SYNTHETIC organic compounds widely different in structures have been found to be carcinogenic, but among these polycyclic hydrocarbons containing the phenanthrene nucleus are of special interest and have been exhaustively studied. Robinson has suggested that the weight of evidence as regards the essential structural requirement for carcinogenicity indicates the possibility of reaction at an "activated phenanthrene bridge" in the great majority of cases, and the present paper is an elaboration of this idea.

The 1:2-benzanthracene molecule is present in the majority of active carcinogens, and is also remarkable because whereas it is inactive, substitution in the 9:10-positions by methyl groups gives (I) which is highly carcinogenic. The two centres of reactivity in 1:2-benzanthracene are: the 3:4-double bond (the 9:10-positions in the phenanthrene half of the molecule, the "phenanthrene bridge") and the meso positions. In view of the fact that a large number of physiologically active substances are structurally related to phenanthrene, the phenanthrene bridge (shown in I and in other carcinogenic compounds by asterisk) in 1:2-benzanthracene and its derivatives probably plays a significant role in the physiological activity of these compounds. The reactivity of the 9:10-phenanthrene double bond may be enhanced (or competitive reactivity reduced) by suitable substitution elsewhere, particularly in the meso positions, which are thus deactivated.

The above hypothesis provides a simple explanation of the high carcinogenic activity of (I) as against the inactivity of 1:2-benzanthracene. It also accounts for the inactivity of 1:2:3:4-dibenzanthracene (where the reactive phenanthrene double bond is blocked) and also shows that the meso positions of anthracene are not evolved in carcinogenesis. Haddow has drawn attention to other examples which confirm the importance of the phenanthrene bridge in carcinogenesis. Whereas there are certain exceptions to the above hypothesis and more than one mechanism may be involved, there are several striking examples which substantiate the hypothesis, which forms the basis of the present work.
With the view to collect evidence as regards the role of the phenanthrene bridge in carcinogenesis, compounds analogous to carcinogenic hydrocarbons in which an appropriate benzene ring is replaced by the isosteric thiophene nucleus were synthesised. In 9:10-dimethyl-1:2-benzanthracene (I) there are three benzene nuclei which might be so replaced and three such sulphur isosters, in which the end benzene ring (A or D) is replaced, have been found to be carcinogenic.\(^6\), \(^6\), \(^7\) Since the isosteric replacement does not affect the reactive centres in (I), the activity of these compounds is to be expected. The third isomer 4:9-dimethyl-2:3-benzothiophanthrene (II), in which the ring C is replaced by a thiophene ring, has now been synthesised and is of greater significance because of the removal of the phenanthrene bridge.

\[\text{CH}_3\]

\[\text{CH}_3\]

\[\text{CH}_3\]

2:3-Benzoo-4:9-thiophanthrenequinone (III) was prepared according to Mayer\(^8\) by condensation of \(\omega\)-chloroacetophenone and thioisatin in aqueous alkali and the cyclisation of the resulting 2-benzoylethonaphthene-3-carboxylic acid. The above synthesis of (III) was preferred to the alternative synthesis\(^9\) starting from thionaphthene and phthalic anhydride that was initially employed. Thionaphthene required for the latter route to (III) was prepared by cyclization of thiophenoxyacetaldehyde dimethyl acetal and represents a new synthesis of thionaphthene which has general application in the syntheses of other thiophenes and thiapyrans.\(^9\) Whereas Mayer cyclized 2-benzoylethonaphthene-3-carboxylic acid by the action of anhydrous aluminium chloride on its acid chloride, it has now been converted to (III) by the action of benzoyl chloride and sulphuric acid.\(^8\) The sulphur isoster (II) was prepared from the quinone (III) through the iodomethyl derivative according to Fieser and Hershberg\(^6\) and was purified through the picrate, chromatographic separation and finally by recrystallization from acetone.

Compound (II) proved to be non-carcinogenic by subcutaneous injection in mice and weakly active on painting. While not unduly stressing the importance of this result, it seems probable that the phenanthrene double bond in (I) is essential for its carcinogenic activity. A highly significant fact in support of the above conclusion is that high activity again
emerges in the benzo derivatives (IX) and (X) (see later), where the phenanthrene double bond is once again introduced.

