Polycyclic Aromatic Hydrocarbons.

PEDLER LECTURE DELIVERED BEFORE THE SOCIETY IN LONDON ON FEBRUARY 16TH, 1950.

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AN invitation to give a Pedler Lecture is one of the greatest compliments that the Council can pay to an organic chemist, and when I received the invitation to give this lecture I accepted the honour with gratitude. Although I selected a very general title, I do not intend to attempt to survey more than a few selected topics within the field of investigations on polycyclic aromatic hydrocarbons.

In a sense, this lecture marks the coming-of-age of the Pedler Lectureship. For it was nearly twenty-one years ago, in 1929, that the first Pedler Lecture was given by W. H. Perkin, jun. I still have very vivid memories of that occasion, as indeed I have of most of the other Pedler lectures.

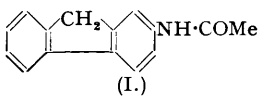
It was in the year of the first Pedler lecture that I began work on carcinogenic hydrocarbons of the benzanthracene group, having served my apprenticeship with Dr. E. de Barry Barnett, with whom I carried out a series of researches on anthracene derivatives. These served to lay the foundation on which much of my future work was to be built. During that period, the work of Barnett and Matthews [1] made available the tetracyclic hydrocarbon, 1 : 2-benzanthracene. The fluorescence spectrum of a sample of their compound was found by Hieger to bear a striking resemblance to the spectra of certain cancer-producing tars, the powerful characteristic fluorescence of which had impressed Mayneord. This focussed attention on hydrocarbons related to 1 : 2-benzanthracene and led Kennaway to the discovery of the carcinogenic properties of 1 : 2 : 5 : 6-dibenzanthracene.[2] It was the publication by Clar,[3] in 1929, of a simple new synthesis of this hydrocarbon that provided the incentive to prepare and test this particular derivative of 1 : 2-benzanthracene. Thus a series of rather fortuitous circumstances led to 1 : 2 : 5 : 6-dibenzanthracene being the first chemical compound found to have cancer-producing activity. But once the fluorescence relations had been noted the discovery of the benzanthracene group of carcinogenic hydrocarbons was probably a foregone conclusion. At any rate, even before the biological results with dibenzanthracene had been obtained I had begun work, inspired by the fluorescence results, on the synthesis of a series of new hydrocarbons derived from benzanthracene. One of these, 6-*iso*propyl-1 : 2-benzanthracene, proved to be a carcinogenic hydrocarbon of moderate potency.[4] This was followed, within a short time, by the isolation from coal tar, and the synthetic preparation, of 3 : 4-benzpyrene, a highly active, strongly fluorescent, carcinogenic hydrocarbon of the benzanthracene group which had exactly the fluorescence spectrum associated with the crude active tars.[5]

From these modest beginnings a major branch of cancer research has been built up. Its bibliography has grown to considerable dimensions. By 1939 nearly 900 papers dealing with chemical carcinogenic agents had been published [6] and this number has been greatly augmented during the ensuing decade. According to Hieger [7] about 300 synthetic carcinogenic compounds are now known. A substantial proportion of these was prepared by my very able group of collaborators at the Royal Cancer Hospital in the period 1929—39. I should like to take this opportunity to pay tribute not only to them, but also to Sir Ernest Kennaway whose scientific imagination and inspiring enthusiasm encouraged us to succeed in many ventures which otherwise we should have thought too difficult to attempt. I propose in this Lecture to recall some of the results obtained during that period and to see how they look in the light of our later knowledge, and also to discuss some of the recent work on carcinogenic and related hydrocarbons carried out in my laboratory in Glasgow. It would be superfluous as well as impracticable to attempt a general review of carcinogenic chemical compounds. This has been done on several occasions. In this connection perhaps I may direct attention to three publications, namely, the lecture which I gave to the Royal Institute of Chemistry in 1943, the very interesting special number of the *British Medical Bulletin* on "Chemical Carcinogenesis," published in 1947, and the excellent discussion of the relation between carcinogenic activity and chemical constitution published more recently by my former colleague, Dr. G. M. Badger.[8]

Classes of Carcinogenic Compounds.—I have referred to the large number of chemical compounds which have been shown to produce malignant tumours in laboratory animals. Perhaps I should reassure you by adding that there are much larger numbers which have been tested and found inactive. To avoid laying myself open to a charge of misrepresentation I

ought also to point out that by no means all of the active compounds come within the category of polycyclic aromatic hydrocarbons. Outside this class, the group which has been most extensively investigated is that which comprises a number of azo-dyes.[9] The discovery of their carcinogenic properties was due to Japanese workers. They are slow in action, they require large doses to produce tumours, and for the most part they cause cancer only in the liver. These facts suggest that the real carcinogens are, not the azo-dyes, but compounds formed from them by transformation in the liver cells.

