106. *The Isolation of a Cancer-producing Hydrocarbon from Coal Tar. Parts I, II, and III.*

By J. W. Cook, C. L. Hewett, and I. Hieger.

Part I. Concentration of the Active Substance. By I. Hieger.

The first experimental production of cancer by the products of distillation of coal was achieved in 1915 by Yamagiwa and Ichikawa (*Mitteil. med. Facultät, kaiser. Univ. Tokyo*, 1915, **15**, 295), and the first enquiry into the chemical basis of tar cancer was that of Bloch and Dreifuss (*Schweiz. med. Woch.*, 1921, **2**, 1033), who concluded from their observations that the substance responsible must be a high-boiling, neutral, non-nitrogenous compound which forms a stable picrate. The production of strongly carcinogenic tars by pyrolysis of isoprene or acetylene in a current of hydrogen (Kennaway, *J. Path. Bact.*, 1924, **27**, 233; *Brit. Med. J.*, 1925, ii, 1) showed that the agent present in these tars must be a hydrocarbon. A large number of known constituents of fractions of coal and other tars boiling above 270° were tested in this Institute with negative results. The use of the fluorescence spectrum, first applied to this problem by Mayneord in 1927, was continued by the present author (*Biochem. J.*, 1930, **24**, 505) with observations on a specific fluorescence spectrum (bands at 4000, 4180, 4400 Å.) common to the tars and mineral oils which produce cancer of the skin either as an industrial disease or in animal experiments.

These spectra suggested an attempt to isolate the fluorescing substance, whether this were the cancer-producing factor itself or some substance which closely accompanies this factor. The results of spectrographic and biological tests have now proved to be in complete agreement. Thus the preparations derived from pitch by a variety of solvent extraction methods, the fractions separated by formation of picrates, distillation and crystallisation, and finally the hydrocarbon (1 : 2-benzpyrene) have proved carcinogenic and have given in each case the fluorescence spectrum with bands at 4000, 4180, 4400 Å. The treatment of pitch described here has yielded fractions which are far superior to pitch as cancer-producing agents and at the same time possess the specific fluorescence spectrum in an enhanced degree.

Hence the fluorescence test can predict the behaviour of fractions of pitch when tested on animals, although it must be pointed out that certain cancer-producing hydrocarbons prepared synthetically (*e.g.*, 1 : 2 : 5 : 6-dibenzanthracene) have fluorescence spectra different from those of active pitch fractions.

Experimental.

Two tons of a medium soft pitch (see fluorescence spectrum I) which was shown to be cancer-producing (see below) were distilled from a cast-iron retort. The distillate above 510° (thermometer in liquid) resembled the deep red glassy distillate of pitch which was found some years ago to be carcinogenic (Kennaway, *Brit. Med. J.*, 1924, i, 564). The fraction, b.p. 500—510°, resembled very soft pitch and was much more sol. in ordinary solvents than the fraction, b. p. above 510°. A fraction with the same physical characteristics distilled at 475—500° (pyrometer instead of thermometer); yield, about 5% of the charge of pitch. From this fraction, boiling EtOH (20 vols.) (cf. Twort and Fulton, *J. Path. Bact.*, 1930, **33**, 119) extracted (paddle stirrer and reflux condenser) about one-third, which was distilled under 3·5 mm. press. The fraction, b. p. 250—290°, when tested on mice, proved to be strongly carcinogenic and its fluorescence spectrum showed the three bands already referred to. On redistillation three fractions were collected at 3·5 mm.: A, b. p. 250—260°; B, 260—278°; C, 278—290°. Fraction A, on purification, was rapidly freed from the substance responsible for the characteristic fluorescence spectrum of the original distillate; fraction B, which gave this spectrum (spectrum II) and was strongly carcinogenic, was found most convenient for the subsequent operations; while fraction C, although strongly carcinogenic, was discarded on account of its unsuitable physical properties.

Fraction B was dissolved, together with 1 part of picric acid, in 3·5 parts of AcOH, the picrates recrystallised twice from C_6H_6 (2 parts), the mother-liquors of which were decomposed by

shaking with dil. Na_2CO_3 aq., and the product again distilled in vac. The distillate crystallised on standing, and after recrystn. from light petroleum had m. p. 114—117°, raised to about 160° by further crystn. from C_6H_6–EtOH.

The yields obtained in the various stages were subject to considerable fluctuation, but were on the average as follows: distillation of pitch, 5%; alcohol extraction, 30%; fractional distillation in vac., 20%; isolation of cryst. material (m. p. 160°), 1%.

A 3% solution in C_6H_6 of the original pitch applied to a series of 20 mice produced cancer in 8 of the 9 mice which lived for more than 300 days. Hence it appears unlikely that the carcinogenic compound obtained from this pitch was formed by heating in the course of the initial distillation. The approximate yields given above indicate that the cryst. substance, m. p. 160° (crude 1 : 2-benzpyrene), would be present in the original pitch in an amount of not less than 0·003%; the actual proportion is probably greater, as the experimental losses are naturally large.

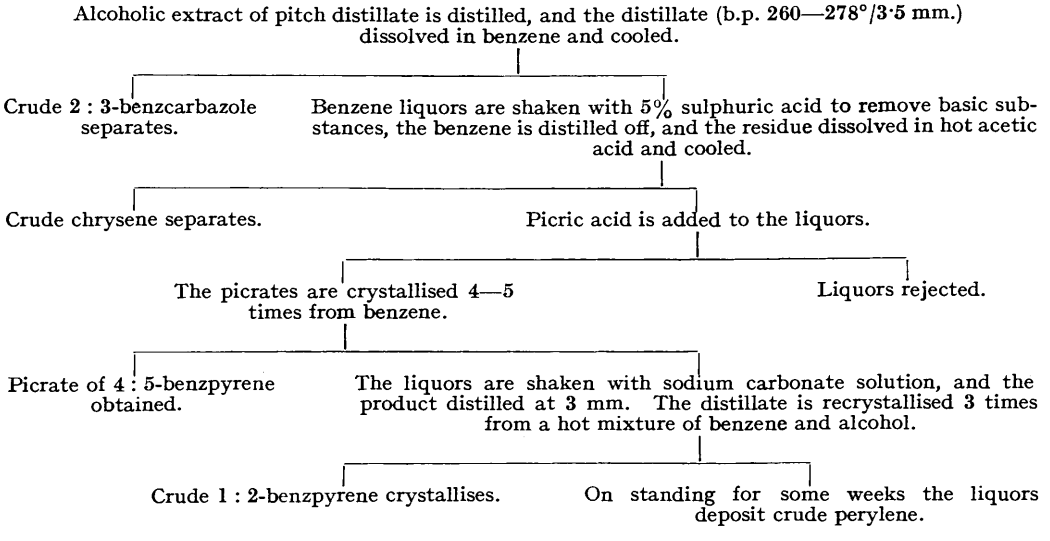
The fluorescence spectra of the products at various stages (see plate) show clearly how the typical bands of the spectrum of the original carcinogenic mixtures become more well-defined with increasing concn. of the active material.