Heterocyclic compounds analogous to the weakly carcinogenic hydrocarbons 1:2:5:6- and 1:2:7:8-dibenzanthracenes and containing nitrogen as a part of carbazole, phenazine or acridine ring systems have been synthesised and many of them show definite though weak carcinogenic activity. Notable among these is 3:4:5:6-dibenzocarbazole which however is a powerful carcinogen. The corresponding compounds containing oxygen and sulphur have been reported, but their activity was not known when the work was in progress. 1:1′-Dinaphthalene-2:2′-sulphide (IV) has been prepared by Barber and Smiles from 1-iodonaphthalene-2-sulphonic acid by a five-step synthesis. It has now been prepared in two steps from β-naphthol by converting it to β-dinaphthol and by the action of phosphorous pentasulphide on the latter under drastic conditions. Both β-dinaphthalene oxide and (IV), which is isosteric with the inactive hydrocarbon 3:4-(2′:1′-naphtho)-phenanthrene, proved non-carcinogenic.

1:2:5:6-Dibenzanthracene (V), and to a less degree 1:2:7:8-dibenzanthracene (VII) are carcinogenic. The corresponding meso-dimethyl derivatives (VI) and (VIII) are also carcinogenic; (VIII) being more active than (VII). There are three reactive centres in (V) and (VII): the two phenanthrene double bonds and the meso positions. Substitution of the meso positions by methyl groups and one of the phenanthrene bridges by a sulphur atom leads to the sulphur isosters (IX) and (X), which should
prove highly carcinogenic due to the increased activation (decreased competitive reactivity) of the remaining reactive centre.

Condensation of α-bromoacetylnaphthalene and thioisatin in aqueous sodium hydroxide gave 2-(1'-naphthoyl)-thionaphthene-3-carboxylic acid (XI) which on ring-closure gave 2:3:7:8-dibenzo-4:9-thiophenanthrenequinone (XII). The latter was converted to 4:9-dimethyl-2:3:7:8-dibenzothiophanthrene (IX) which was purified through its di-picrate.

The reaction of β-bromoacetylnaphthalene with thioisatin similarly gave 2-(2'-naphthoyl)-thionaphthene-3-carboxylic acid (XIII). Cyclization of this acid would lead to 2:3:5:6-dibenzo-4:9-thionaphthenequinone (XIV) as the major product and to the isomeric 2:3:6:7-dibenzo-4:9-thiophanthrenequinone (XVII). In actual fact only the quinone (XIV) was the
sole product of the cyclization. The quinone was converted to 4:9-dimethyl-2:3:5:6-dibenzothiophanthrene (X) as in the case of (IX).

Both (IX) and (X) proved to be highly carcinogenic both by subcutaneous injection and also by painting technique, the activity being comparable with (I).

Mayer obtained a mixture of two quinones constituted by him as (XII) and (XIV) by the cyclization of the mixture of 3-(2'-naphthyl)-thionaphthene-2-carboxylic acid (XV) and 3-(1'-naphthoyl)-thionaphthene-2-carboxylic acid (XVI) resulting from the condensation of naphthalene with thionaphthene-2:3-dicarboxylic anhydride. The mixture of the quinones, on fractional crystallization from benzene, gave the quinone, m.p. 157° (major product) and the less soluble quinone, m.p. 257°. Cyclization of the acid (XV) would lead to the quinone (XII) or to 2:3:6:7-dibenzo-4:9-thiophanthrenequinone (XVII), whereas the acid (XVI) would give (XIV) or the quinone (XVIII). The quinones (XII) and (XIV) have now been unambiguously synthesized and melt at 255–56° and 261–62.5°. The higher melting quinone, m.p. 257° obtained by Mayer could therefore be either of the above quinones. The quinone (XVII), synthesised by Mayer, melts at 301°. The lower melting quinone, m.p. 157°, obtained by Mayer may therefore be (XVIII). The quinones described by Mayer are being prepared to establish the identity of the quinone, m.p. 257°.

