photochemical iodine-sensitized reaction. Comparison of the temperature coefficients of the thermal and photochemical reactions leads to a calculation of the energy of dissociation of iodine into atoms in benzene solution and gives further evidence in favor of the supposition of an iodine atom catalysis.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Hydroxy Derivatives of 3,4-Benzpyrene[1] and 1,2-Benzanthracene

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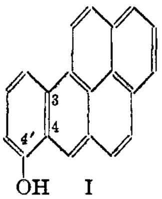
The study of hydroxy derivatives of carcinogenically active hydrocarbons was undertaken partly because it is of interest to determine the influence of hydroxyl and modified hydroxyl substituents on the cancer-producing properties of the hydrocarbons, and partly in the hope of broadening the field of biological experimentation. If suitable functional groups can be introduced without complete loss of carcinogenic activity, it should be possible to attach to the carcinogens side chains of such a character as to vary the solubility relations in different directions. Hydroxy compounds would be of further interest, as Cook and his associates[4] have observed, because polynuclear aromatic compounds arising in the body as products of the metabolism of steroids might be expected to appear in an hydroxylated form comparable with oestrone and equilenin.

From the information now available, the most potent of the known carcinogenic hydrocarbons can be classified as either 10-alkyl-1,2-benzanthracenes or as compounds containing the 1,2-benzanthracene ring system with a carbon substituent in the alternate meso position 9.[5] Methylcholanthrene, cholanthrene, 9,10-dimethyl-1,2-benzanthracene and 10-methyl-1,2-benzanthracene are the most powerful agents of the first type, while 3,4-benzpyrene is the most active representative of the second type of hydrocarbon. On the basis of this provisional classification, a systematic investigation has been undertaken to determine the influence of hydroxyl, amino and other groups at various positions on the carcinogenic properties of these hydrocarbons. The results presented in this paper are incidental observations which constitute a start in the direction indicated.

The synthesis of 4′-hydroxy-3,4-benzpyrene (I) was reported in a preliminary communication,[6] and further details of the preparation of the compound and of various derivatives are given in this paper. The hydroxyl group of this compound occupies position 5 in the 1,2-benzanthracene ring system present in the molecule. Dr. M. J. Shear injected the crystalline hydroxy compound into mice and obtained no tumors at the site of injection after sixteen months. Negative results were obtained also with 4′-ketotetrahydro-3,4-benzpyrene, from which I is prepared by dehydrogenation. The action of 4′-methoxy-3,4-benzpyrene suggested that it may prove to be weakly carcinogenic, although it is too early to make a definite decision. Experiments with the acetate, benzoate, *p*-aminobenzoate, and carboxymethyl derivative are in progress.



I

Although 1,2-benzanthracene itself is almost completely devoid of cancer-producing properties,[7] the fact that some of the simple alkyl derivatives of the hydrocarbon are potent carcinogenic agents suggested the idea of testing samples of the 3-hydroxy and 3-methoxy compounds which were available from an earlier research

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[1] Revised numbering system, see Note 7, THIS JOURNAL, **58**, 2377 (1936).

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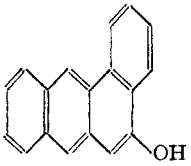
[4] Cook, Haslewood, Hewett, Hieger, Kennaway and Mayneord, "Reports of the II International Congress of Scientific and Social Campaign against Cancer," **1**, 1 (1936); *Am. J. Cancer*, **29**, 219 (1937).

[5] Fieser and Hershberg, THIS JOURNAL, **59**, 394 (1937); L. F. Fieser, M. Fieser, Hershberg, Newman, Seligman and Shear, *Am. J. Cancer*, **29**, 260 (1937).

[6] Fieser, Hershberg and Newman, THIS JOURNAL, **57**, 1509 (1935).