Part II. Isolation of 1 : 2- *and* 4 : 5-*Benzpyrenes, Perylene, and* 1 : 2-*Benzanthracene.*

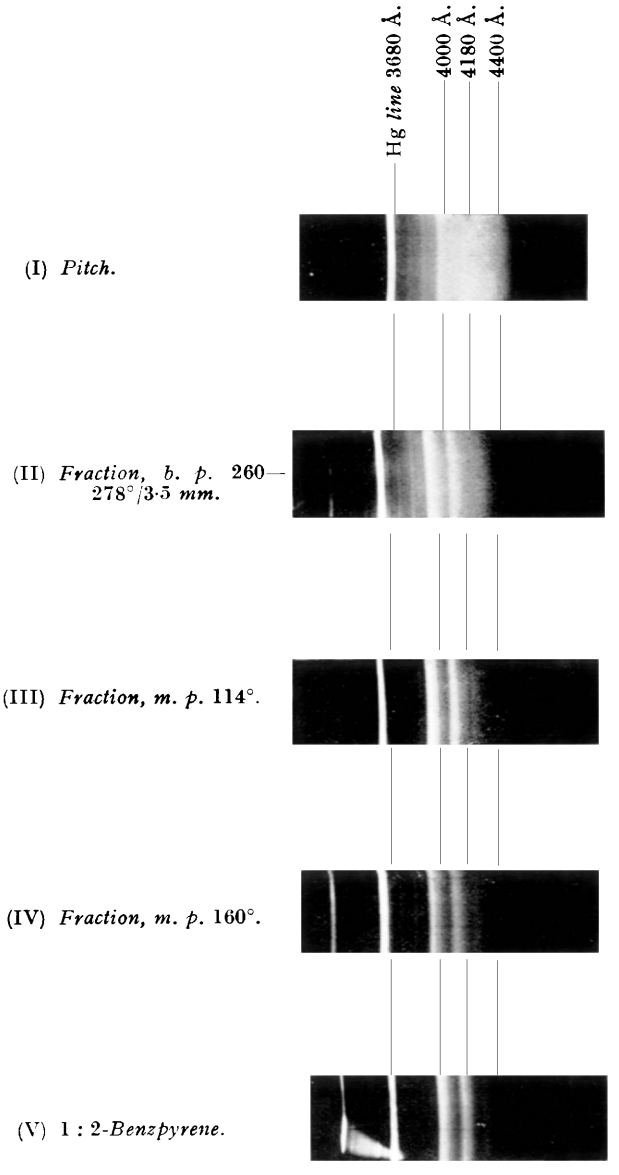
By J. W. Cook, C. L. Hewett, and I. Hieger.

When the crystalline material, m. p. *ca.* 160°, prepared as described in Part I, was obtained, it was difficult to form an opinion as to whether it was essentially a single substance or a complex mixture. Unfruitful attempts were made to devise chemical methods of separation by means of bromo- and nitro-derivatives, and of products of oxidation and reduction. It was then found that by repeated crystallisation of the picrate from benzene, a picrate, m. p. 198°, could be obtained which yielded a hydrocarbon, m. p. 175·5—176·5°, shown by comparison with the synthetic compound (Part III) to be pure 1 : 2-benzpyrene. This pure hydrocarbon was strongly carcinogenic and still retained the typical fluorescence spectrum to which reference has been made.

For the isolation of this and other constituents of the alcoholic extract of distilled pitch, a procedure was ultimately evolved which is briefly illustrated by the following scheme :—



The identification of the 2 : 3-benzcarbazole was effected by analysis and by the preparation of characteristic derivatives. This benzcarbazole was first isolated from crude anthracene by Graebe and Knecht (*Ber.*, 1879, **12**, 341; *Annalen*, 1880, **202**, 1) and was afterwards synthesised by Bucherer and Sonnenburg (*J. pr. Chem.*, 1910, **81**, 1). Chrysene and perylene were identified by their properties and by comparison with authentic


Hg *line* 3080 Å.
4000 Å.
4180 Å.
4400 Å.
(I) *Pitch.*
(II) *Fraction, b. p.* 260—278°/3·5 *mm.*
(III) *Fraction, m. p.* 114°.
(IV) *Fraction, m. p.* 160°.
(V) 1 : 2-*Benzpyrene.*

[*To face p.* 396.

samples. The presence in coal tar of perylene, the parent substance of a large group of important vat dyes, does not appear to have been recorded previously.

The picrate of 4 : 5-benzpyrene, obtained as shown in the scheme, had m. p. 235°, and yielded the hydrocarbon, m. p. 181—187°, which was identified by synthesis (Part III) as 4 : 5-benzpyrene. It has been applied to mice for ten months without producing tumours.

1 : 2-Benzanthracene was isolated from the chrysene fraction of coal tar by making use of the fact that it forms an acidic adduct with maleic anhydride (Clar, *Ber.*, 1932, **65**, 519), whereas chrysene does not. The purified 1 : 2-benzanthracene-9 : 10-*endo*-αβ-succinic anhydride agreed in its properties with the compound obtained by Clar. By vacuum sublimation it was dissociated into its components, the 1 : 2-benzanthracene being identified by comparison with an authentic sample and by oxidation to the quinone. This isolation of 1 : 2-benzanthracene from coal tar is of interest, for, although it has hardly any cancer-producing action itself, it is the parent substance of a considerable group of carcinogenic hydrocarbons.

Experimental.

Separation of Pitch Constituents.—A hot solution of the distillates (400 g.) from the original alc. extract (see scheme) in C_6H_6 (1 l.) was cooled, and the resulting crystals (about 5—10 g.) were collected and recrystallised several times from xylene. A solution of the product in xylene was boiled for 40 min. with maleic anhydride, by which means most of the colour was removed, the 2 : 3-benzcarbazole being obtained as colourless leaflets, m. p. 339—340° (from xylene). This substance, which contained nitrogen (Found : C, 88·4; H, 5·4. Calc. : C, 88·4; H, 5·2%), gave with 2 : 7-dinitroanthraquinone a characteristic *compound* which separated from xylene in dark green needles, m. p. 291—294° (decomp.) (Found : C, 69·6; H, 3·6; N, 7·9. $C_{30}H_{17}O_6N_3$ requires C, 69·9; H, 3·3; N, 8·2%). The identification of 2 : 3-benzcarbazole was completed by the preparation of the acetyl derivative described by Graebe and Knecht (*loc. cit.*).

The C_6H_6 liquors containing the bulk of the pitch distillate were diluted to 2·5 l. and agitated with 5% H_2SO_4, whereby much tarry basic material was removed. The C_6H_6 was distilled off, and the residue dissolved in AcOH (2 l.). On cooling, a small amount of cryst. material separated. Pure chrysene (about 1·5 g.) could be isolated by partial oxidation of this substance (1 pt.) with $Na_2Cr_2O_7$ (2 pts.) in boiling AcOH (50 pts.) for 1 hr., the contaminating benzcarbazole being destroyed by oxidation. After crystn. from C_6H_6 the chrysene was obtained as colourless leaflets, m. p. 248—249° alone or mixed with an authentic sample. Fresh treatment with $Na_2Cr_2O_7$ in AcOH for 7 hr. gave 1 : 2-chrysenequinone.

The main AcOH solution was treated with picric acid (400 g.) and the picrates were collected, dried, and recrystallised 5 times from a large vol. of C_6H_6. The solid picrates were reduced in this way to a very small amount of material, which was treated with aq. Na_2CO_3, and the product (about 5 g.) distilled in vac. and recrystallised from xylene–EtOH and then from C_6H_{12} (Found : C, 95·2, 95·25; H, 4·6, 4·7; *M*, Rast method, 254, 273. $C_{20}H_{12}$ requires C, 95·2; H, 4·8%; *M*, 252). This substance formed small yellow plates, m. p. 181—187°, was unchanged by boiling in xylene solution with maleic anhydride, and was shown by comparison with a synthetic sample to be 4 : 5-*benzpyrene*. The indefinite m. p. was due to a trace of high-melting impurity (possibly perylene) which could not be removed. The *picrate* formed reddish-brown needles (from C_6H_6), m. p. 235° (Found : C, 65·7; H, 3·1; N, 8·4. $C_{20}H_{12},C_6H_3O_7N_3$ requires C, 64·9; H, 3·1; N, 8·7%).