Attempts to reduce the quinones (XII) and (XIV) to the corresponding thiophanthrene derivatives by the Clar method gave 2:3:7:8-dibenzo-(XIX) and 2:3:5:6-dibenzothiophanthrones (XX), which are being further studied.
2: 3-Benz-4: 9-thiophanthrenequinone (III).—Thiocatin (4.9 g.), prepared from thioindoxyl according to Mayer, was dissolved in warm 2N sodium hydroxide (30 c.c.), and sodium iodide (1 g.), and \( \omega \)-chloroaceto-phenone were added. The mixture was boiled under reflux for one hour, cooled, filtered and acidified with dilute hydrochloric acid when 2-benzoyl-thionaphthene-3-carboxylic acid, m.p. 238-41° (6.9 g.) separated. It gave colourless needles from glacial acetic acid, m.p. 245-47° (5.3 g.). Mayer prepared this acid by employing \( \omega \)-bromonacetophenone without addition of sodium iodide and gives m.p. 240-41°. The acid (3 g.) was suspended in benzoyl chloride (30 c.c.), warmed in boiling water-bath, concentrated sulphuric acid (0.6 c.c.) was added and after five minutes, the contents were diluted in 2N sodium hydroxide, and the mixture was boiled when the quinone (VI) was obtained, m.p. 190-200° (2.9 g.). A solution of the product in benzene was passed through a column of activated alumina, and the quinone finally crystallized from benzene-petroleum ether when it gave yellow needles, m.p. 214-16° (2.0 g.) (Mayer gives m.p. 212-13°).

4: 9-Dimethyl-2: 3-benzothiophanthrene (II).—A solution of the quinone (III) (4.2 g.) in dry thiophene-free benzene (75 c.c.) was added to the Grignard reagent from magnesium (1.62 g.), methyl iodide (4.3 c.c.) and dry ether (90 c.c.) and the contents were boiled at 70-80° for one hour. The reaction mixture was cooled in ice, and gradually added to an ice-cooled solution of hydriodic acid, sp. gr. 1.7 (52.4 c.c.), in acetic acid (90 c.c.). Ether and benzene in the reaction mixture were removed in vacuum at 10-15° (2 hours), the iodomethyl compound, which separated as a greenish yellow solid, was collected, and washed with 1:1 aqueous acetic acid. It was dissolved in dioxan (360 c.c.), and reduced with a solution of hydrated stannous chloride (34.6 g.) in concentrated hydrochloric acid (105 c.c.) and dioxan (170 c.c.), by boiling under reflux for 30 minutes. The solvent was distilled under reduced pressure and the concentrated solution on cooling gave pale olive flakes of the dimethyl derivative, m.p. 145-52° (1.96 g.). It was treated with a saturated alcoholic solution of picric acid (3 g.), and the picrate was decomposed with 1% aqueous ammonia and the mixture was extracted with benzene. The benzene solution was washed, dried (Na\(_2\)SO\(_4\)), and after concentration passed through an activated alumina column. The fraction giving bright bluish violet fluorescence was collected, and the pale yellow solid (1.78 g.), obtained by removal of the solvent, was crystallized from
acetone when it gave pale yellow rhombic prisms (0·96 g.), m.p. 154-55°, raised to 154·5-55·5° by recrystallization from the same solvent (Found: C, 82·3; H, 5·3; S, 11·7. \( \text{C}_{19}\text{H}_{10}\text{S} \) requires C, 82·5; H, 5·3; S, 12·2%). The picrate gave bronze-coloured flakes from absolute alcohol, m.p. 157-58° (Found: N, 8·4. \( \text{C}_{21}\text{H}_{18}\text{N}_{2}\text{O}_{7}\text{S} \) requires N, 8·5%).