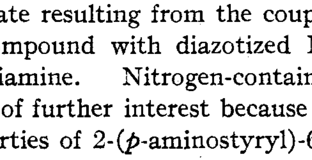
[7] Barry, Cook, Haslewood, Hewett, Hieger and Kennaway, *Proc. Roy. Soc.* (London), **B117**, 318 (1935).

of one of us with Dietz.[8] As the preliminary tests of Dr. Shear gave interesting results, the experiments were repeated with fresh samples of the compounds and a few additional derivatives were prepared. 3-Hydroxy-1,2-benzanthracene (II) gave entirely negative results in one experiment with eleven mice, but in another experiment tumors were obtained at the site of injection in two of ten mice after eight months. These mice were males and the tumors were in immediate contact with injected material still present. 3-Methoxy-1,2-benzanthracene has given tumors at the site of injection in several animals and the tumors have been found to be transplantable. Both the hydroxy compound and the ether appear to have carcinogenic properties, although the action in each case is slow and irregular. No tumors have been obtained as yet with the benzoate (eight months) or stearate (six months). It is of interest that 9-methoxy-1,2,5,6-dibenzanthracene has been found to possess carcinogenic activity.[7]



II

In view of the discovery by Yoshida[9] that *o*-aminoazotoluene has carcinogenic properties, it seemed of interest to investigate the *p*-aminobenzeneazo derivative of 3-hydroxy-1,2-benzanthracene, III. A product presumably containing this compound was obtained in an amorphous condition by the alkaline hydrolysis of the crystalline acetate resulting from the coupling of the hydroxy compound with diazotized N-acetyl-*p*-phenylenediamine. Nitrogen-containing compounds are of further interest because of carcinogenic properties of 2-(*p*-aminostyryl)-6-(*p*-acetylaminobenzoylamino) - quinoline methoacetate,[10] because of the biological changes produced by 3,4,5,6-dibenzcarbazole,[4] and because of the indications that aromatic amines may be responsible for cases of bladder-cancer among workers engaged in the manufacture of dyestuff intermediates.[4] For this reason, 3-hydroxy-1,2-benzanthracene was converted into 3-amino- and 3-methylamino-1,2-benzanthracene by the Bucherer reaction, and tests with the compounds are now in progress.



III

Experimental Part[11]

4' - Keto - 1',2',3',4' - tetrahydrobenzpyrene.—Sannié and Poremski[12] have pointed out that in preparing this ketone by the method very briefly described by Fieser and Fieser[13] it is advisable to employ carefully purified γ-3-pyrenylbutyric acid and to remove the excess thionyl chloride as completely as possible. These specifications were included in the original directions but they are properly emphasized. Although the acid should be of good quality (from xylene[13]), purification through the ester[14] is not necessary. The importance of carefully eliminating the excess thionyl chloride was established by adding some of the reagent to one of two portions of a satisfactory sample of the acid chloride in carbon bisulfide solution. On subsequently adding stannic chloride, a copious black precipitate separated at once and the final product was of an inferior grade. In the parallel test there was very little immediate precipitation of black material and the bright purple-red complex slowly formed. It is important to avoid overheating the acid chloride during the preparation. The reaction of the acid with thionyl chloride can be hastened safely by warming the mixture in a water-bath maintained at 40°, and in removing the ether at reduced pressure this bath temperature should not be exceeded or the material may be spoiled. The first evaporation leaves a yellow oil which largely solidifies on adding fresh ether. On removing the solvent again and distributing the product over the walls of the flask, the acid chloride forms a hard, nearly colorless cake streaked in places with yellow. After evaporating fresh portions of ether and of carbon bisulfide, the yellow streaks disappear and the product is obtained as a light gray solid, m. p. 76–77°. The cyclization can be conducted satisfactorily at 25°, followed by refluxing. On using four times the amount of stannic chloride specified, the yield of ketone dropped to 46–49%. Water may be used at the end of the reaction in place of ice and, as the red complex is unusually stable, steaming should be continued for some time. The crude ketone, which has been obtained repeatedly in a good condition in 80–85% yield, can be purified by distillation in vacuum or, very satisfactorily, by crystallization from dioxane. The purest sample obtained melted at 174–174.5°.