The main C_6H_6 liquors containing the bulk of the picrates were shaken with aq. Na_2CO_3, and the product distilled in vac. The distillate, after 3 recrystns. from C_6H –EtOH, gave 24 g. of crude 1 : 2-benzpyrene, m. p. about 160°.

This substance (10 g.) was recovered unchanged after a solution in xylene had been boiled for 5 hr. with maleic anhydride, and was treated with picric acid (10 g.) in C_6H_6. The *picrate*, m. p. 188—192°, was recrystallised 9 times from C_6H_6 and then formed dark purplish-brown needles (1 g.), m. p. 198° (Found : C, 65·1; H, 3·2; N, 9·0. $C_{20}H_{12},C_6H_3O_7N_3$ requires C, 64·9; H, 3·1; N, 8·7%). 1 : 2-*Benzpyrene*, obtained by shaking a C_6H_6 solution of this picrate with aq. Na_2CO_3, was recrystallised from C_6H_6–EtOH and formed yellow leaflets (from conc. solution) or needles (from dil. solution), m. p. 175·5—176·5° (Found : C, 95·0; H, 4·8; *M*, Rast method, 288, 269. $C_{20}H_{12}$ requires C, 95·2; H, 4·8%; *M*, 252).

After several weeks the combined C_6H_6–EtOH liquors from the crude 1 : 2-benzpyrene deposited crystals which, recryst. twice from xylene, formed canary-yellow needles (0·2 g.), m. p. 263—266°, not depressed by an authentic sample of perylene. The identification with perylene was completed by comparison of the colour reactions with H_2SO_4, and of the fluorescence spectra.

Isolation of 1 : 2-*Benzanthracene* (Experiments by F. GOULDEN).—The crude mixture of solid substances which separated from a tar fraction, b. p. above 400°, supplied by the South Metropolitan Gas Company (cf. Cook, J., 1931, 498) was recrystallised 3 times from xylene, whereby a cryst. substance was obtained which consisted chiefly of chrysene. The xylene liquors were concentrated in vac., the solid which crystallised was dissolved in C_7H_8, and the solution shaken several times with conc. H_2SO_4. The material recovered from the C_7H_8 liquors (20 g.) was heated under reflux for 2 hr. with maleic anhydride (5 g.) in xylene (250 c.c.). The xylene was removed in steam in presence of excess NaOH, and the filtered solution acidified. The ppt. was collected and recrystallised from AcOH, forming a cryst. colourless powder, m. p. 236° (cf. Clar, *loc. cit.*). This anhydride was sublimed at 300°/5 mm., and the sublimate recrystallised from EtOH, forming colourless leaflets, m. p. 158·5—159·5°, not depressed by 1 : 2-benzanthracene. Oxidation with $Na_2Cr_2O_7$ in AcOH gave orange needles of 1 : 2-benzanthraquinone.

Part III. Synthesis of 1 : 2- *and* 4 : 5-*Benzpyrenes.* By J. W. COOK and C. L. HEWETT.

THE condensed-ring aromatic hydrocarbons studied in this Institute during recent years have been mostly of the anthracene or phenanthrene type, the former giving acidic adducts with maleic anhydride, the coloured derivatives of *lin.*-benzanthracene reacting with remarkable facility, and passing on oxidation into *p*-quinones which give deep red vats with alkaline reducing agents. Hydrocarbons of the phenanthrene type, on the other hand, are oxidised to *o*-quinones and do not react with maleic anhydride. From this standpoint, the behaviour of the yellow carcinogenic hydrocarbon isolated from coal tar pitch was anomalous, for it was oxidised to a dark-coloured amorphous substance, apparently a mixture of an acid with a quinone which gave an orange vat, whereas maleic anhydride was without action on the hydrocarbon. These and other properties recalled the behaviour of perylene, for at that time we believed that maleic anhydride did not react with perylene, although Clar (*Ber.*, 1932, **65**, 846) has subsequently shown that it does so under suitable conditions. In addition, analysis of the pure carcinogenic hydrocarbon and its picrate pointed to an isomeride of perylene. If all of the rings are benzenoid, there are only two possible isomerides, namely, the benzpyrenes. As the amount of material at our disposal for study of degradation products was small, and as we wished in any case to compare these pentacyclic compounds with the 10 known out of the 15 possible hydrocarbons composed of 5 benzene rings, which we have already examined for carcinogenic activity, we decided to synthesise the two benzpyrenes. This was accomplished, and 1 : 2-benzpyrene was identical with the carcinogenic hydrocarbon isolated from pitch, while 4 : 5-benzpyrene was identical with the other new hydrocarbon which we had obtained from pitch.

In our opinion, the hydrocarbon, m. p. 183°, isolated by Meyer and Taeger (*Ber.*, 1920, **53**, 1261) from the products of pyrogenic synthesis from acetylene, for which they suggested the formula $C_{17}H_{10}$ or $C_{17}H_{12}$, was also 4 : 5-benzpyrene; the properties and analyses which they record are in agreement with our suggestion, although their value for the molecular weight was somewhat low.

The identification of the carcinogenic hydrocarbon from pitch with synthetic 1 : 2-benzpyrene was established by comparison of the hydrocarbons and of their picrates, and also of the fluorescence and absorption spectra of the hydrocarbons. We are indebted to Mr. W. V. Mayneord and Miss Woodroffe for the absorption curves (absorption maxima at 2580, 2843, 2965, 3636, and 3821 Å.), which will be published elsewhere. It is perhaps significant that the fluorescence spectrum of 1 : 2-benzpyrene is of exactly the same type as that of 1 : 2-benzanthracene, which is the parent substance of the group of carcinogenic hydrocarbons already described (Cook, Hieger, Kennaway, and Mayneord, *Proc. Roy. Soc.*, *B*, 1932, **111**, 455; Cook, *ibid.*, p. 485), but is different in character from the spectra

of chrysene and pyrene (Hieger, unpublished experiments), although the 1 : 2-benzpyrene molecule contains the ring system of each of these three hydrocarbons.

The synthetic 1 : 2-benzpyrene gave malignant tumours of the skin of mice just as rapidly as the material isolated from pitch. Thus, in a preliminary series of 10 mice painted with the synthetic material, 5 died very early and the remaining 5 all developed tumours (4 epitheliomas and 1 papilloma); in one mouse there was a glandular metastasis in the right axilla. These and subsequent series of experiments on mice, for which we are indebted to Professor E. L. Kennaway and which will be described elsewhere, suggested that 1 : 2-benzpyrene produces tumours in about half the time required by 1 : 2 : 5 : 6-dibenzanthracene, so that 1 : 2-benzpyrene is the most active carcinogenic compound yet known.

