to the 9 : 10-bond of 9-bromophenanthrene, to give a complex which, on hydrolysis, yields phenanthraquinone.

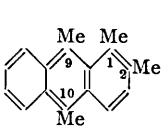
A cyano-group was found to deactivate the point of addition, for 10-cyano-1 : 2-benzanthracene reacted with osmium tetroxide very much more slowly than benzanthracene. Furthermore, 10-cyano-9-methyl-1 : 2-benzanthracene (XI), which presents the interesting combination of an electron-donating and an electron-attracting group, was found to react slightly less rapidly than benzanthracene. The deactivating influence of the cyano-group in the 10-position is therefore somewhat stronger than the activating influence of the methyl group in the 9-position.

6-Methyl-3 : 4-benzphenanthrene and 1 : 2 : 5 : 6-dibenzphenanthrene reacted with osmium tetroxide only slowly. It is possible that these compounds are less polarisable than benzanthracene derivatives, and in this connection it is noteworthy that Badger and Reed (*loc. cit.*) found phenanthrene very much less reactive than benzanthracene. 3 : 4-Benzpyrene (XII) and 1 : 2-dimethylchrysene were found to react moderately rapidly with osmium tetroxide, but 1 : 2 : 5 : 6-dibenzanthracene reacted only slightly more rapidly than 1 : 2-benzanthracene.

It is interesting to compare these results with the work of Eckhardt (*Ber.*, 1940, **73**, 13), who followed the rate of oxidation of some carcinogenic and related compounds with perbenzoic acid. The oxidation products have not been determined, and although the shape of some of the curves indicates that, in some cases, the reaction may be rather more complex than that with osmium tetroxide, it seems probable that the point of attack is the same. As is well known, perbenzoic acid reacts with ethylenic double bonds to form epoxides (Prileschajew, *Ber.*, 1909, **42**, 4811). It should be emphasised, however, that the mechanism of the perbenzoic acid reaction is almost certainly fundamentally different from that of osmium tetroxide addition. Eckhardt observed (i) an increase in the rate of oxidation following the introduction of a methyl group, and (ii) a decrease in the rate of oxidation following the introduction of the deactivating groups, CHO and NO_2.

Discussion.—It has long been recognised that nearly all the carcinogens of the polycyclic aromatic type are derivatives of phenanthrene. Indeed, certain alkyl derivatives of all the benzphenanthrenes (*viz.*, chrysene, 3 : 4-benzphenanthrene, and 1 : 2-benzanthracene), except triphenylene, are carcinogenic, although the position of the alkyl group is also of great importance. It must be admitted that relatively few homologues of triphenylene have been examined, but it does seem to be an exception, and this is of great interest, for in this hydrocarbon the chief feature of the phenanthrene ring system, namely, the 9 : 10-double bond, is absent. Furthermore, Hewett (*J.*, 1940, 293) has pointed out that the potent carcinogens are derivatives of phenanthrene substituted by fused benzene rings, or methyl groups, in three or four of the positions 1, 2, 3, and 4. Further substitution of 2 : 3-benzphenanthrene (*i.e.*, 1 : 2-benzanthracene) in either or both of the remaining 1- and 4-positions gives the highly carcinogenic hydrocarbons 9 : 10-dimethyl-1 : 2-benzanthracene, methylcholanthrene, 3 : 4-benzpyrene, etc. Further substitution of chrysene (1 : 2-benzphenanthrene) or of 3 : 4-benzphenanthrene gives the potent carcinogens 1 : 2-dimethylchrysene and 2-methyl-3 : 4-benzphenanthrene. On the other hand, all derivatives of phenanthrene are not carcinogenic. Of the 15 possible pentacyclic hydrocarbons in which each ring is 6-membered, 13 may be considered as derived from phenanthrene, but only 5 have given tumours; of the 8 non-carcinogenic derivatives, only one lacks a free 9 : 10-double bond (Barry *et al.*, *Proc. Roy. Soc.*, 1935, **117**, *B*, 318).