4'-Hydroxy-3,4-benzpyrene.—The best results were obtained by heating a mixture of 2 g. of the pure ketone (from dioxane) and 0.24 g. of sulfur at 210–215° for seventy to

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[8] Fieser and Dietz, THIS JOURNAL, **51**, 3141 (1929).

[9] See Cook, *et al.*,[4] for references.

[10] Browning, J. B. Cohen, Cooper, Ellingworth and Gulbransen, *Proc. Roy. Soc.* (London), **B113**, 300 (1933).

[11] The melting points are corrected except as noted. Analyses by Mrs. G. M. Wellwood.

[12] Sannié and Poremski, *Bull. soc. chim.*, [5], **3**, 1139 (1936).

[13] Fieser and Fieser, THIS JOURNAL, **57**, 782 (1935).

[14] Fieser, Fieser and Hershberg, *ibid.*, **58**, 1463 (1936).

eighty minutes, distilling the product at reduced pressure, and crystallizing the distillate from xylene; yield, 0.6 g. (30%), m. p. 216–218°. Recrystallized from xylene, the substance formed yellow needles with a bronze luster, m. p. 218–219°.

The **acetate**, prepared by refluxing the hydroxy compound with acetic anhydride and sodium acetate for two hours, was crystallized from acetic acid and then from absolute alcohol. It formed small, yellow needles, m. p. 194–195°.

Anal. Calcd. for $C_{22}H_{14}O_2$: C, 85.14; H, 4.55. Found: C, 84.75; H, 4.77.

The **benzoate** was obtained with the use of benzoyl chloride and pyridine (two hours on the steam bath) and crystallized from benzene–absolute alcohol; flat yellow needles, m. p. 191–192°.

Anal. Calcd. for $C_{27}H_{16}O_2$: C, 87.07; H, 4.33. Found: C, 86.74; H, 4.71.

For the preparation of the **methyl ether,** 0.3 g. of the hydroxy compound was dissolved in a solution of 1 g. of potassium hydroxide in 20 cc. of 50% alcohol and treated in the cold with 2 cc. of dimethyl sulfate. After adding more alkali and more reagent, the ether (0.28 g.) was precipitated with water and crystallized from benzene–absolute alcohol. It formed pale yellow-orange, flat prisms, m. p. 183–184°.

Anal. Calcd. for $C_{21}H_{14}O$: C, 89.33; H, 5.00. Found: C, 89.38; H, 5.14.

The **carboxymethyl derivative** was obtained by the action of excess chloroacetic acid on hydroxybenzpyrene dissolved in aqueous potassium hydroxide and dioxane under varying conditions, but the yield at best was only about 30%. The crude material obtained on acidification of the solution was dried and dissolved in acetone. Benzene was added, and after removing most of the acetone by distillation the product separated as rosets of fine, yellow needles, m. p. 243–244°.

Anal. Calcd. for $C_{21}H_{14}O_3$: C, 80.23; H, 4.52. Found: C, 79.97; H, 4.12.

The ***p*-nitrobenzoate,** prepared as above, crystallized from benzene–ligroin as shiny brown needles, m. p. 252–253°.

Anal. Calcd. for $C_{27}H_{15}O_4N$: C, 77.68; H, 3.62. Found: C, 77.85; H, 3.57.

The ***p*-aminobenzoate** was obtained in excellent yield by the hydrogenation of the nitro compound in warm dioxane solution using Adams catalyst. When the color of the solution had changed from yellow-orange to pale yellow-green, the filtered solution was diluted with water and the crystalline precipitate was crystallized from dioxane–alcohol. The amine formed pale yellow needles, m. p. 268–269°.

