[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Aceanthrene Derivatives Related to Cholanthrene

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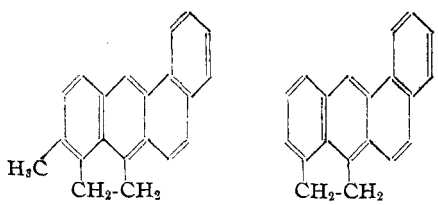
It was reported in a paper by one of us with Seligman[2] that the methyl group of methylcholanthrene (I) is not important in determining the high carcinogenic potency of this hydrocarbon, for cholanthrene (II) produces transplantable tumors only slightly less rapidly than methylcholanthrene.[2,3] Fieser and Newman[4] investigated



I II III IV

further simplifications of the molecule, and it was found that 5,10-dimethyl-1,2-benzanthracene (III) retains the properties characteristic of methylcholanthrene and produces tumors regularly and with great rapidity. Dr. M. J. Shear has confirmed the preliminary report[4] and found that the hydrocarbon has about the same activity as methylcholanthrene, producing in mice transplantable tumors which appear, on the average, after about three months. The five-membered ring present in the cholanthrenes evidently is not important in determining the biological action of the compounds, and it is not even essential for high activity that both methyl groups be retained. Dr. Shear has found that 10-methyl-1,2-benzanthracene[4] (IV) is comparable in carcinogenic activity with the hydrocarbons I–III. Injected subcutaneously into mice in 5-mg. dosage, the hydrocarbon gave tumors which made their first appearance in two months. At the end of four months, tumors had been obtained in fifteen of twenty mice. Three of these tumors were transplanted successfully. In contrast to this powerful agent, 5-methyl-1,2-benzanthracene produces tumors only slowly[5] and is more nearly comparable with 1,2,5,6-dibenzanthracene than with the cholanthrenes.

From these results it is evident that the carcinogenic potency of methylcholanthrene cannot be attributed to a special pentacyclic structure, as was suggested by the early work of Cook and his associates,[3b] or to the fact that the hydrocarbon is a 1,2-benzanthracene derivative with alkyl substituents at the 5- and 6-positions. From the present results it appears that the structural feature of greatest importance is the presence of a simple carbon substituent at the meso position 10 in the 1,2-benzanthracene ring system. In comparison with the effect of such a group, the influence of alkyl substituents at the 5- and 6-positions[5] is of secondary significance. Evidently the size and structure of the substituent at the 10 position is of considerable importance, for the English investigators[5] found that 10-isopropyl- and 10-benzyl-1,2-benzanthracene are devoid of carcinogenic activity. The contrasting effects of methyl and isopropyl groups at position 10 are surprising in view of the observation[5] that 6-methyl-1,2-benzanthracene is somewhat inferior in carcinogenic properties to the 6-isopropyl compound. The 5-*n*-propyl compound, however, was found to give fewer tumors than the 5-methyl derivative.[3a] The relationship between the 10-methyl and 10-isopropyl compounds appears to be comparable also with that between the highly potent methylcholanthrene and the slowly acting 16,20-dimethylcholanthrene.[6] The latter hydrocarbon, which carries a larger and more branched substituent at the meso position, produces tumors which appear in about the same average time[7] as in the case of 1,2,5,6-dibenzanthracene (seven and one-half months). It is clearly important to explore further the relationship of structure and carcinogenic activity in the series of 10-alkyl-1,2-

(1) Lilly Research Fellow.

(2) Fieser and Seligman, THIS JOURNAL, **57**, 2174 (1935).

(3) (a) Cook, *Ber.*, **69A**, 38 (1936); (b) Cook, Haslewood, Hewett, Hieger, Kennaway and Mayneord, Reports of the II International Congress of Scientific and Social Campaign against Cancer, **1**, 1 (1936).

(4) Fieser and Newman, THIS JOURNAL, **58**, 2376 (1936).