In order to determine whether the phenanthrene ring system is essential for carcinogenic activity in compounds of the polycyclic aromatic type, work was initiated, in 1938, on the synthesis of 1 : 2 : 9 : 10-tetramethylanthracene (XVII) and of 1 : 2 : 3 : 4-tetramethyl-



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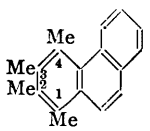

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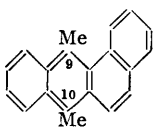

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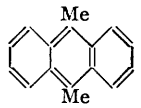

(XX.)

phenanthrene (XVIII). Both hydrocarbons are structurally closely related to the potent carcinogen 9 : 10-dimethyl-1 : 2-benzanthracene (XIX). Tetramethylanthracene proved unexpectedly difficult to synthesise (Badger, Cook, and Goulden, *J.*, 1940, 16; Badger, Goulden, and Warren, *J.*, 1941, 18; Fieser and Webber, *J. Amer. Chem. Soc.*, 1940, **62**, 1360), and when it was eventually prepared (Sandin, Kitchen, and Fieser, *J. Amer. Chem. Soc.*, 1943, **65**, 2018) it was found to be unstable. 9 : 10-Dimethylanthracene (XX) was therefore examined (Badger, Goulden, and Warren, *loc. cit.*). Tetramethylphenanthrene was prepared by Hewett and Martin (*J.*, 1940, 1396). It was eventually shown that the phenanthrene ring system is not an essential requirement of a polycyclic aromatic hydrocarbon for carcinogenic activity, for both 1 : 2 : 3 : 4-tetramethylphenanthrene and 9 : 10-dimethylanthracene were found to be definitely if slightly carcinogenic (Badger *et al.*, *Proc. Roy. Soc.*, 1942, **131**, *B*, 170; Kennaway, Kennaway, and Warren, *Cancer Res.*, 1942, **2**, 157).

Further work, however, has again confirmed the importance of the phenanthrene ring system, and of the 9 : 10-bond in particular. Some interesting results have been obtained, for example, with compounds in which the 9 : 10-bond has been replaced by a sulphur atom (British Empire Cancer Campaign, Annual Report, 1946, 109). Sir Robert Robinson (*Brit. Med. J.*, 1946, i, 945) suggested that, although there are exceptions, an activated phenanthrene-type bridge appears to be implicated in most carcinogens.

In another approach to the problem, Pullman (*Ann. Chim.*, 1947, **2**, 5; Pullman and Pullman, *Experientia*, 1946, **2**, 364; *Rev. Sci.*, 1946, **84**, 145) suggested that carcinogenic activity is associated with an optimum density of π electrons on the phenanthrene-type bridge (hereinafter referred to, in accordance with Pullman, as the " K position "). Alkyl groups, especially methyl groups, act as slight donors of electrons to the ring, so that the substitution of methyl groups in suitable positions may increase the electron density at position K to more than the critical value for the development of carcinogenic activity. Quantum-mechanical calculations of the electron densities of several derivatives of 1 : 2-benzanthracene, 3 : 4-benzphenanthrene, 1 : 2-benzacridine, and 3 : 4-benzacridine were presented in support of the theory. Since osmium tetroxide attacks the K position, apparently without exception (Cook and Schoental, *loc. cit.*), this reaction offered an experimental method of testing the validity of the Pullman calculations.

On the whole, the present work appears to support, at least qualitatively, these calculations. On the other hand, it seems to the author that in assessing the validity of the correlation between

the electronic charge on the K position and carcinogenic activity, too little account has been taken of the numerous exceptions. On the basis of the Pullman theory, methylbenzanthracenes with methyl groups in the angular ring, should be carcinogenic. No calculations for these compounds were given. As a first approximation, however, one might expect that 4′-methyl-1 : 2-benzanthracene should have the same carcinogenic activity as 10-methyl-1 : 2-benzanthracene. In point of fact all the methylbenzanthracenes substituted in positions 1′, 2′, 3′, or 4′ are either completely inactive or have only trace activity. Inactive compounds of this type include : 1′-, 2′-, 3′-, and 4′-methyl-, 1′ : 10-, 2′ : 6-, 2′ : 7-, 3′ : 6-, and 3′ : 7-dimethyl-1 : 2-benzanthracene. It has been demonstrated by the present work that this criticism is valid, for 2′ : 7-dimethyl-1 : 2-benzanthracene was found to react rapidly with osmium tetroxide (see fig.). Furthermore, acenaphthanthracene, which is only slightly carcinogenic, reacted at the same rate as methylcholanthrene, one of the most potent of all the carcinogens.