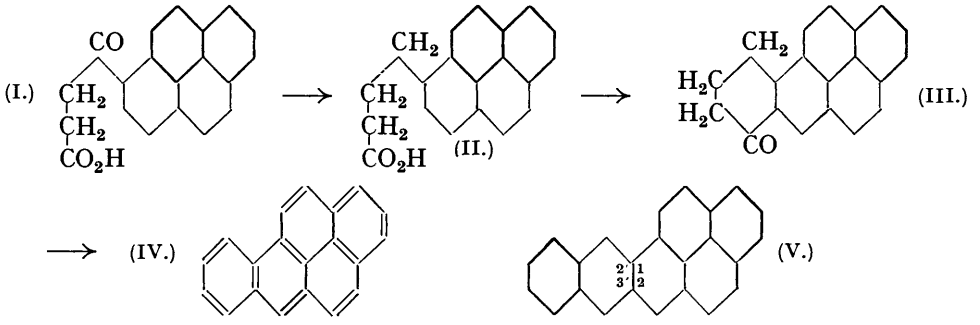
For the synthesis of the 1 : 2-benzpyrene ring system we at first proposed to apply the Scholl *peri*-condensation to a benzoyl derivative of pyrene ketone (Bamberger and Philip, *Annalen*, 1887, **240**, 178) or of 1 : 8-malonylnaphthalene (Fleischer and Retze, *Ber.*, 1922, **55**, 3284), but the methods described for the preparation of these materials gave unpromising results. In the absence, however, of an available source of pyrene, which we ultimately used as our starting point, 1 : 2-benzpyrene could probably be synthesised in the way outlined above, using the dihydropyrene ketone obtained by Mayer and Sieglitz (*Ber.*, 1922, **55**, 1839) from 1-naphthylpropionic acid.

The claim that succinic anhydride condensations with polycyclic compounds give much better yields in nitrobenzene solution (Meister Lucius und Brüning, D.R.-P., 376,635) than in other solvents (Borsche and Sauernheimer, *Ber.*, 1914, **47**, 1645) prompted us to use this solvent for the condensation of pyrene with succinic anhydride, which led to β-1-*pyrenoylpropionic acid* (I). Succinic anhydride condensations in nitrobenzene have more recently been successfully employed by Haworth (J., 1932, 1125) in the case of naphthalene, and by Fieser and Peters (*J. Amer. Chem. Soc.*, 1932, **54**, 4347; cf. I. G. Farbenind. A.-G., *Eng. Pat.* 274,095) in the case of acenaphthene.

The position assigned to the substituent in β-1-pyrenoylpropionic acid is based on analogy with the products formed from pyrene and aroyl chlorides (Scholl and Seer, *Annalen*, 1912, **394**, 160). This assumption is substantiated by the fact that the benzpyrene subsequently obtained from the pyrenoylpropionic acid was unquestionably the 1 : 2-compound, being different from 4 : 5-benzpyrene prepared by a method which established its structure. It is important that in some cases (*e.g.*, 2-methyl- and 2 : 3-dimethyl-naphthalenes) the position of substitution in Friedel–Crafts reactions depends upon the nature of the acid chloride or anhydride. With the aroyl chlorides and anhydrides the 1-position is exclusively attacked (Scholl and Tritsch, *Monatsh.*, 1911, **32**, 999; Clar, *Ber.*, 1929, **62**, 350; Cook, J., 1932, 456), whereas with acyl chlorides and anhydrides the substituent mainly enters position 6 (Haworth, Letsky, and Mavin, J., 1932, 1784; Dziewoński and Brand, *Rocz. Chem.*, 1932, **12**, 693). By selecting a number of test cases which need not be enumerated, we have found that this difference in behaviour is not due to the solvent employed. Probably it is determined by the enhanced mobility of acyl groups, position 1 being primarily attacked in all cases, acyl groups then migrating to position 6. Support for this view is afforded by the observations of Fieser and Peters (*loc. cit.*) that β-1-naphthoylpropionic acid is converted into the 2-isomeride by melting with aluminium chloride–sodium chloride, conditions which suffice for the migration of the less mobile aroyl group from position 1 to positions 6 and 7 in the case of 1-aroyl derivatives of 2-methylnaphthalene (Fieser and Peters, *J. Amer. Chem. Soc.*, 1932, **54**, 3742).

The sodium salt of 1-pyrenoylpropionic acid (I) was rapidly reduced by zinc dust and ammonia to the hydroxy-acid (isolated as the *γ-butyrolactone*), which by prolonged reduction passed into γ-1-*pyrenylbutyric acid* (II). After many unsuccessful attempts by other means, ring closure of the butyric acid to 4′-*keto*-1′ : 2′ : 3′ : 4′-*tetrahydro*-1 : 2-*benzpyrene* (III) was achieved by zinc chloride at 180°. The yields, however, were very poor and uncertain, and much better results were obtained by the action of anhydrous stannic chloride at 115—120°. This reagent has frequently been used for dehydrations, but as far as we are aware has not hitherto been employed for ring closures of this type.

The keto-compound (III) gave 1 : 2-*benzpyrene* (IV) when heated with selenium at 320—340°. The reaction is essentially dehydration, and the selenium plays the part of an intramolecular hydrogen carrier, since benzpyrene was not isolated when the keto-compound was heated alone. The conversion of the keto-compound into the hydrocarbon without previous reduction contrasts with the observation of Lévy (*Compt. rend.*, 1932, **194**, 1749), who obtained 2-ethyl-1-naphthol and not 2-ethylnaphthalene by selenium dehydrogenation of 1-keto-2-ethyl-1 : 2 : 3 : 4-tetrahydronaphthalene. A better yield of 1 : 2-benzpyrene was obtained by reduction prior to the selenium treatment.



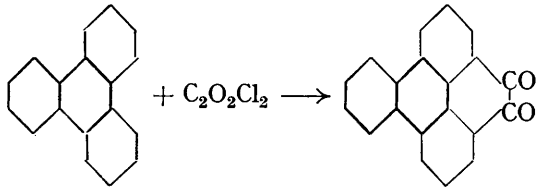
The average yields obtained in the four stages of this synthesis were 61, 72, 19, and 35% respectively, so that the yield of 1 : 2-benzpyrene was 2·9% with reference to the original pyrene.

By fusion of the keto-acid (I) with zinc chloride, the side chain was eliminated and pyrene formed, and fission was also effected by boiling with 10% sulphuric acid in acetic acid. This lability is similar to that shown by the *meso*-ketones of anthracene (Krollpfeiffer, *Ber.*, 1923, **56**, 2364; Cook, J., 1926, 1287, 2163) and suggests that position 1 of the pyrene ring system is associated with a considerably higher degree of reactivity than is normal for an aromatic ring. Even the butyric acid (II) gave evidence of lability, for pyrene was isolated from the mother-liquors of the keto-compound (III) obtained by dehydration with stannic chloride.

2′ : 3′-*Naphtha*-1 : 2-*pyrene* (V) was prepared for comparison with 1 : 2-benzpyrene, and although this hydrocarbon has been applied to mice for 8 months it has not so far yielded any tumours, with 18 out of the original 20 mice still living. This influence of an additional benzene ring in suppressing carcinogenic activity finds a counterpart in certain benz-derivatives of 1 : 2 : 5 : 6-dibenzanthracene (Cook, Hieger, Kennaway, and Mayneord, *loc. cit.*). The hexacyclic hydrocarbon (V) was obtained from pyrene and phthalic anhydride, the *phthaloylic acid* being reduced to 1-o-*carboxybenzylpyrene*, which was dehydrated to the corresponding *anthrone*, the latter giving the hydrocarbon on reduction. The same naphthapyrene (V) was also formed by pyrolysis of the crude mixture of ketones formed from pyrene and *o*-toluoyl chloride. This naphthapyrene, in common with other naphthacene compounds, was deep orange in colour, and reacted with maleic anhydride in boiling xylene to give 2′ : 3′-*naphtha*-1 : 2-*pyrene*-1′ : 4′-endo-αβ-*succinic anhydride*.