(5) Barry, Cook, Haslewood, Hewett, Hieger and Mayneord, *Proc. Roy. Soc.* (London), **B117**, 318 (1935).

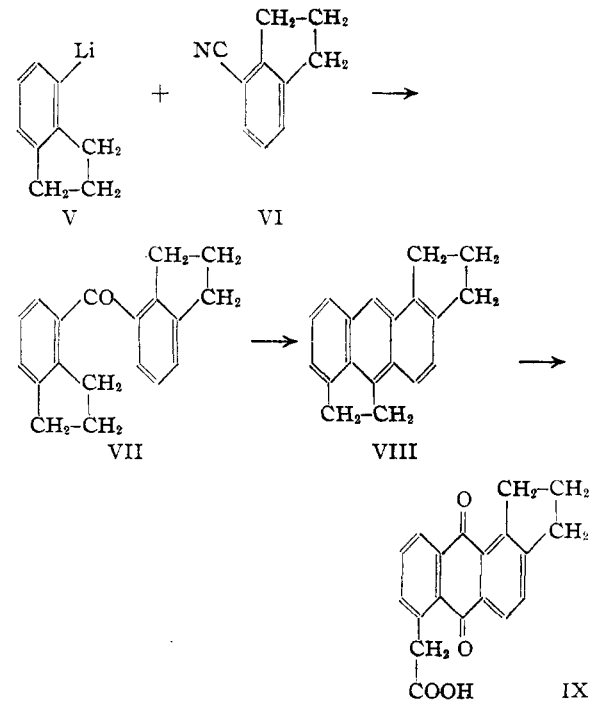
(6) Fieser and Seligman, THIS JOURNAL, **57**, 1377 (1935).

(7) Shear, *Am. J. Cancer*, **28**, 334 (1936).

benzanthracenes, and such work is in progress.[8] It is interesting that the powerfully carcinogenic 3,4-benzpyrene may be regarded as a 1,2-benzanthracene with a carbon substituent at the alternate meso position 9, and that 8,9-dimethylene-1,2-benzanthracene[2] shows some activity, producing tumors in an average time of about seven months.[7] The influence of a methyl group at the 9-position is being investigated in independent work by Dr. M. S. Newman.

It is hoped that by defining the features of structure associated with high carcinogenic activity, some clue may be found which will help to reveal the nature of the action of cancer-producing hydrocarbons on cellular tissue. The recognition that the potency of methylcholanthrene is retained in compounds of much less complicated structure may serve at least to clarify the problem. In order to provide as sound a basis for speculation as possible, it seemed desirable to determine whether 10-methyl-1,2-benzanthracene represents a limiting structure beyond which the process of simplification cannot be carried without great loss in activity. The 10-methyl group clearly is important, for the parent hydrocarbon is almost completely inactive.[5] It is conceivable that the angular aromatic ring attached to the anthracene system in the 1,2-position may be subject to modification, for the 5,6-dimethyl- and 5,6-cyclopenteno derivatives of 1,2-benzanthracene are comparable with 1,2,5,6-dibenzanthracene in carcinogenic activity.[5] In order to test this point we have synthesized for comparison with cholanthrene one hydrocarbon differing from this substance in having two methyl groups in place of the angular benzenoid ring, and another one having a cyclopenteno ring in the 1,2-position.

For the synthesis of 1,2-cyclopenteno-5,10-aceanthrene (VIII), 4-chlorohydrindene was prepared from *o*-chlorobenzaldehyde through the known 4-chlorohydrindone-1[9] and a part of the material was converted into the lithium derivative V, while a part was transformed into 4-cyanohydrindene (VI) by interaction with cuprous cyanide in pyridine solution. By the condensation of V and VI and hydrolysis of the resulting ketimine, the ketone VII was obtained in a crystalline condition. Since the ketone is symmetrical, the