Again a few carcinogens have been prepared which consist of a polycyclic structure substituted with a deactivating group. The cyano-group deactivates the ring, and this has been confirmed in the present instance by the demonstration that both 10-cyano-1 : 2-benzanthracene and 10-cyano-9-methyl-1 : 2-benzanthracene react with osmium tetroxide more slowly than does 1 : 2-benzanthracene. Nevertheless, the former compound is slightly carcinogenic and the latter is a particularly potent carcinogen.

The theory also takes no account of the fact, confirmed many times, that methyl derivatives of 3 : 4-benzphenanthrene, and of chrysene, are often potent when applied to the skin of mice, but show only feeble activity—or none at all—when administered by subcutaneous injection. The carcinogenic " grading " of such compounds is therefore a matter for some discussion. In any case, the grading used by Pullman (*loc. cit.*) for many of these derivatives appears to be unjustifiably high. 6-Methyl-3 : 4-benzphenanthrene is given ++, although it gave only one papilloma when applied to the skin of 20 mice in an experiment lasting nearly 2 years. Similar criticism applies to the 7-methyl- and 8-methyl-3 : 4-benzphenanthrenes, which have been found to be only very slightly active when applied to the skin, and completely inactive when administered by injection. (For these and other carcinogenic activities, the series of papers by Cook, Kennaway, and associates should be consulted : *Proc. Roy. Soc.*, 1932, **111**, *B*, 455, 485; 1935, **117**, *B*, 318; 1937, **123**, *B*, 343; 1940, **129**, *B*, 439; 1942, **131**, *B*, 170. For further references see Hartwell, " Survey of compounds which have been tested for carcinogenic activity," 1941.) When re-graded in the light of these criticisms, these compounds become serious exceptions to the theory.

Experimental.

Materials.—(a) *Chloroform.* Chloroform B.P. was purified by shaking with concentrated sulphuric acid (at least twice), with water, dilute sodium hydroxide, and with several changes of water. After drying over calcium chloride, it was twice distilled, large head and tail fractions being discarded. These operations were carried out during one day, the purified chloroform being used for the preparation of standard solutions, and the reaction mixtures, on the following morning.

(b) *Osmium tetroxide.* This was used as obtained from the manufacturers, in 1 g. ampoules. Standard solutions were prepared by dissolving the contents of one ampoule (accurately weighed) in purified chloroform, at 20°, and making up to 50·0 ml. Such standard solutions, which were protected as much as possible from the light, were used within 48 hours.

(c) *Pyridine.* This was purified by prolonged boiling with potassium permanganate, followed by distillation over potassium hydroxide.

(d) *Hydrocarbons.* Pure synthetic materials were used.

(e) *Hydrocarbon–osmium tetroxide–pyridine complexes.* The complexes were prepared, in benzene, as described by Cook and Schoental (*loc. cit.*), and recrystallised by dissolving in chloroform containing a little pyridine, filtering, and adding light petroleum or benzene. The crystalline complexes separated on prolonged standing. (This method of recrystallisation was first used by Dr. R. Schoental.) A few complexes were also successfully recrystallised from boiling benzene.

Stability of the Benzanthracene Osmate–Pyridine Complex.—This was investigated by preparing standard solutions of the complex as follows : (i) in pure chloroform; (ii) in chloroform which had been purified and then exposed to air and light for a week (odour of carbonyl chloride very distinct); (iii) in chloroform B.P. from a recently opened bottle; (iv) as (i) but containing 4% of pyridine; (v) as (ii) but containing 4% of pyridine; (vi) as (iii) but containing 4% of pyridine. Samples (1 ml.) were removed as soon as the solution was made up, and diluted with a mixture of pyridine and chloroform (1 : 9) to 25·0 ml. Colour intensities were then estimated with the Spekker absorptiometer. Further samples were removed at convenient intervals. A significant change in " Spekker " reading indicated the instability of the solution. Solutions (i), (iv), (v), and (vi) were unchanged up to 24 hours. Solution (ii) was slightly turbid, gave a higher reading than the remainder, and darkened on standing. Solution (iii) gave increasing " Spekker " readings during 4 hours. When hydrogen chloride was passed into solution (i) it rapidly darkened.