A very interesting carcinogenic compound of another class is 2-acetamidofluorene (I). This has been used as an insecticide and its carcinogenic effects were first described in 1941.[10] Its capacity to form malignant new growths in great variety, mostly remote from the site of injection, has been fully confirmed in several laboratories.[11] Carcinogenic properties of analogues of 2-acetamidofluorene (*viz.*, II, III, IV) have been reported recently.[12] In these, the methylene bridge of the fluorene system is replaced by sulphur or oxygen, or is entirely absent. The



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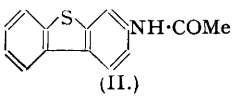

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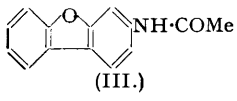

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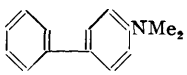
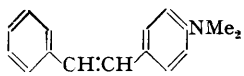




(V.)

activity of the last compound, 4-dimethylaminodiphenyl (IV), is of especial interest as it seems to form a link between 2-acetamidofluorene (I) and some basic stilbenes such as 4-dimethylaminostilbene (V) which Haddow and his collaborators [13] have shown to produce tumours resembling in their distribution those produced by the fluorene derivative.

Yet another class of nitrogenous carcinogenic compounds is that of the nitrogen mustards, two examples of which [methyl-di-(2-chloroethyl)amine and tri-(2-chloroethyl)amine] have been reported recently by Boyland and Horning [14] to produce tumours in mice. The substances were administered by subcutaneous injection of aqueous solutions of their hydrochlorides, and the resulting tumours were mostly lung tumours. It has also been shown that lung tumours arise in rats and mice after administration, in various ways, of urethane,[15] but not of other alkyl carbamates or other hypnotics.[16] The literature also contains reference to isolated examples of other types of compounds, the administration of which to experimental animals has been followed by tumours.

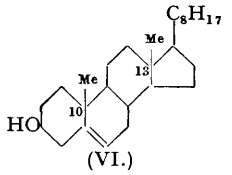
These various classes of carcinogenic compounds are of considerable interest and importance, but it has always seemed to me that they are not strictly comparable with the carcinogenic polycyclic hydrocarbons. The members of the latter group give mainly local tumours at the site of application. They do this in a great variety of different organs in several species of animal, and the more potent compounds are active in very minute doses and give tumours after a relatively short latent period.

In considering the possibility of chemical causes of human cancer it is well to recognise that relatively large amounts of feebly active compounds may be just as effective as very small amounts of more potent compounds. In this connection it is also necessary to appreciate that the biological test procedure becomes progressively less certain as the carcinogenic potency diminishes. This is due partly to biological variation. With a weakly active compound tumours may arise in a small proportion only of the treated animals and it is then difficult to decide whether the tumours are due to abnormal sensitivity of the animals, or whether they are " spontaneous " tumours which would arise irrespective of any external stimulus.

It is against this background that one must attempt to assess the precise significance of the extraordinarily suggestive results obtained by Hieger and others in the course of a series of researches on the carcinogenic effects of the unsaponifiable fractions of the lipoids of human tissues (mostly liver). In his latest publication [17] Hieger states that 63 sarcomas (*i.e.*, tumours of connective tissue) were obtained in approximately 2000 mice after injections of unsaponifiable fractions derived from tissues of human subjects (cancerous and non-cancerous) and from cattle. The average latent period was about 18 months. He also obtained sarcomas in about 5% of 213 mice after injection of commercial cholesterol in lard. In the time at my disposal it is impossible to elaborate on or discuss this work, but I may say that I am unable to subscribe to the suggestion that the carcinogenic factor present in the unsaponifiable fractions may be cholesterol itself.