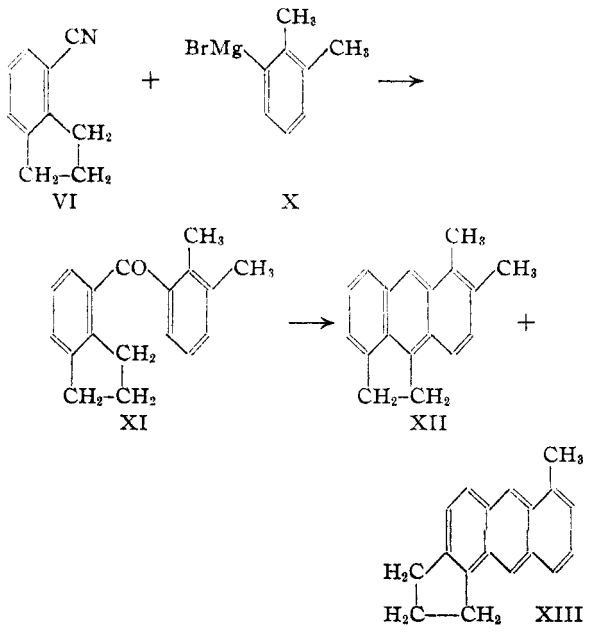
Elbs reaction theoretically can proceed in only one direction, and the sole product found in the reaction mixture was the hydrocarbon VIII. The yield of nearly pure material was 21%, which is considerably better than is usually obtained in the Elbs condensation of *o*-methylated diphenyl ketones. On the other hand, the pyrolysis proceeds less smoothly than in the methylcholanthrene synthesis,[10] where the cyclopenteno ring involved in the cyclization interacts with a naphthoyl, rather than a benzoyl, group. The structure of the hydrocarbon was established by oxidation in glacial acetic acid solution to the anthraquinone acetic acid derivative IX.

1,2-Dimethyl-5,10-aceanthrene (XII) was obtained along with the isomeric 1-methyl-5,6-cyclopentenoanthracene (XIII) by the pyrolysis of the ketone (XI) resulting from the condensation of 4-cyanohydrindene with the Grignard reagent (X) from *vic.*-bromo-*o*-xylene. The hydrocarbons were isolated in a satisfactory condition of purity only in very small amounts and oxidation experiments were completed only in the case of the lower melting isomer. This yielded a neutral anthraquinone having the full complement of carbon atoms, and therefore it must have come from 1-methyl-5,6-cyclopentenoanthracene (XIII). The other hydrocarbon probably has the alternate

(8) 10-Methyl-1,2-benzanthracene has been prepared from 1,2-benz-10-anthrone and methylmagnesium iodide, a method previously investigated without success by Cook, *J. Chem. Soc.*, 1089 (1930). The 10-ethyl compound, m. p. 113.5–114°, corr., has been prepared in a similar manner.

(9) Mayer, Philipps, Ruppert and Schmitt, *Ber.*, **61**, 1966 (1928).

(10) Fieser and Seligman, (a) THIS JOURNAL, **57**, 942 (1935); (b) **58**, 2482 (1936).



structure XII, but this was not established rigidly. An acidic substance giving a strong vat test was formed on oxidation, but it was not obtained in a pure condition.

In tests conducted by Dr. M. J. Shear 1,2-cyclopenteno-5,10-aceanthrene (VIII), injected subcutaneously into mice, has given no tumors in four months. Tests with the hydrocarbons XII and XIII have been negative after two months. No tumors have been obtained with aceanthrene[11] in one and one-half years.