For the synthesis of 4 : 5-benzpyrene we first attempted to prepare 4-phenylphenanthrene, which should be convertible into the pentacyclic hydrocarbon by the Scholl *peri*-dehydrogenation (*e.g.*, with aluminium chloride or selenium). In no way, however, could we effect condensation between 4-keto-1 : 2 : 3 : 4-tetrahydrophenanthrene and phenylmagnesium bromide or *cyclo*hexylmagnesium chloride, all attempts leading to reduction of the ketone to the corresponding *carbinol*. This is analogous to the observation of Sabatier and Mailhe (*Compt. rend.*, 1905, **141**, 298) that benzophenone and di*cyclo*hexyl ketone are reduced to the carbinols by *cyclo*hexylmagnesium chloride, but is nevertheless surprising, for Haworth (J., 1932, 1125) has shown that 4-keto-1 : 2 : 3 : 4-tetrahydrophenanthrene reacts normally with methylmagnesium iodide, a finding which we confirmed.

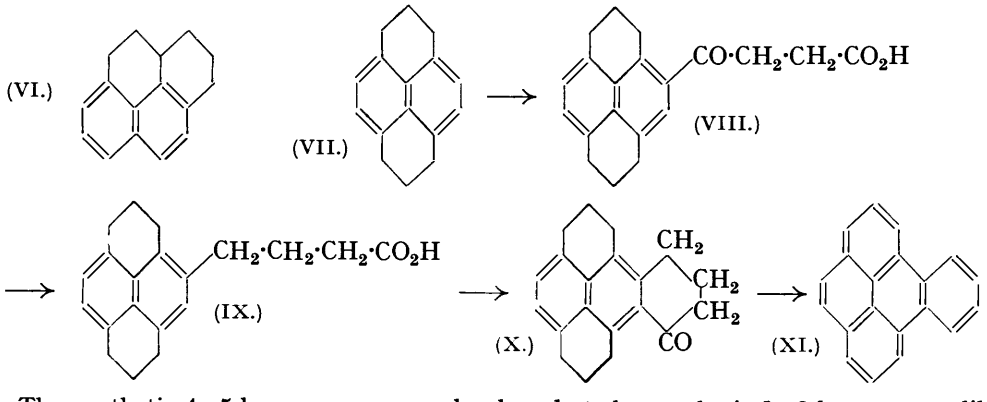
Another mode of synthesis which suggested itself for the 4 : 5-benzpyrene ring system consisted in the action of oxalyl chloride on triphenylene :



Unfortunately, this reaction led only to a *triphenylenecarboxylic acid.*

We were about to investigate other possible methods of synthesis from suitable derivatives of phenanthrene or benzanthrone when Dr. E. de Barry Barnett, to whom we express our indebtedness, suggested to us that the hexahydropyrene of Graebe (*Annalen*, 1871, **158**, 297) and Goldschmiedt (*Annalen*, 1907, **351**, 226) was the symmetrical compound and would therefore condense with succinic anhydride in position 4 of the pyrene ring system. Neither Graebe nor Goldschmiedt adduced evidence of the symmetrical nature of their hexahydropyrene, although Langstein (*Monatsh.*, 1910, **31**, 870) assumed this from the fact that it gave a picrate and hence must still contain a naphthalene system. This argument is fallacious, for we obtained, by reduction of pyrene, not only the hexahydropyrene in question, but also another *hexahydropyrene* which, moreover, gave a stable *picrate.* Nevertheless, the hexahydropyrene of Graebe and Goldschmiedt is the symmetrical compound, for it was oxidised by alkaline permanganate to naphthalene-1 : 4 : 5 : 8-tetracarboxylic acid. The new hexahydropyrene was more resistant to permanganate and we could not obtain a naphthalenetricarboxylic acid from it; it must have the unsymmetrical structure (VI), for there is no possibility of stereoisomerism with the symmetrical compound (VII).

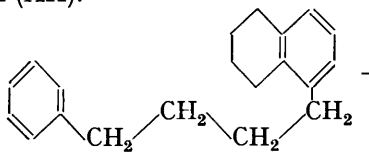
s.-Hexahydropyrene condensed smoothly with succinic anhydride to give β-1 : 2 : 3 : 6 : 7 : 8-*hexahydro-4-pyrenoylpropionic acid* (VIII), which was unattacked by zinc and ammonia or by amalgamated zinc and hydrochloric acid, but was reduced to the hydroxy-acid (isolated as the *lactone*) by sodium amalgam and water. Reduction of the keto-acid (VIII) to γ-1 : 2 : 3 : 6 : 7 : 8-*hexahydro-4-pyrenylbutyric acid* (IX) was achieved by the Kishner–Wolff method, and in this connexion it is surprising that the *semicarbazone* of β-1-pyrenoylpropionic acid (I) was recovered unchanged after heating with sodium ethoxide at 200°. The hexahydropyrenylbutyric acid (IX) was cyclised by 80% sulphuric acid at 100°, the resulting 1′-keto-1 : 2 : 3 : 6 : 7 : 8 : 1′ : 2′ : 3′ : 4′-*decahydro*-4 : 5-*benzpyrene* (X) being converted by reduction with sodium and alcohol, and subsequent dehydrogenation with selenium, into 4 : 5-benzpyrene (XI) :



The synthetic 4 : 5-benzpyrene was colourless, but the synthetic 1 : 2-benzpyrene, like the material isolated from pitch, was pale yellow, and the colour was not removed by maleic anhydride in boiling xylene, nor by shaking a benzene solution with sulphuric acid,

treatments which decolorise other higher hydrocarbons contaminated with coloured impurities. The sulphuric acid treatment led to rapid and complete removal of the benzpyrene from its benzene solution, a property which is shared by the highly reactive, orange 2 : 3-benzanthracene. It thus seems that the colour of 1 : 2-benzpyrene is a definite attribute of the compound itself.

The fluorescence spectrum of 1 : 2-benzpyrene is identical with that of the carcinogenic mixture formed by the action of aluminium chloride on tetrahydronaphthalene (Kennaway, *Biochem. J.*, 1930, **24**, 497; Hieger, *ibid.*, p. 505; cf. Schroeter, *Ber.*, 1924, **57**, 1990). The presence of α-phenyl-δ-2-tetralylbutane in such mixtures was shown by Schroeter, and it may well be that 1 : 2-benzpyrene arises by dehydrogenation of the corresponding 1-tetralyl compound (XII).

(XII.)  → (IV) + 12H

Dehydrogenation of some of the constituents of the mixture must occur to furnish the perhydroanthracene isolated by Schroeter. The amount formed of the active carcinogenic fraction is so small that attempted isolation of 1 : 2-benzpyrene was not feasible.

Experimental.

β-1-*Pyrenoylpropionic Acid* (I).—Anhyd. $AlCl_3$ (330 g.) was added slowly, with cooling and agitation, to a solution of succinic anhydride (125 g.) in $PhNO_2$ (1250 c.c.). Pyrene (supplied by the Gesellschaft für Teerverwertung) (250 g.) was then gradually introduced, and the whole kept at room temp. for 24 hr. with occasional shaking. Ice and HCl aq. were added to the product, the $PhNO_2$ removed in steam, and the residue dissolved in dil. Na_2CO_3 aq. The sparingly sol. sodium salt of β-1-pyrenoylpropionic acid was twice recrystallised from H_2O, and the free acid liberated and recrystallised from AcOH and then AcOH–EtOH (charcoal); it formed bright yellow crystals (230 g.), m. p. 183° (Found: C, 79·3; H, 4·7. $C_{20}H_{14}O_3$ requires C, 79·45; H, 4·7%). This *keto-acid* gave a *semicarbazone*, m. p. 203—205° (Found: N, 11·3. $C_{21}H_{17}O_3N_3$ requires N, 11·7%), which was recovered unchanged after heating for 2½ hr. at 200° with NaOEt (1 g. semicarbazone, 1 g. Na, 15 c.c. EtOH).