Aromatic Hydrocarbons related to the Sterols.—Kennaway also has entertained the possibility

that cholesterol (VI) might be implicated in some way in the causation of cancer, and he showed, in 1928, that at very high temperatures cholesterol is converted into a cancer-producing tar.[18] Undue importance was not attached to this, in view of the pyrolytic nature of the process, but, when shortly afterwards we found that several polycyclic aromatic hydrocarbons were endowed with cancer-producing properties, we became interested in the hydrocarbons of this class which Diels had obtained by the selenium dehydrogenation of cholesterol.[19] Consideration of Diels's paper raised considerable doubts in my mind as to the correctness of the structures which he proposed for the two crystalline hydrocarbons which he had isolated ($C_{18}H_{16}$ and $C_{25}H_{24}$). In order to obtain further evidence, I had already carried out some experiments on the dehydrogenation of cholesterol when Rosenheim and King, in 1932, announced their new ring structure for the sterols and bile acids. We appreciated at once that this new formulation opened up the possibility of conversion of sterols and bile acids, by cyclisation of the side chain and then dehydrogenation, into hydrocarbons of the benzanthracene group. Kennaway and I published a Note to this effect.[20]



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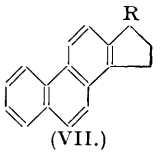

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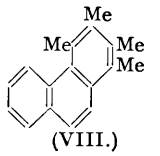

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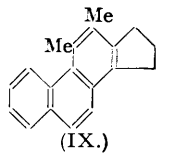

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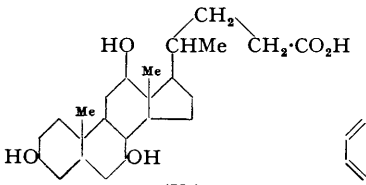

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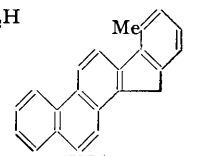

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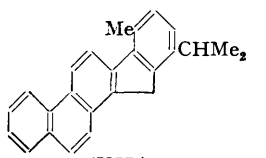

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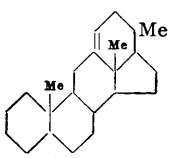

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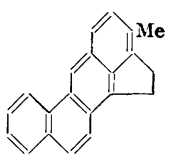

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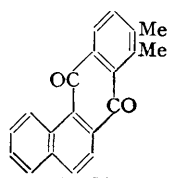

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It would be inappropriate to recapitulate here in detail the investigations which led to the clarification of the reactions involved in the dehydrogenation of sterols and bile acids. They are admirably summarised by Fieser and Fieser in their book.[21] There are, however, certain features which I should like briefly to discuss. The major crystalline product formed by selenium dehydrogenation ($C_{18}H_{16}$) consists essentially of 3'-methyl-1 : 2-*cyclo*pentenophenanthrene (VII; R = Me), formed by elimination of the side chain and migration of the methyl group at $C_{(13)}$ before dehydrogenation.[22] But the crude material is clearly a mixture and I have very little doubt that it contains also the parent 1 : 2-*cyclo*pentenophenanthrene (VII; R = H), which has in fact been obtained by dehydrogenation of *iso*rubijervine, one of the *Veratrum* alkaloids.[23] Biological tests with 1 : 2-*cyclo*pentenophenanthrene showed, as we expected, that this hydrocarbon was devoid of carcinogenic action.[24]

At a later stage in our investigations consideration of the structural relations among carcinogenic hydrocarbons derived from chrysene, 3 : 4-benzphenanthrene, and 1 : 2-benzanthracene led Hewett[25] to synthesise 1 : 2 : 3 : 4-tetramethylphenanthrene (VIII) which indeed proved to be weakly carcinogenic.[26] If one concedes the possibility of migration of methyl groups from $C_{(10)}$ and $C_{(13)}$ of the sterol molecule into the six-membered rings during dehydrogenation,* then it is possible to visualise the formation of 3 : 4-dimethyl-1 : 2-*cyclo*pentenophenanthrene (IX). This hydrocarbon might well have some carcinogenic activity and it would be of interest to synthesise it for biological test.

* For an example of migration of the methyl group at $C_{(10)}$ of a steroid under conditions of selenium dehydrogenation, see Inhoffen, Stoeck, and Kölling, *Chem. Ber.*, 1949, **82**, 263.