3-Methoxy-1,2-benzanthracene.—The reduction of crude 3-methoxy-1,2-benz-10-anthrone with aluminum amalgam and alcoholic ammonia was reported by Fieser and Dietz[8] to give somewhat irregular results, the yields varying from 25 to 65%. The reaction seems to be particularly sensitive to the nature of the aluminum metal, and in the present work we had little success using the aluminum turnings on hand and only slightly better results with aluminum foil. A more reliable method of reduction was found in the use of activated zinc dust, sodium hydroxide, and toluene.[15]

Fifty grams of zinc dust was shaken with a solution of 2.5 g. of copper sulfate in 100 cc. of water and the supernatant liquor was decanted. The crude anthrone from 20 g. of 4-methoxy-1-naphthylphenylmethane-2′-carboxylic acid was added, together with 800 cc. of 1 *N* sodium hydroxide solution and 300 cc. of toluene. In some experiments the anthrone was employed in the moist condition originally obtained;[8] in others the moist material was suspended in water, the acid present was neutralized with ammonia and the product was collected and dried before use in the reduction. The reaction mixture was boiled under reflux for eight hours, during which time the aqueous layer changed in color from dark brown to red to red-brown while the toluene layer remained yellow. The liquor was filtered hot into a separatory funnel and a separation was made at once. The toluene solution deposited nearly colorless needles of 3-methoxy-1,2-benzanthracene, and additional material was obtained from the mother liquor, which was combined with benzene extracts of the aqueous layer and of the zinc residue. The total yield of material of average melting point 160–161°, uncorr., was 11 g. (63%). Repeated crystallization from glacial acetic acid gave colorless needles, m. p. 167–168°.

3-Hydroxy-1,2-benzanthracene Benzoate.—The hydroxy compound, prepared according to Fieser and Dietz,[8] was heated with benzoyl chloride in pyridine solution at the boiling point for a few minutes. On dilution with water and cooling, the benzoate separated as long, pale yellow needles. Recrystallization from alcohol gave long, colorless needles, m. p. 174–174.5°; it is readily soluble in dioxane, moderately so in benzene, sparingly soluble in alcohol.

Anal. Calcd. for $C_{25}H_{16}O_2$: C, 86.24; H, 4.63. Found: C, 85.82; H, 4.94.

3-Hydroxy-1,2-benzanthracene Stearate.—A solution of 0.5 g. of hydroxybenzanthracene and 0.8 g. of stearyl chloride (from the acid with thionyl chloride, b. p. 179–182° at 4 mm.) in 5 cc. of dry pyridine was heated for two hours on the steam bath and cooled. On adding 5–6 drops of 0.5 *N* potassium hydroxide solution, an amorphous white precipitate separated. Washed with 50% pyridine solution and dried, the crude material (1.2 g.) melted at 72–78°. After digestion with small portions of methanol, in which the substance is only moderately soluble, the bulk of the material was crystallized from this solvent and obtained as small, colorless needles, m. p. 87–89° (0.5 g.). The melting point was not changed on further crystallization.

Anal. Calcd. for $C_{36}H_{46}O_2$: C, 84.65; H, 9.09. Found: C, 84.33; H, 8.84.

Carboxymethyl Derivative of 3-Hydroxy-1,2-benzanthracene.—A mixture of 0.5 g. of hydroxybenzanthracene, 1.8 cc. of 33% aqueous sodium hydroxide solution, 2 cc. of dioxane, and 2 cc. of water was warmed until solution was complete, cooled (two layers), treated with 1.3 cc. of 50% aqueous chloroacetic acid solution and heated at 100° for three hours. The solution was diluted with water,

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[15] Martin, This Journal, 58, 1438 (1936).

made acid to Congo red paper and the product was extracted with ether, taken into dilute soda solution and precipitated by acidification. Crystallization from toluene, using decolorizing carbon, gave colorless needles, m. p. 216–217°.

Anal. Calcd. for $C_{20}H_{14}O_3$: C, 79.20; H, 4.99. Found: C, 78.93; H, 4.89.