Ethyl β-1-*pyrenoylpropionate*, prepared by the HCl–EtOH method, formed lemon-yellow needles, m. p. 90—91° (Found: C, 79·8; H, 5·5. $C_{22}H_{18}O_3$ requires C, 80·0; H, 5·5%).

Fission of β-1-*Pyrenoylpropionic Acid.*—(a) A mixture of the keto-acid (5 g.) and anhyd. $ZnCl_2$ (15 g.) was heated at 160—170° for 10 min. The melt was cooled, extracted with H_2O and dil. Na_2CO_3 aq., and recrystallised from C_6H_6–EtOH. The product had m. p. 149°, alone or mixed with pyrene.

(b) A solution of the keto-acid (5 g.) in AcOH (50 c.c.) and conc. H_2SO_4 (5 g.) was boiled for ¾ hr. and gave pyrene.

Reduction of β-1-*Pyrenoylpropionic Acid.*—This could not be effected by Clemmensen's method, and Zn and HCl in AcOH gave resinous products. A solution of the keto-acid (50 g.) in 2*N*-NaOH (150 c.c.), H_2O (500 c.c.), and conc. aq. NH_3 (500 c.c.) was boiled under reflux for 48 hr. with Zn dust (150 g.). After filtration, the Zn residues were extracted several times with boiling dil. NaOH aq., and the combined filtrates acidified. The ppt. was dried and its conc. solution in xylene boiled for 1 hr. This treatment was necessary to convert the hydroxy-acid present in the mixture into its lactone. The crystals which separated on cooling were extracted with boiling dil. Na_2CO_3 aq., and the γ-1-*pyrenyl*-γ-*butyrolactone* which remained undissolved was recrystallised from AcOH, forming almost colourless microscopic rhombs, m. p. 178° (Found: C, 83·85; H, 5·0. $C_{20}H_{14}O_2$ requires C, 83·9; H, 4·9%).

The Na_2CO_3 extract, on acidification, yielded γ-1-*pyrenylbutyric acid* (II), which separated from xylene in colourless leaflets (36 g.), m. p. 185—186°. This butyric acid gave a yellow solution in conc. H_2SO_4, whereas the corresponding keto-acid and lactone both gave red colours (Found: C, 83·3; H, 5·5. $C_{20}H_{16}O_2$ requires C, 83·3; H, 5·6%). A further quantity of the butyric acid was obtained by submitting a solution of the lactone in aq. NaOH to the renewed action of Zn dust and NH_3. In this way, 240 g. of keto-acid were converted into 165 g. of γ-1-pyrenylbutyric acid.

4'-Keto-1' : 2' : 3' : 4'-tetrahydro-1 : 2-benzpyrene (III).—Unsuccessful attempts to effect ring closure of pyrenylbutyric acid included a study of the action of P_2O_5 in $PhNO_2$, of 80% H_2SO_4 at 100° (which led mostly to sulphonation), of $POCl_3$, and of $AlCl_3$ on the acid chloride. Success was attained when a melt of pyrenylbutyric acid (10 g.) and anhyd. $ZnCl_2$ (30 g.) was stirred for ½ hr. at 180°. After cooling, the powdered solid was extracted with H_2O, then dil. Na_2CO_3 aq., and finally with Et_2O. A large amount of black amorphous material remained undissolved. The Et_2O extract gave 1·5 g. of *4'-keto-1' : 2' : 3' : 4'-tetrahydro-1 : 2-benzpyrene* (III), which crystallised from C_6H_6–EtOH in yellow leaflets, m. p. 171·5—173·5°, and gave a pink solution in conc. H_2SO_4 (Found : C, 88·6; H, 6·1. $C_{20}H_{14}O$ requires C, 88·8; H, 5·2%). The *oxime* separated from C_6H_6 in almost colourless needles, m. p. 222—224° (decomp.) after slight sintering (Found : N, 4·9. $C_{20}H_{15}ON$ requires N, 4·9%).

Repetition of the above expt. gave poorer yields, and better results were obtained by using $SnCl_4$ for cyclisation. A mixture of pyrenylbutyric acid (40 g.) and anhyd. $SnCl_4$ (30 c.c.) was heated at 115—120° for 1 hr., the product dissolved in acetone, and the solution diluted with C_6H_6, extracted with dil. HCl aq., then with dil. Na_2CO_3 aq., and concentrated. MeOH was added, the solution boiled with charcoal, and the ketotetrahydrobenzpyrene allowed to crystallise (yield, 7 g. In all, 30 g. were obtained from 165 g. of pyrenylbutyric acid). The material from the mother-liquors was distilled at 4 mm. Crystn. of the higher-boiling fraction gave a further quantity of the keto-compound, and the lower-boiling fraction gave pyrene.

1 : 2-Benzpyrene (IV).—This hydrocarbon (1·7 g.) was obtained by heating the aforesaid ketone (III; 10 g.) with Se (3 g.) at 300—340° for 6 hr. (4-Keto-1 : 2 : 3 : 4-tetrahydrophenanthrene was converted into phenanthrene merely by heating with Se.) An improved yield of 1 : 2-benzpyrene was obtained by preliminary reduction of the ketone (III; 13 g.) with Na (5 g.) in boiling EtOH (260 c.c.). The product was dehydrogenated with Se (6·5 g.) at 320—340° for 16 hr., extracted with C_6H_6, and distilled in vac. The distillate (6·5 g.; b. p. 310—312°/10 mm.) crystallised from C_6H_6–MeOH in pale yellow needles (4·4 g.), m. p. 174—175°.

1 : 2-Benzpyrene picrate, prepared in C_6H_6 and recryst. to const. m. p., formed long purplish-black needles, m. p. 197—198°, not depressed by the picrate of the carcinogenic hydrocarbon isolated from pitch. *1 : 2-Benzpyrene* (IV), regenerated from the pure picrate, crystallised from C_6H_6–MeOH in long, pale yellow needles, m. p. 176·5—177·5° (Found : C, 95·2; H, 4·8. $C_{20}H_{12}$ requires C, 95·2; H, 4·8%). The orange-red solution in conc. H_2SO_4 had a strong green fluorescence, and dil. solutions in C_6H_6 showed an intense violet fluorescence.

Nitro-1 : 2-benzpyrene.—When a suspension of 1 : 2-benzpyrene (0·1 g.) in AcOH (5 c.c.) and conc. HNO_3 (0·1 c.c.) was kept at room temp. over-night, there was formed a substance which, after recrystn. from AcOH and then C_6H_6, formed a light brown microcryst. powder, m. p. 233—236°. This consisted essentially of a *mononitro*-compound, although contaminated with a substance of higher nitrogen content (Found : C, 79·2; H, 3·6; N, 5·4. $C_{20}H_{11}O_2N$ requires C, 80·8; H, 3·7; N, 4·7%).