The C_{25} hydrocarbon which Diels [19] had obtained by dehydrogenation of cholesterol was clearly pentacyclic, the fifth ring being formed from the side chain; but it was evidently not a benzanthracene derivative. Ruzicka *et al.*[27] isolated a very similar hydrocarbon as a minor product of the selenium dehydrogenation of cholic acid (X), and we were able to show by synthesis that this was 5-methylnaphtho-(2′ : 1′-1 : 2)fluorene (XI).[28] This is evidently formed by cyclisation of the side chain on to the five-membered ring. If the C_{25} hydrocarbon from cholesterol were produced in the same way then it should be 5-methyl-8-*iso*propylnaphtho-(2′ : 1′-1 : 2)fluorene (XII). This was not the case as the cholesterol hydrocarbon was different from a hydrocarbon of this structure which we also synthesised.[29] But the two hydrocarbons were very similar in properties and in their absorption spectra, and the cholesterol hydrocarbon is obviously a naphthofluorene homologue. Its detailed structure still awaits elucidation.[30]

Success in our quest for a benzanthracene hydrocarbon related to the sterols came from another method of approach. Wieland, in 1925, had described the conversion of deoxycholic acid into a hydrocarbon, dehydronorcholene, which on the new sterol formulation was evidently represented by the formula (XIII).[31] At a Discussion Meeting of the Royal Society, held in the summer of 1933, I pointed out [32] that dehydrogenation of dehydronorcholene should lead to the hydrocarbon (XIV), which represents a benzanthracene derivative with substituents in positions favourable for carcinogenic activity. The experiment was made by my collaborator Haslewood, who found that with the side chain anchored at a second point dehydrogenation of dehydronorcholene took place smoothly to give the fully aromatic hydrocarbon (XIV) in very much better yield than is the case with hydrocarbons formed by direct dehydrogenation of sterols and bile acids. We established the structure of this new hydrocarbon by degrading it to 5 : 6-dimethyl-1 : 2-benzanthraquinone (XV), which we synthesised.[33] In the publication of our work we were to some extent anticipated by Wieland and Dane,[34] who also dehydrogenated dehydronorcholene to the same hydrocarbon (XIV) which they called methylcholanthrene.

The formation of methylcholanthrene in this way, and the establishment of its structure, furnished the first chemical proof of the position of the side chain in the sterols and bile acids. It was obtained also by a similar series of reactions from the more abundant cholic acid by Fieser,[35] who also synthesised it.[36] Another interesting method of formation was found by Rossner [37] who isolated methylcholanthrene in about 1% yield from the selenium dehydrogenation of a product of condensation of cholestanone with phenylhydrazine. This suggests that cholanthrene derivatives may also be formed in the direct dehydrogenation of cholesterol and I think that it would be worth while to examine the products further.

Chemical Carcinogens and Human Cancer.—Biological tests showed methylcholanthrene to be a very potent carcinogen. It is in fact one of the most active compounds known and has been extensively used by cancer research workers. It is very interesting that substances of such common occurrence in the animal body as cholesterol and the bile acids should be convertible by relatively simple, if somewhat drastic, reactions into a highly potent cancer-producing compound. Whether methylcholanthrene or some similar compound is responsible for initiating the spontaneous tumours in man and other animals is an open question. So far the presence of such substances in the body has not been demonstrated, but the possibility is being kept in mind. Inhoffen, for example, has recently discussed the possible stages of biochemical transformation of sterols and bile acids into cholanthrene derivatives.[38]

We do not know, of course, that methylcholanthrene is carcinogenic in man. It has been shown to produce tumours in a number of different species of animal, but there is known to be a wide species difference in the response to carcinogenic influences. Strong [39] has recently reported that in New Haven, rhesus monkeys have been injected with methylcholanthrene for 15 years without producing a single neoplastic tumour. This is the kind of factor that needs to be considered in attempting to assess the significance of the chemical carcinogens in relation to the general problem of cancer. We know, of course, that coal tar can cause skin cancer in man, as it does in mice, but not at all readily in rats. 3 : 4-Benzpyrene, which is a constituent of tar, can produce malignant tumours in mice, and it is, therefore, a reasonable presumption that it can do so in man. But there is no direct proof of this. The chemical carcinogens have been of great value in many investigations on cancer, but it is unlikely that this represents the full extent of their importance. It is very suggestive that by their use it is possible to produce at will, in a majority of treated animals, malignant tumours which simulate all the characteristics of human cancer. It has not been shown that chemical compounds are concerned in the origin of cancer of the internal organs of man, but the experimental work points to the possibility of this. How the compounds bring about the transformation of normal cells into malignant cells is entirely unknown, in spite of the attention that has been devoted to this problem. Once

this transformation has taken place the compound which has stimulated it is no longer necessary for the continued growth of the malignant cells.