4 - *p* - Acetaminobenzeneazo - 3 - hydroxy - 1,2 - benzanthracene.—A solution of 0.615 g. of N-acetyl-*p*-phenylenediamine, prepared according to Nietzki,[16] and purified by vacuum distillation and crystallization from water, in 5.1 cc. of glacial acetic acid was treated with 0.4 cc. of concentrated sulfuric acid, which precipitated the sulfate, cooled to 17°, and treated slowly with 1.2 cc. of ice-cold isopropyl nitrite. To the resulting red solution 20 cc. of absolute ether was added, which precipitated the diazonium salt as an oil. After decanting the cloudy liquid, the oil was dissolved in 5 cc. of glacial acetic acid and on adding ether the diazonium salt was precipitated in a crystalline, somewhat pink, condition. The salt, suspended in 10 cc. of glacial acetic acid (in which the crystalline material is sparingly soluble) was added to a mechanically stirred solution of 0.5 g. of 3-hydroxy-1,2-benzanthracene in 47 cc. of glacial acetic acid at 17°. A deep red suspension of the azo compound was soon obtained, and after stirring for one and one-half hours the mixture was diluted with 50 cc. of water, stirred for fifteen minutes, and the product was collected. The crude azo compound melted at 275–279°, uncorr.; yield 0.72 g. (95%). Crystallized from a large volume of benzene, the substance formed small, lustrous red needles, m. p. 278–279°, uncorr. It is sparingly soluble in all ordinary solvents.

Anal. Calcd. for $C_{26}H_{19}O_2N_3$: C, 77.00; H, 4.73. Found: C, 77.10; H, 4.50.

4 - *p* - Aminobenzeneazo - 3 - hydroxy - 1,2 - benzanthracene.—The hydrolysis of the acetate presented difficulties and the amine was not obtained in a completely pure condition. Of a number of methods of hydrolysis with acids and alkalies investigated, the following was the most satisfactory. The acetate (1.4 g.) was refluxed with 420 cc. of a 10% solution of potassium hydroxide in absolute alcohol for nineteen hours and the hot solution was filtered from a small amount of brown residue and diluted with 300 cc. of hot water. The material which separated on cooling was washed with 50% alcohol, and then washed with water until free from alkali. The amine was obtained in this way as an amorphous, deep brownish-purple powder melting at 211–213°, uncorr.; yield 0.54 g. (43%). It is practically insoluble in all solvents tried except pyridine, and it did not crystallize from the solvent. Analyses of the amorphous material indicated the presence of impurities (Calcd. for $C_{24}H_{17}ON_3$: C, 79.3; H, 4.7; N, 11.6. Found: C, 80.5; H, 5.2; N, 9.2).

3-Amino-1,2-benzanthracene.—A mixture of 1 g. of 3-hydroxy-1,2-benzanthracene, 5 g. of sodium bisulfite in 10 cc. of water, 10 cc. of concentrated ammonia solution, and 5 cc. of dioxane was heated in a sealed tube at 180–190° for ten hours. After dilution with water, the crystalline product was collected, dried and crystallized from absolute alcohol; yield 0.65 g. (65%), m. p. 209–210°. After further crystallizations the substance formed fan-shaped clusters of yellow needles, m. p. 211.5–212.5°. The amine is very slightly soluble in hot, dilute hydrochloric acid.

Anal. Calcd. for $C_{18}H_{13}N$: N, 5.76. Found: N, 5.86.

3 - Methylamino - 1,2 - benzanthracene.—This derivative was prepared exactly as described above except that 33% aqueous methylamine solution was substituted for the ammonia solution; the yield of pure material was 38%. Crystallized from alcohol, in which it is much more soluble than the primary amine, the compound formed rosets of short, flat, yellow needles, m. p. 115.5–116.5°.

Anal. Calcd. for $C_{19}H_{15}N$: N, 5.45. Found: N, 5.73.

Summary

The preparation of various functional derivatives of 4′-hydroxy-3,4-benzpyrene and of 3-hydroxy-1,2-benzanthracene is described and it is reported that 3-hydroxy- and 3-methoxy-1,2-benzanthracene have weak carcinogenic properties.

CONVERSE MEMORIAL LABORATORY
CAMBRIDGE, MASS.
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[16] Nietzki, *Ber.*, **17**, 343 (1884).