1 : 2-Benzpyrenequinone.—A suspension of finely powdered 1 : 2-benzpyrene (1 g.) in H_2O (25 c.c.) and conc. H_2SO_4 (2·5 c.c.) was boiled for ¾ hr. with $K_2Cr_2O_7$ (4 g.). The product was twice recrystallised from C_5H_5N and then from xylene; it formed a brick-red microcryst. powder, m. p. 242—244°, and gave an orange-brown vat with Zn dust and dil. NaOH aq. This was essentially a *monoquinone*, but an analytically pure compound could not be obtained (Found : C, 84·2; H, 3·6. $C_{20}H_{10}O_2$ requires C, 85·1; H, 3·6%).

1-Pyrenoyl-o-benzoic Acid.—To a solution of phthalic anhydride (30 g.) in C_6H_6 (200 c.c.) was added anhyd. $AlCl_3$ (27 g.) and then pyrene (40 g.). After 6 hr. at room temp. with occasional shaking, ice and HCl aq. were added, the C_6H_6 removed in steam, and the residue extracted with dil. Na_2CO_3 aq. The *phthaloylic acid*, pptd. by addition of HCl aq., crystallised from EtOH in small yellow crystals (33 g.), m. p. 224·5—225·5° (Found : C, 82·1; H, 4·0. $C_{24}H_{14}O_3$ requires C, 82·3; H, 4·0%).

1-o-Carboxybenzylpyrene.—A solution of the foregoing phthaloylic acid (30 g.) in 2*N*-NaOH (300 c.c.) was boiled for 3 hr. with Zn dust (60 g.) activated with Cu. The filtered solution was acidified, and the pptd. acid repptd. from its solution in dil. Na_2CO_3 aq. and recrystallised from AcOH (yield, 18 g.). 1-o-*Carboxybenzylpyrene* formed colourless crystals, m. p. 217—218° (Found : C, 85·65; H, 4·95. $C_{24}H_{16}O_2$ requires C, 85·7; H, 4·8%).

2' : 3'-Naphtha-1 : 2-pyrene (V).—(a) Cyclisation of 1-*o*-carboxybenzylpyrene (11 g.) with anhyd. $ZnCl_2$ (33 g.) at 200° was complete in 20 min. The crude anthrone-like product was reduced for 3 hr. by Zn dust (20 g.) and boiling 1·5*N*-NaOH (300 c.c.). The solid in suspension was collected, excess Zn dissolved in HCl aq., and the *2' : 3'-naphtha-1 : 2-pyrene* (V) recrystallised from C_6H_6. It formed deep orange leaflets, m. p. 273° (Found : C, 95·3; H, 4·75. $C_{24}H_{14}$

requires C, 95·3; H, 4·7%). No pure quinone could be obtained by oxidation with $Na_2Cr_2O_7$ in AcOH.

(b) An ice-cold mixture of pyrene (60 g.), *o*-toluoyl chloride (46 g.), and CS_2 (150 c.c.) or $PhNO_2$ (300 c.c.) was treated gradually with $AlCl_3$ (40 g.). After keeping in ice for 6 hr., the products were decomposed with ice and HCl aq. and worked up in the usual way. In neither case could a pure ketone be isolated. The crude ketones were heated in pyrex glass retorts at 420—440° for 2 hr., the residues sublimed in vac., and the sublimates recrystallised from xylene. In both cases the products were identical with that prepared as described under (a).

2′ : 3′-Naphtha-1 : 2-pyrene-1′ : 4′-endo-αβ-succinic Anhydride.—A solution of 2′ : 3′-naphtha-1 : 2-pyrene (0·25 g.) and maleic anhydride (0·25 g.) in xylene (30 c.c.) was boiled for ¾ hr. On cooling, the *adduct* separated as an almost colourless cryst. powder, decomp. without melting, 270—280° (Found: C, 83·75; H, 4·3. $\cdot C_{28}H_{16}O_3$ requires C, 84·0; H, 4·0%).

4-Hydroxy-1 : 2 : 3 : 4-tetrahydrophenanthrene.—This was the chief product of the action of PhMgBr or C_6H_{11}·MgCl on 4-keto-1 : 2 : 3 : 4-tetrahydrophenanthrene (Schroeter, Müller, and Huang, *Ber.*, 1929, **62**, 657). Many expts. were made by altering such factors as the temp. of reaction, the proportions of reacting substances, by adding the Grignard solution to the ketone and vice versa, but in no case was there any evidence of the introduction of a substituent into the phenanthrene ring system. The following example is typical:—

An ethereal solution of 4-keto-1 : 2 : 3 : 4-tetrahydrophenanthrene (6·5 g.) was slowly added to an ice-cold filtered Grignard solution prepared from $C_6H_{11}Cl$ (5·9 g.) and Mg (1·3 g.). After 1 hr. at 0° and ½ hr. at room temp. the product was decomp. with ice and NH_4Cl. The crystals insol. in Et_2O (1·2 g.) were collected, a further 1 g. being obtained by addition of MeOH to the semi-solid mass remaining after removal of the Et_2O. The *carbinol* was recrystallised from C_6H_6–MeOH and formed colourless plates, m. p. 140—141°, which gradually became dehydrated in a vac. desiccator as shown by successive analyses of the same sample (Found: C, 85·7; H, 6·4; *M*, Rast method, 196, 208. $C_{14}H_{14}O$ requires C, 84·8; H, 7·1%; *M*, 198). A pure olefin was not obtained by dehydration with picric acid in boiling EtOH.

The residual oil from the isolation of this carbinol was dehydrogenated with Se and distilled at 4 mm. The lower fraction, b. p. to 200°, gave phenanthrene, and the higher fraction, b. p. 360—380°, gave a sparingly sol. substance which crystallised from C_6H_6 in small yellowish needles, m. p. 312°. This compound was also formed as a by-product in the Se dehydrogenation of 4-keto- or 4-hydroxy-1 : 2 : 3 : 4-tetrahydrophenanthrene, and analysis suggested that it was a diphenanthrafuran (Found: C, 91·0; H, 4·5. $C_{28}H_{16}O$ requires C, 91·3; H, 4·4%).

Triphenylenecarboxylic Acid.—Anhyd. $AlCl_3$ (5 g.) was added to an ice-cold suspension of triphenylene (4 g.) in CS_2 (25 c.c.) and oxalyl chloride (5 c.c.). After keeping at room temp. for 24 hr., the product was decomposed with ice and HCl aq., and the CS_2 removed with steam. The residue was extracted with dil. Na_2CO_3 aq.; 2·5 g. of unchanged triphenylene remained. The Na_2CO_3 extract, on cooling, deposited the sparingly sol. sodium salt of *triphenylenecarboxylic acid*. The free acid (1·4 g.) separated from $PhNO_2$ in almost colourless needles, m. p. 325—326° (decomp.) (Found: C, 83·8; H, 4·1. $C_{19}H_{12}O_2$ requires C, 83·8; H, 4·4%). The *methyl* ester, prepared from the sodium salt and Me_2SO_4, formed colourless needles (from EtOH), m. p. 122—124° (Found: C, 84·0; H, 5·3. $C_{20}H_{14}O_2$ requires C, 83·9; H, 4·9%).