There is a school of pathologists who find it necessary to postulate the need, not only for the "incitor" or "remote cause," but also for a "continuing cause" of cancer, which is thought to be required to maintain the growth of malignant tumours. Those who hold this view postulate further that this "cause" is a pathogenic virus, but hitherto have been singularly unsuccessful in demonstrating the presence of such a virus in mammalian cancers, if one excepts the Bittner milk factor, which is in a special category. Very considerable interest therefore attaches to the recent work of Gye and his collaborators [40] who found that tumours could be produced by inoculation of mice with mouse tumour tissue which had been kept at −79° and then dried. Gye claims that such preparations cannot contain living cells (which if inoculated would continue to grow) and that the new tumours are therefore produced by a virus. Dr. Gye's collaborator and successor, Dr. Craigie, is rightly much more cautious in his interpretation and stresses the need for more exact knowledge of the ability of the tumour cell to survive under various conditions.[41] This, I think, is where we must leave this matter, with the existence of the cancer virus still unproven.

Metabolism of Polycyclic Hydrocarbons.—I turn now to another phase in the study of carcinogenic hydrocarbons. This is the investigation of their fate in the animal body. Such work was originally undertaken in the hope that it would provide some clue as to the mechanism of carcinogenic action. It seems now that this hope is unlikely to be realised, but even so the results are of chemical interest. Partly because of the unavailability of the carcinogenic hydrocarbons in adequate quantity, parallel studies have been made with related non-carcinogenic hydrocarbons. The pioneer worker in this field was Boyland, but important contributions have also been made by Chalmers, by Berenblum and Schoental, and by Young, in the United Kingdom, and by Rhoads and his collaborators in the United States.

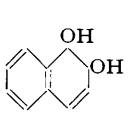
When polycyclic aromatic hydrocarbons are introduced into the animal body they may be partly excreted unchanged, but are mostly transformed into other compounds. Many of these have been isolated and identified, but they represent only a small proportion of the compound administered. The balance of the material is more extensively degraded, partly to unidentified acids.[42] The compounds which have been identified include diols formed by addition of hydroxyl groups to two adjacent positions, phenols which may or may not be formed through the intermediary of such diols, glucuronic acids derived from both these types, and mercapturic acids. The products which I shall discuss belong to the first two classes.

Diols have been isolated in the cases of naphthalene, anthracene, and phenanthrene. Young [43] isolated a lævorotatory diol (XVI) from the urine of rats after administration of naphthalene. It has been shown in my laboratory that this is a *trans*-diol.[44] We synthesised the compound (XVII) which is formed from it by hydrogenation. The diol (XVIII) from anthracene has been obtained in two forms, one of them optically active and the other inactive. Opinion has varied as to their configuration, but Booth and Boyland [45] have now concluded that they are both *trans*-diols. Our own work [44] supports this conclusion for the melting points of our synthetic (±)-*trans*-dihydroxytetrahydroanthracene and its diacetate agree with the values recorded by Boyland and Levi [46] for the dihydride of their inactive metabolite and its diacetate. In the case of phenanthrene, hydroxylation takes place at two bonds in the molecule. One product is dehydrated to 9-phenanthrol and appears to be the *trans*-9 : 10-diol (XIX); the other is dehydrated to 1-phenanthrol and is presumably the 1 : 2-diol (XX).[47] The evidence, therefore, indicates that these metabolic diols all have the *trans*-configuration. This is a point of some importance in connection with the mechanism of biochemical oxidation.

With the more complex hydrocarbons diols have not been isolated. They may be formed intermediately and dehydrated either *in vivo* or during extraction, but it is also possible that the phenols which have been isolated are formed by direct replacement of hydrogen. The structures established for the products are shown in the formulæ and show an interesting degree of regularity in the positions of attack. 1 : 2-Benzanthracene gives (XXI); [48] chrysene gives (XXII); [49] 1 : 2 : 5 : 6-dibenzanthracene in rats and mice gives (XXIII),[50] but a different, unidentified, diol in rabbits; [51] 3 : 4-benzpyrene gives (XXIV) and (XXV).[52, 54] The most interesting feature is that the points of attack are not the positions which nearly all chemical reagents show to be the most reactive.