1:1'-Dinaphthalene-2:2'-sulphide (IV).—\( \beta \)-Dinaphthol was prepared by oxidation of \( \beta \)-napthol with dilute aqueous ferric chloride.\(^{16}\) A mixture of \( \beta \)-dinaphthol (25 g.) and phosphorous pentasulphide (9·75 g.) was heated in a 250 c.c. Claisen flask under reduced pressure (0·1 mm.) first gradually and then strongly with a free flame. Considerable frothing occurred due to the evolution of vapours having a repugnant odour. Short yellow needles collected on the cooler portions of the flask and the residue in the flask turned black. After cooling, the residue and the small quantity of the distillate were repeatedly extracted with boiling benzene (total 150 c.c.). The benzene extract was left overnight in the refrigerator and the yellow precipitate obtained, m.p. 195-205° (0·9 g.), was dissolved in benzene and passed through an activated alumina column. The fraction showing strong blue fluorescence after concentration gave lustrous pale yellow flakes (benzene) of 1:1'-dinaphthalene-2:2'-sulphide, m.p. 269-10° (0·35 g.), unaltered by further recrystallization. Barber and Smiles\(^{13}\) give m.p. 202° (Found: C, 84·6; H, 4·4; S, 11·6. Calc. for \( \text{C}_{20}\text{H}_{13}\text{S} \): C, 84·5; H, 4·3; S, 11·3%).

2-(1'-Naphthoyl)-thionaphthene-3-carboxylic acid (XI).—\( \alpha \)-Acetylnaphthalene was isolated free from the \( \beta \)-acetyl derivative through its less soluble picrate (m.p. 116-18°), and brominated to give \( \alpha \)-bromoacetylnaphthalene.\(^{17}\) A hot solution of thioisatin (3·1 g.) and sodium iodide (0·7 g.) in 2N sodium hydroxide (20 c.c.) was added to \( \alpha \)-bromoacetylnaphthalene (5·1 g.), and the mixture was boiled under reflux for 30 minutes. After dilution with water, the mixture was clarified (Norit), filtered, cooled and the solution acidified with dilute hydrochloric acid. The precipitate (5·0 g.) was crystallized from glacial acetic acid when colourless flat needles of the ketoacid (XI) separated, m.p. 185-200° (3 g.). The acid was purified by two further recrystallizations, and after washing with acetic acid was dried at 145°/10 mm. for 1½ hours; softens above 180° and melts at 199-201°; unaltered by further recrystallizations (Found: C, 72·2; H, 3·6; S, 10·3. \( \text{C}_{20}\text{H}_{14}\text{O}_{6}\text{S} \) requires C, 72·3; H, 3·6; S, 9·6%).

2:3;7;8-Dibenzo-4:9-thiophanthrenequinone (XII).—A mixture of the above ketoacid (0·5 g.) and benzoyl chloride (5 c.c.) was heated in water-bath, and acidified with a drop of concentrated sulphuric acid. The bright
green solution which changed rapidly to deep yellow, was further heated for two minutes, diluted in 2N sodium hydroxide, and the mixture boiled. The quinone separated as a red precipitate, m.p. 225-35° (0.45 g.) and crystallized from benzene in needles, m.p. 248-51°; raised to 255-56° by two further recrystallizations from the same solvent (Found: C, 76.5; H, 3.3; S, 10.9. C_{20}H_{16}O_{2}S requires C, 76.4; H, 3.2; S, 10.2%).

4:9-Dimethyl-2:3:7:8-dibenzoanthrene (IX).—The quinone (XII) (0.95 g.) was added to the Grignard compound from magnesium (1 g.) and methyl iodide (4 c.c.), dissolved in ether (20 c.c.) and thiophene-free benzene (20 c.c.). The solution was heated under reflux for one hour and after cooling in ice was added gradually to a cold solution of hydriodic acid sp. gr. 1.7 (12 c.c.) in methyl alcohol (20 c.c.), when a yellow precipitate of the iodomethyl compound separated. Acetic acid (20 c.c.) was added to the mixture and after removing ether and benzene under reduced pressure below 10°, the precipitate was separated, dissolved in dioxan (75 c.c.), and boiled with a solution of stannous chloride (7.5 g.) in concentrated hydrochloric acid (22.5 c.c.) and dioxan (40 c.c.) for 15 minutes. The reaction mixture was distilled under reduced pressure, and after collecting about 90 c.c. the residue was cooled gradually to room temperature, and finally in a freezing mixture when a cream-coloured precipitate was obtained, m.p. 155-67° (0.38 g.), which after four crystallizations from benzene-light petroleum (60-80°), gave colourless flakes of the dimethyl compound, m.p. 175°. The mother-liquor gave a sticky yellow solid after dilution with water. The latter was dissolved in benzene and chromatographed. The pale blue fluorescent fraction was concentrated and treated with excess of picric acid. The lustrous dark red needles of the dipicrate separated, m.p. 185-87° raised to 186-87° on further recrystallization from dilute alcoholic solution of picric acid (Found: C, 53.5; H, 3.0; N, 11.4. C_{34}H_{22}N_6O_{14}S requires C, 53.0; H, 2.9; N, 10.9%). The dipicrate was decomposed with 1% aqueous ammonia, and the product boiled with alcohol (10 c.c.) and cooled. The cream-coloured crystalline powder, m.p. 170-74°, gave lustrous colourless flakes of the dimethyl derivative after two recrystallizations from benzene-light petroleum (60-80°), m.p. 175° (Found: C, 84.2; H, 5.2; S, 10.6. C_{22}H_{18}S requires C, 84.6; H, 5.1; S, 10.3%).