Action of Osmium Tetroxide on Pyridine.—A solution of osmium tetroxide (0·5 g.) in pyridine (50 c.c.) was kept at room temperature, the yellow solution gradually becoming brown. After a week, crystals began to separate. After a month, the deposit (0·1 g.) was collected. The *complex* was insoluble in

chloroform, but was recrystallised from pyridine, forming light brown needles, or elongated prisms, which gradually decomposed, without melting (Found : Os, 45·75, 46·5. $OsO_4,2C_5H_5N$ requires Os, 46·15%). The same complex was obtained when solutions of osmium tetroxide and pyridine in chloroform were allowed to interact for a week or more.

Reaction.—The calculated quantity of the hydrocarbon was weighed, and washed into a standard flask with pure chloroform, at 20°. Pyridine, to give 4% in the reaction mixture, was then added. The flask was clamped in the thermostat, and the desired quantity of standard osmium tetroxide solution, at 20°, added. The mixture was quickly made up to the mark with chloroform, shaken, and the time taken. At suitable intervals 1-ml. samples were removed, and diluted to 25·0 ml. with a solution prepared from pyridine (50 ml.) and chloroform (450 ml.). The colour intensity of this solution was then estimated on the Spekker absorptiometer, green filters being used (Adam Hilger Ltd., " Spectrum Green, H, 604 "). The concentration of the complex was obtained from a calibration curve prepared from known concentrations of the complex. The effect of unreacted osmium tetroxide was estimated from a similar calibration curve of osmium tetroxide–pyridine at various concentrations. No correction was applied, however, for the reaction of osmium tetroxide with pyridine to give the brown complex.

For the comparison of the rates of addition to the carcinogenic compounds, standard conditions were used : hydrocarbon, 0·020M; osmium tetroxide, 0·0154M. 2′ : 7-Dimethyl-1 : 2-benzanthracene and 1 : 2 : 5 : 6-dibenzanthracene are sparingly soluble, and a completely homogeneous solution was not obtained at this concentration for the first 1—2 hours. The error due to this circumstance is not, however, thought to be large.

Identification of the Benzanthracene Reaction Product.—The reaction mixture remaining after the investigations with 1 : 2-benzanthracene was hydrolysed by shaking with an aqueous solution of potassium hydroxide (1%) and mannitol (10%). After 2 hours, the crude diol, m. p. 202—204° (decomp.) (lit., 202—204°) was filtered off. A portion was converted into the diacetoxy-compound by boiling with acetic anhydride and pyridine. After recrystallisation from light petroleum (b. p. 100—120°), 3 : 4-diacetoxy-3 : 4-dihydro-1 : 2-benzanthracene had m. p. 139—141°, not depressed by admixture with an authentic specimen, m. p. 142—143·5°.

Reaction with Acenaphthanthracene.—The complex was prepared as usual, in benzene, and hydrolysed with 1% aqueous potassium hydroxide containing 10% of mannitol, by shaking, in methylene chloride, for 2 hours. The *diol* (XIV) separated from benzene in colourless micro-needles, m. p. 215° (decomp.) (Found : C, 83·25; H, 5·6. $C_{20}H_{16}O_2$ requires C, 83·3; H, 5·6%). The *monoacetate* (XV) was prepared by 3 minutes' boiling with acetic anhydride containing a little pyridine. It separated from light petroleum (b. p. 100—120°) as fern-like clusters of colourless plates, m. p. 169—171° (Found : C, 80·0; H, 5·2. $C_{22}H_{18}O_3$ requires C, 80·0; H, 5·5%). The diol was dehydrated by brief boiling in acetic acid containing a few drops of hydrochloric acid. The crude phenol was immediately methylated with methyl sulphate and sodium hydroxide, at 100°. 4-*Methoxy*-4′ : 3-*ace*-1 : 2-*benzanthracene* (XVI) separated from light petroleum (b. p. 100—120°) in very pale yellow needles or plates, m. p. 169—170° (Found : C, 88·6; H, 5·7. $C_{21}H_{16}O$ requires C, 88·7; H, 5·7%).

My thanks are due to Professor J. W. Cook, F.R.S., for his interest in this work, and for gifts of most of the hydrocarbons used. I am also grateful to Dr. R. I. Reed for many helpful discussions, to Mr. L. Hunter for the osmium analysis, and to Miss R. H. Kennaway for the other analyses. This work has been carried out during the tenure of an I.C.I. Research Fellowship.

UNIVERSITY OF GLASGOW. [*Received, May 25th,* 1948.]