The structures assigned by the biochemical workers to the benzpyrene metabolites (XXIV and XXV), which were different from the known 5-hydroxy-3 : 4-benzpyrene, were based on the relationship of these phenols to the red 5 : 8-quinone (XXVI) and the yellow 5 : 10-quinone (XXVII) which are formed by chromic acid oxidation of the hydrocarbon. These quinones,

which were also isolated from the animal excreta, were first prepared by Vollmann and his collaborators [53] who based their structures on analogy with the colours of the pyrenequinones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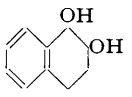
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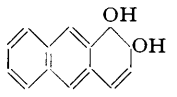
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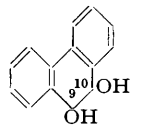
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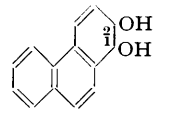
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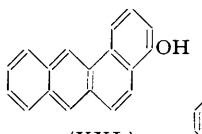
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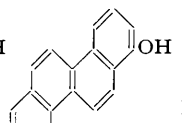
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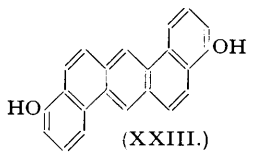
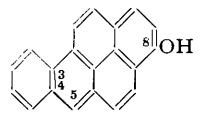

(XXIII.)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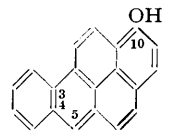
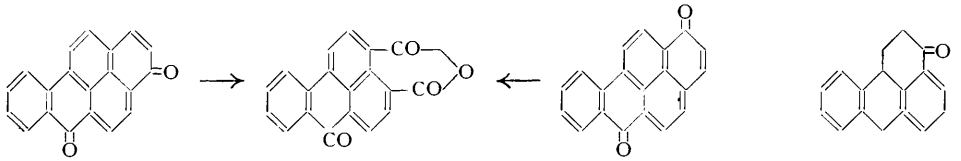
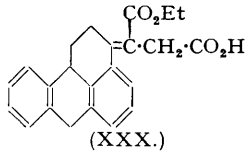
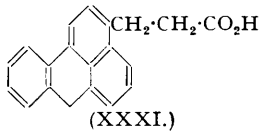

(XXV.)

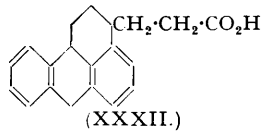
and on the fact that both benzpyrenequinones were oxidised to the same *meso*benzanthrone-3 : 4-dicarboxyanhydride (XXVIII). In view of the element of uncertainty about these results it was decided to synthesise 8-hydroxybenzypyrene (XXIV) by an unambiguous route. This has been successfully completed by Professor Ludwiczak and Dr. Schoental, under my supervision,[54] and the oxidation of the synthetic product to the red quinone (XXVI) has placed the results of the metabolic work on a surer foundation and has also confirmed the structures assigned to a series of 10-substituted benzpyrenes which have been related to the yellow quinone (XXVII).

A new type of synthesis of the benzpyrene ring system was necessary. The starting point was anthracene, which was converted, through 9-anthraldehyde, into the tetracyclic ketone


(XXVI.) (XXVIII.) (XXVII.) (XXIX.)


(XXX.)


(XXXI.)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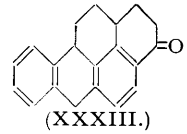
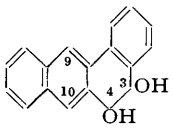

(XXXIII.)

(XXIX). This was condensed with ethyl succinate to give a half-ester (XXX) which underwent disproportionation and decarbethoxylation when heated with hydrobromic acid in acetic acid. The products were *meso*benzanthrenepropionic acid (XXXI) and its tetrahydride (XXXII). The value of hydroaromatic compounds as intermediates in the synthesis of polycyclic aromatic compounds is well illustrated here. The benzanthrene compound (XXXI) resisted cyclisation, probably on account of its ready oxidation to the corresponding *meso*benzanthrone, but the tetrahydro-compound (XXXII) was smoothly cyclised by hydrogen fluoride to the pentacyclic ketone (XXXIII). From this, by dehydrogenation with palladium-black in boiling 1-methylnaphthalene, the metabolic 8-hydroxybenzpyrene (XXIV) was obtained in excellent yield.