2-(2'-Naphthoyl)-thionaphthene-3-carboxylic acid (XIII).—β-Bromoacetyl-naphthalene (5.1 g.) was heated under reflux with thioisatin (3.1 g.), sodium iodide (0.7 g.) and 2N sodium hydroxide (20 c.c.) for one hour. The crude ketoacid was isolated in the same manner as (XI), and gave colourless needles from acetic acid, m.p. 250-70° (partial decom.) (3.2 g.). The mother-liquor after concentration gave a second crop of the acid (0.6 g.).
After three recrystallizations, the ketoacid gave colourless needles which softened above 250° and melted with partial decomposition at 274-76° (Found: C, 71.8; H, 3.8; S, 9.5. C_{20}H_{12}O_3S requires C, 72.3; H, 3.6; S, 9.6%).

2:3:5:6-Dibenzo-4:9-thiophanthrenequinone (XIV).—The above ketoacid (3.0 g.) was treated with benzoyl chloride and concentrated sulphuric acid as in the preparation of (XI1). The crude quinone (2.9 g.) gave lustrous orange felted needles from benzene (350 c.c.), m.p. 260-61° (1.75 g.). The mother-liquor after concentration gave a second crop of the quinone, m.p. 256-58° (0.46 g.). A portion of the quinone when dissolved in benzene and passed through a column of activated alumina, gave a uniform chromatogram indicating its homogeneity and the quinone thus purified had the same m.p. It was sublimed at 280-90°/0.2 mm., and the sublimed product after crystallization gave slender orange needles (benzene), m.p. 261-62.5° (Found: C, 75.9; H, 3.1; S, 10.2. C_{20}H_{10}O_2S requires C, 76.4; H, 3.2; S, 10.3%).

4:9-Dimethyl-2:3:5:6-dibenzothiophanthrene (X).—The quinone (XIV) (0.95 g.) was treated with magnesium methyl iodide as in the preparation of (IX), using double the quantities of acetic acid and methanol. After reduction of the iodomethyl compound, the dioxan solution was concentrated under reduced pressure to about 30 c.c. and cooled in ice, when pale yellow needles of (X) separated, m.p. 148-50° (0.52 g.). It crystallized from benzene-alcohol in lustrous felted slender pale yellow needles, m.p. 150-50.5° (Found: C, 85.1; H, 5.2; S, 10.2. C_{22}H_{16}S requires C, 84.6; H, 5.1; S, 10.3%). The dimethyl compound (40 mg.) was treated with sym-trinitrobenzene (40 mg.) in benzene, and the derivative thus obtained was crystallized from benzene-alcohol when it gave bright red stout needles, m.p. 160-62°, raised to 162-63° by further recrystallization from dilute solution of sym-trinitrobenzene in the same solvents (Found: C, 64.0; H, 3.5; N, 7.8. C_{28}H_{16}N_3O_8 requires C, 64.0; H, 3.2; N, 8.0%).