Experimental Part[12]

4-Chlorohydrindene.—*o*-Chlorocinnamic acid was prepared from *o*-chlorobenzaldehyde, acetic anhydride, and potassium acetate according to the procedure of Lasch.[13] In experiments in which the heating was conducted for eight hours and for fourteen hours, the yields of recrystallized material, m. p. 207–209°, were 62 and 56%, respectively. The product was accompanied by a resinous substance insoluble in sodium carbonate solution. *o*-Chlorohydrocinnamic acid[14] was prepared by the catalytic hydrogenation of 77 g. of the unsaturated acid in 500 cc. of warm dioxane, using a total of 1 g. of Adams catalyst. The reaction proceeded to completion in about ten hours, the course of the reduction being followed most satisfactorily by precipitating the material with water from a test portion of the solution and determining the melting point. A small amount of unchanged acid raises the melting point and is detected easily. Continued hydrogenation beyond the desired point leads to an inferior product. The yield of satisfactory material, m. p. 93–95°, was 95%. A sample recrystallized from benzene–ligroin melted at 94.5–95.5°. For conversion to β-(*o*-chlorophenyl)-propionyl chloride[9] 70 g. of the acid was refluxed for three hours with 136 g. of purified thionyl chloride, the excess reagent was distilled, and the product was obtained as a colorless liquid, b. p. 147.5–149° (26 mm.); yield 73 g. (95%). A solution of this material (73 g.) in 1800 cc. of carbon bisulfide was cooled to 5° and 66 g. of aluminum chloride was added with vigorous mechanical stirring. The resulting light yellow solution was allowed to come to room temperature in one and one-half hours, during which time a white complex separated. After refluxing for fifteen minutes the product was collected in the usual way and taken into ether–benzene. The solution was washed in succession with dilute acid, dilute alkali, and saturated sodium chloride solution, and after removal of the solvent the product was distilled. The yield of colorless 4-chlorohydrindone-1,[9] b. p. 123–124° (5 mm.), m. p. 89–90.5° (recrystallized, 90–90.5°), was 55.5 g. (93%).

The Clemmensen reduction of the ketone (114 g.) was conducted exactly as described for the preparation of 4-methyl-7-chlorohydrindene.[10] The yield of 4-chlorohydrindene, b. p. 108–110° (24 mm.), was 93 g. (89%). Material for use in the formation of the lithium derivative was further purified by shaking it repeatedly with successive, equal, portions of concentrated sulfuric acid until an impurity which at first imparted to the acid a brilliant scarlet coloration was largely removed. Little material was lost in the process.

4-Cyanohydrindene.—Following the procedure described in an analogous case,[10b] 46 g. of the 4-chlorohydrindene heated with 27.8 g. of cuprous cyanide and 40 cc. of pyridine at 220° for seventeen hours, gave 34.5 g. (80%) of redistilled nitrile, b. p. 139–141° (22 mm.). The higher boiling material remaining after distillation of the nitrile boiled at about 120–180° (22 mm.) and proved to be largely the corresponding **amide.** Crystallized from alcohol, it formed long, flat, colorless needles, m. p. 173–173.5°, and it was identical with a sample prepared by the reaction of the acid chloride with aqueous ammonia solution.

Anal. Calcd. for $C_{10}H_{10}NO$: C, 74.49; H, 6.89. Found: C, 74.09; H, 6.60.

The nitrile proved very resistant to acid hydrolysis, refluxing with 6 *N* hydrochloric acid for two days giving only a small amount of the acid. When 1 g. of the nitrile was heated with 15 cc. of concentrated hydrochloric acid for ten hours at 180–200° there was some carbonization and 0.8 g. of **hydrindene-4-carboxylic acid** was obtained. Recrystallized from dilute alcohol, the acid formed flat needles, m. p. 152.5–153.5°.

Anal. Calcd. for $C_{10}H_{10}O_2$: C, 74.03; H, 6.24. Found: C, 73.69; H, 6.16.

Di-(4-hydrindyl) Ketone (VII).—For the manipulation of lithium metal it was found convenient to prepare wire of uniform diameter in a sodium press equipped with a 3-mm. die. The wire is collected under heavy paraffin oil and cut into uniform 12-cm. lengths, which are placed in a test-tube having a stopcock at the bottom and a nitrogen inlet near the stopper. The sticks of metal are washed with successive portions of benzene and ether under nitrogen pressure, draining the wash liquor through the stop-

(11) Fieser and Peters, THIS JOURNAL, **54**, 4373 (1932).