New Chemical Methods of Oxidation.—We have seen that biochemical oxidation of polycyclic hydrocarbons takes place at positions in the molecule which are not the most reactive ones, and may give diols by addition of hydroxyl groups. By the use of Criegee's osmium tetroxide reagent [55] Dr. Schoental and I have found a method which more closely resembles biochemical oxidation than any method previously studied. This reagent leads to addition at positions which are not those normally attacked by other oxidising agents or by cationoid reagents, to give esters of osmic acid which may be hydrolysed to diols. In two important respects the results of this process are different from those of biochemical oxidation. The positions of attack are different, and the diols, from Criegee's work, must have the *cis*-configuration whereas the

metabolic diols are *trans*-diols. This work has been published [56] and I need only add that in all the hydrocarbons which contain a phenanthrene system addition occurs at the *meso*-positions of this system. In the case of 1 : 2-benzanthracene, for instance, the diol formed by hydrolysis of the osmic ester has the structure (XXXIV). It might be thought that stereochemical factors prevent addition to the more reactive anthracene *meso*-positions, but this explanation will not hold in the case of pyrene, which gives the 1 : 2-diol (XXXV). In all other known reactions of pyrene except hydrogenation,[57] the first point of attack is position 3, and there is no stereochemical reason why osmium tetroxide should not add at the 3 : 4-bond. The presence of a phenanthrene system is not essential for the osmium tetroxide reaction. Anthracene gives a diester, which is hydrolysed to the tetrol (XXXVI).



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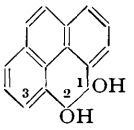

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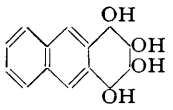

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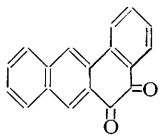
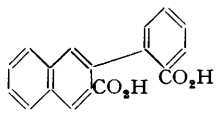


(XXXVII.)



(XXXVIII.)

We have extended this work on the oxidation of polycyclic aromatic hydrocarbons, and have examined the effect of the Milas reagent, which consists of hydrogen peroxide in *tert.*-butyl alcohol, catalysed by osmium tetroxide.[58] Here the reaction was much more complex and mixtures of products were formed. For example, in the case of 1 : 2-benzanthracene we isolated not only the diol (XXXIV), but also the 3 : 4-quinone (XXXVII), the dicarboxylic acid (XXXVIII) which it gives on further oxidation, the 9 : 10-quinone, and phthalic acid. The considerable quantities of products which involve primary attack at the 3 : 4-positions indicate the intermediate formation of a cyclic osmic ester. Simultaneous formation of the 9 : 10-quinone indicates clearly that part of the Milas reagent attacks the hydrocarbon molecule by another mechanism. This may be an oxidation by means of free radicals, but there is at present no specific evidence to support this view.

Recent work by Weiss and Waters and their respective collaborators [59] has shown that free hydroxyl radicals can hydroxylate aromatic nuclei to give phenols as is indeed also the case with the Milas reagent.[60] So far, however, there is no evidence that free hydroxyl radicals can add in pairs to an aromatic double bond to give diols such as are formed in biochemical oxidation.

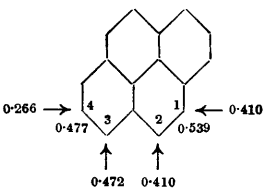
Oxidation of olefins with hydrogen peroxide in presence of certain catalysts has been reported to give *trans*-α-diols. This result has been obtained with selenium dioxide,[61] pervanadic acid,[62] and pertungstic acid.[62a] Oxidation may take place through the intermediary of epoxides, which are known to give *trans*-diols on hydrolysis. Epoxides are formed by oxidation of olefins with organic per-acids, and Eckhardt [63] has shown that many polycyclic aromatic hydrocarbons are oxidised by perbenzoic acid. But recent re-investigation of this reaction by Waters [64] has given no evidence of the formation of epoxides. The products isolated were the result of oxidation at the usual reactive centres; for example, 1 : 2-benzanthracene gave the 9 : 10-quinone and there was no evidence of oxidation at the 3 : 4-positions. A purely chemical method for the formation of *trans*-diols by oxidation of aromatic hydrocarbons has yet to be found. Our knowledge of the biochemical oxidation of these hydrocarbons is still rudimentary.