2:3:7:8-Dibenzothiophanthrone (XIX).—The quinone (XII) (1 g.), zinc dust (1 g.), anhydrous zinc chloride (2.5 g.), and sodium chloride (1 g.) were intimately powdered together and heated to 220°. The mixture was raised to 280° and heated at this temperature for 15 minutes under stirring. The mixture was cooled, powdered and boiled with 10% hydrochloric acid (150 c.c.). The precipitate was washed, dried, and the olive grey powder dissolved in benzene, and the solution passed through an activated alumina column. An orange fraction of the unchanged quinone (0.04 g.) was eluted leaving a strong adsorbed substance on the alumina. The latter was
eluted by boiling with alcohol, and gave after concentration an olive precipi-
tate, m.p. 240-46°, which was sublimed at 260-80°/0-08 mm. The pale
yellow sublimate on crystallization from benzene gave the thiophanthrone
in lustrous aggregates of pale olive-coloured silky needles, which soften
above 230° and melt at 241-43° (partial decomp.) (Found: C, 79.7; H,
4.0; S, 10.7. C$_{20}$H$_{12}$OS requires C, 80.0; H, 4.0; S, 10.7%). The thiophanthrone gave a greenish yellow solution in alcoholic potassium hydroxide,
and was partially soluble in aqueous alkali.

2:3:5:6-Dibenzothiophanthrone (XX).—An intimate mixture of the
quinone (XIV) (0.5 g.), zinc dust (0.5 g.), anhydrous zinc chloride (1.25 g.)
and sodium chloride (0.5 g.) was heated at 280-90° for 15 minutes. The
reaction mixture was powdered, boiled with 10% hydrochloric acid, and the
precipitate was collected. It was extracted with benzene, the solution was
decolourised (Norit) and after concentration gave small colourless needles
of the thiophanthrone, pale grey fluffy powder with silvery reflex, m.p. 224-25°
decomposition) (Found: C, 79.7; H, 4.1. C$_{20}$H$_{12}$OS requires C, 80.0; H,
4.0%). The thiophanthrone gave a pale yellow solution in alcoholic potas-
sium hydroxide which gave yellowish orange needles after cooling. It was
also soluble in boiling aqueous alkali.

**SUMMARY**

With the view to test the significance of an "activated phenanthrene
bridge" in carcinogenic hydrocarbons, syntheses of analogues in which
an appropriate benzene ring is replaced by the isosteric thiophene nucleus
were carried out. The replacement of the ring (C) in the highly carcinogenic
hydrocarbon (I), led to 4:9-dimethyl-2:3-benzothiophanthrene (II) which
proved to be inactive presumably due to the removal of the necessary phenan-
threne double bond. The benzo derivatives of (II), 4:9-dimethyl-2:3:7:8-
dibenzothiophanthrene (IX) and 4:9-dimethyl-2:3:5:6-dibenzothiophan-
the (X), wherein the phenanthrene bridge is once again introduced, are
highly carcinogenic. 1:1'-Dinaphthalene-2:2'-sulphide, an isoster of the
inactive hydrocarbon 3:4-(2':1'-naphtho)-phenanthrene, was prepared by
a shorter synthesis and proved to be non-carcinogenic.

Condensation of thioisatin with $\alpha$-chloroacetophenone, $\alpha$-bromoacetyl-
and $\beta$-bromoacetyl-naphthalenes in alkaline solution followed by cycliza-
tion of the intermediate 2-aroylthionaphthene-3-carboxylic acids gave (III),
2:3:7:8-dibenzo-4:9-thiophanthrenequinone (XII) and 2:3:5:6-dibenzo-
4:9-thiophanthrenequinone (XIV) respectively. The quinones were con-
verted to the corresponding 4:9-dimethylthiophanthrenes, (II) (IX) and (X).
Reduction of (XII) and (XIV) gave $2:3:7:8$-dibenzo-(XIX) and $2:3:5:6$-dibenzothiophanthrones (XX).

I am indebted to Sir Robert Robinson, P.R.S., O.M., for his kind interest in the work, which was carried out in the Dyson Perrins Laboratory, Oxford, during 1944–46. I am also grateful to Dr. I. Berenblum for physiological tests.

REFERENCES