Reduction of Pyrene.—Catalytic hydrogenation in tetralin solution with a Ni catalyst gave the *s.*-hexahydropyrene of Goldschmiedt (*loc. cit.*) together with a mixture of more sol. products which were not investigated; it was found more convenient to use Goldschmiedt's reduction method. For this purpose, Na (480 g.) was added gradually to a solution of pyrene (300 g.) in boiling C_5H_{11}·OH (9 l.), in 6 batches. After being washed with H_2O, the solution was concentrated. The crystals which separated were recrystallised from EtOH (2·5 l.) and yielded 71 g. of pure *s.*-hexahydropyrene (VII), long colourless needles, m. p. 132—133° (Goldschmiedt gives 127°; Langstein, *loc. cit.*, gives 129—130°). The C_5H_{11}·OH mother-liquors were distilled in steam, and the residue dried and distilled at 3 mm. (b. p. 165—170°). The product (120 g.; m. p. *ca.* 60°), together with the material (64 g.) recovered from the above EtOH liquors, was treated with an equal wt. of picric acid in EtOH, and the picrate recrystallised to const. m. p. from 5% picric acid in EtOH. A sample for analysis was recrystallised from EtOH and formed reddish-orange needles, m. p. 147·5—148° (Found: C, 60·0; H, 4·8. $C_{16}H_{16},C_6H_3O_7N_3$ requires C, 60·4; H, 4·4%). as.-*Hexahydropyrene* (VI), obtained by shaking this *picrate* in C_6H_6 with aq. Na_2CO_3, crystallised from EtOH in colourless leaflets (23 g.), m. p. 105—105·5° (Found: C, 92·3; H, 7·8. $C_{16}H_{16}$ requires C, 92·3; H, 7·7%). A further 6 g. of *s.*-hexahydropyrene was obtained from the hydrocarbons recovered from the picrate liquors, but no other pure product could be isolated.

Constitution of s.-*Hexahydropyrene*.—A solution of the hydrocarbon (4·2 g.) in acetone was poured into H_2O (350 c.c.), and the acetone boiled off. To the boiling suspension formed was added 50% KOH aq. (25 c.c.), followed, during several hr., by finely powdered $KMnO_4$ (34 g.). Oxidation was complete in about 4 hr. Naphthalene-1 : 4 : 5 : 8-tetracarboxylic acid (0·9 g.) crystallised from the acidified filtrate. It was purified by further treatment with alkaline $KMnO_4$, recrystallised from H_2O, and converted into the dianhydride by heating at 140—160°. The dianhydride was recrystallised from $PhNO_2$ (Found : C, 62·8; H, 1·8. Calc. : C, 62·7; H, 1·5%). The properties of the acid and of the dianhydride agreed with those given in the literature.

β-1 : 2 : 3 : 6 : 7 : 8-*Hexahydro-4-pyrenoylpropionic Acid* (VIII).—This was prepared from s.-hexahydropyrene (39 g.), succinic anhydride (18·6 g.), and anhyd. $AlCl_3$ (52 g.) in $PhNO_2$ (180 c.c.) as described for the pyrene condensation. After 3 recrystns. from C_6H_6 the *keto-acid* (VIII), which gave a deep purple solution in conc. H_2SO_4, formed small colourless crystals (39 g.), m. p. 173·5° (Found : C, 78·0; H, 6·3. $C_{20}H_{20}O_3$ requires C, 77·9; H, 6·5%).

The *semicarbazone*, prepared in EtOH from the keto-acid, semicarbazide hydrochloride, and NaOAc, formed a colourless microcryst. powder, m. p. 221—222° (Found : N, 11·3. $C_{21}H_{23}O_3N_3$ requires N, 11·5%).

γ-1 : 2 : 3 : 6 : 7 : 8-*Hexahydro-4-pyrenyl-γ-butyrolactone*.—A solution of the keto-acid (VIII; 1 g.) in *N*-NaOH (40 c.c.) was treated, at 100°, with 3% Na amalgam (30 g.). The sodium salt which separated on cooling was collected and converted into the free acid, which was heated with boiling xylene for a few min. The cold solution deposited a small amt. of unchanged acid. The *γ-butyrolactone* was obtained from the liquors and recrystallised from EtOH and then C_6H_{12}. It formed small colourless crystals, m. p. 150—154° (Found : C, 82·3; H, 6·9. $C_{20}H_{20}O_2$ requires C, 82·15; H, 6·9%).

γ-1 : 2 : 3 : 6 : 7 : 8-*Hexahydro-4-pyrenylbutyric Acid* (IX).—A solution of the semicarbazone (12 g.) of the keto-acid (VIII) in alc. NaOEt (12 g. Na; 140 c.c. EtOH) was heated at 180—190° for 5½ hr. The cryst. sodium salt was collected, washed with EtOH, and recrystallised from H_2O, and the free acid liberated. The dry acid was extracted with C_6H_{12} (200 c.c.), in which a small amount of dark-coloured material was insol. The C_6H_{12} was removed on the water-bath, and the residual resin twice recrystallised from MeOH, from which *hexahydro-pyrenylbutyric acid* (IX; 5 g.) separated slowly as colourless microscopic needles, m. p. 133—134° (Found : C, 81·6; H, 7·8. $C_{20}H_{22}O_2$ requires C, 81·6; H, 7·5%).

1′-*Keto*-1 : 2 : 3 : 6 : 7 : 8 : 1′ : 2′ : 3′ : 4′-*decahydro*-4 : 5-*benzpyrene* (X).—Cyclisation of the butyric acid (IX; 4 g.) by 80% H_2SO_4 (40 c.c.) at 100° was complete in 40 min. The blood-red solution was cooled, diluted with H_2O, and extracted with Et_2O. The Et_2O solution was washed with dil. Na_2CO_3 aq. and H_2O, and dried with anhyd. Na_2SO_4, and the Et_2O removed. The cyclic *ketone* (X) crystallised from MeOH in long yellowish needles (1·1 g.), m. p. 147—148° (Found : C, 86·7; H, 7·5. $C_{20}H_{20}O$ requires C, 86·9; H, 7·3%).

4 : 5-*Benzpyrene* (XI).—The aforesaid cyclic ketone (1 g.) was reduced with Na (0·5 g.) in boiling EtOH (25 c.c.), and the product dehydrogenated with Se (1·5 g.) at 320—340° for 20 hr. The product was sublimed at 250°/3—4 mm., and the sublimate treated with picric acid (0·8 g.) in C_6H_6. After 3 recrystns. from C_6H_6 the *picrate* formed ruby-red needles of const. m. p. 229—230°, not depressed by the picrate of the second unknown hydrocarbon isolated from pitch (Found : N, 8·6. $C_{20}H_{12},C_6H_3O_7N_3$ requires N, 8·7%). 4 : 5-*Benzpyrene* (XI) was obtained by shaking the pure picrate with C_6H_6 and aq. Na_2CO_3. It crystallised from C_6H_6, in which it was easily sol., in colourless prisms, m. p. 178—179°, not depressed by the compound from pitch (yield, 0·2 g.) (Found : C, 95·25; H, 4·8; *M*, Rast method, 267, 273. $C_{20}H_{12}$ requires C, 95·2; H, 4·8%; *M*, 252).

The preliminary treatment of pitch was carried out at the Products Works of the Gas Light and Coke Company. We are greatly indebted to the Directors for providing facilities, to Mr. W. Gordon Adam for co-operation which has been invaluable in this and earlier investigations, and to Mr. F. M. Potter and Mr. D. G. Murdoch for technical advice and supervision. We are also indebted to the South Metropolitan Gas Company for four grants to the Cancer Hospital Research Fund, and to Professor E. L. Kennaway for supervision of the animal experiments. One of us (I. Hieger) has been working under a grant from the British Empire Cancer Campaign.

THE RESEARCH INSTITUTE OF THE CANCER HOSPITAL (FREE),
LONDON, S.W. 3.

[*Received, February 7th*, 1933.]