Electron Distribution in Polycyclic Aromatic Hydrocarbons.—It has been suggested by the Pullmans [65] that the carcinogenic activity of polycyclic aromatic hydrocarbons is dependent on an optimum density of π-electrons at the phenanthrene *meso*-positions, which they term the "*K*" region. A similar idea seems to have been entertained by Sir Robert Robinson,[66] but he has not published a detailed account of his views or of the experiments carried out in his laboratory to test them.[67] By the application of quantum-mechanical pinciples the Pullmans calculated the electron densities at the "*K*" positions of a series of carcinogenic compounds and related non-carcinogenic substances, and suggested that the values so obtained could be correlated with the carcinogenic potencies. Badger [8, 69] has discussed their data and has drawn attention to numerous anomalies. It seems to me that it is difficult to come to any conclusion as to the validity of these correlations. There are so many factors which can lead to error. I have already remarked on the uncertainties in the assessment of carcinogenic potency, and there is also the circumstance that many compounds have been assayed with too few animals for the estimate of carcinogenic potency to have statistical significance. There are also elements of uncertainty in the calculation of electron densities. The values obtained vary with the method of calculation, and in each of these it is necessary to make approximations. In fact,

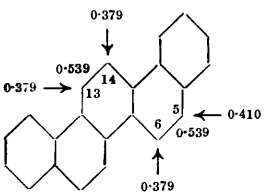
Daudel [68] has remarked, rather naively, that " it seems that the accord between theory and experiment is less good in the case in which the evaluation of the charge is more correct."

I think that the most important outcome of the work on the addition of osmium tetroxide to aromatic hydrocarbons is that it has provided a method of quantitative estimation of chemical reactivity at the " *K* " region of the hydrocarbons which contain a phenanthrene system. For it is at these positions that addition takes place. The osmic esters are highly coloured and their pyridine complexes are soluble in many organic solvents. These properties have been utilised by Badger and Reed [69] to follow colorimetrically the rates of reaction with osmium tetroxide and thus to obtain comparative values of the chemical reactivity at the " *K* " region. These values may be compared with the theoretical calculations of electron densities.

Using a new method of calculation [70] Daudel and his collaborators [71] have sought to correlate the calculated values of indexes of free valency and bond orders of the various positions of a series of polycyclic aromatic hydrocarbons with their positions of chemical reactivity. The results are very suggestive. They interpret, for example, the circumstance that in pyrene (XXXIX) substitution takes place at position 3, which is endowed with the highest index of free valency, whereas addition takes place at the 1 : 2-bond. This bond has the highest bond-order, and the index of free valency at these positions is also relatively high. It is interesting that in all the examples cited the phenanthrene 9 : 10-bond is the one with the highest bond-order, and as we have seen, this is the position at which addition of osmium tetroxide invariably takes place. Another noteworthy example is picene (XL). The French authors had evidently overlooked my work [72] which showed that picenequinone is the 5 : 6-quinone, which their calculations would predict, and not the 13 : 14-quinone, which it was formerly thought to be.

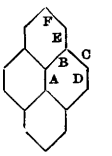


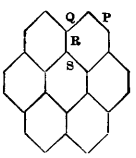
(XXXIX.)



(XL.)

The electron-density calculations also enable computations to be made of the interatomic distances. As many of these have been determined experimentally with great accuracy from *X*-ray crystallographic data they provide another means of checking the results of theoretical calculation. The following table gives in column II the values of bond-lengths, determined by Robertson and White for pyrene [73] and coronene.[74] Column III gives the values calculated by Vroelant and Daudel,[70] and column IV the values calculated by Moffit and Coulson,[75] using the method of molecular orbitals. The agreement is very satisfactory.





Bond.	II.	III.	IV.
A	1·45	1·425	1·412
B	1·39	1·435	1·415
C	1·45	1·415	1·42
D	1·39	1·38	1·37
E	1·42	1·415	1·401
F	1·39	1·39	1·388
P	1·385	1·38	1·372
Q	1·415	1·415	1·411
R	1·43	1·435	1·411
S	1·43	1·425	1·415

Conclusion.—I have attempted in this lecture to give an account of some of the respects in which the study of polycyclic aromatic hydrocarbons has contributed to general chemical knowledge and to the furtherance of research on cancer. Many important developments have been taking place to which I have been unable to refer. The work of Dr. Clar, now working in my laboratory in Glasgow, has established some very interesting correlations between the structures of polycyclic hydrocarbons and their absorption spectra. He has recently summarised this

work.[76] I should have liked to have been able to discuss some of the problems associated with "intramolecular overcrowding" in certain polycyclic structures. I drew attention to some of these problems in an article in the *Annual Reports* for 1942. Recently there have been interesting developments, notably as a result of the work of Newman [77] and Bell [78] who have demonstrated the existence of molecular asymmetry due to distortion of sterically hindered molecules.

The field of polycyclic aromatic hydrocarbons is somewhat specialised. I am sure that it is one in which there are still rich new harvests to be garnered.

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