(12) All melting points are corrected. Analyses by Mrs. G. M. Wellwood.

(13) Lasch, *Monatsh.*, **34**, 1653 (1913).

(14) Gabriel and Herzberg, *Ber.*, **16**, 2037 (1883).

cock. The wire is calibrated by weighing one of the sections, or a part thereof. The lithium is introduced to the reaction flask after this has been flushed with nitrogen and with the gas streaming from the widest opening of the flask. While flushing the opened lithium container with nitrogen, the test-tube is held at right angles to the mouth of the flask, a length of lithium wire is withdrawn and short cylinders are cut with scissors and allowed to drop into the flask. Fresh, silvery surfaces are thus exposed and protected until the other reactants are added.

The reaction between 4-chlorohydrindene (12.1 g.) and lithium (1.1 g.) in ether (75 cc.) started at once without the use of catalysts but proceeded slowly owing to the formation of an adherent coating on the metal. After stirring the mixture under nitrogen for eighteen hours, titration of a 1-cc. portion indicated 71% conversion to the aryl lithium. There was little change at the end of forty hours (75% conversion), and the solution was cooled to −70° and 10 g. of 4-cyanohydrindene in 25 cc. of ether was added rapidly. The solution was allowed to come to room temperature and refluxed for one hour to complete the reaction. The mixture was decomposed with dilute hydrochloric acid, using in all about 100 cc. of 3 *N* acid, the ether was evaporated, 5 cc. of glacial acetic acid was added to facilitate the hydrolysis of the ketimine hydrochloride, and the mixture was refluxed for four hours. The ketone was taken into ether and the solution was filtered from a little suspended material and distilled, affording 10.5 g. (51%) of ketone, b. p. 206–209° (4 mm.). The distillate solidified completely and on crystallization from petroleum ether the compound formed large, colorless prisms, m. p. 77–78°.

Anal. Calcd. for $C_{19}H_{18}O$: C, 87.33; H, 6.93. Found: C, 86.88; H, 7.00.

1,2-Cyclopenteno-5,10-aceanthrene (VIII).—Two lots of the ketone VII, totaling 13.3 g., were pyrolyzed separately at 415–420° for thirty minutes and the product was distilled in vacuum and crystallized from ligroin. The crude, yellow hydrocarbon obtained melted at 160–170° and weighed 2.6 g. (21%). For purification a solution of the material in benzene–ligroin was passed through an adsorption tower of activated alumina, which removed some foreign material. The process was repeated and the material from the first portion of the filtrate after two crystallizations from ligroin formed pale yellow needles melting at 175.5–176° (0.3 g.). The bulk of the material, similarly crystallized, melted at 174–176° (1.7 g.). The hydrocarbon forms a light yellow, fluorescent solution in concentrated sulfuric acid.

Anal. Calcd. for $C_{19}H_{16}$: C, 93.40; H, 6.60. Found: C, 93.70; H, 6.99.

The picrate crystallized from ligroin in the form of purplish black needles, m. p. 140.5–141.5°. The regenerated hydrocarbon was unchanged in melting point.

Anal. Calcd. for $C_{19}H_{16}\cdot C_6H_3O_7N_3$: N, 8.88. Found: N, 8.69.

1,2 - Cyclopenteno - 9,10 - anthraquinone - 5 - acetic Acid (IX).—A solution of 0.65 g. of 1,2-cyclopenteno-5,10-aceanthrene and 3.5 g. of sodium dichromate in 35 cc. of glacial acetic acid was refluxed for two hours and the solution was diluted with a large volume of water. The precipitated material (0.35 g.) formed pale yellow microplates on crystallization from xylene. When heated rapidly, the substance melts with decomposition at 284–285°. It is soluble in hot, dilute soda solution and gives a red vat with alkaline hydrosulfite.

Anal. Calcd. for $C_{19}H_{14}O_4$: C, 74.54; H, 4.61. Found: C, 74.75; H, 4.38.

On attempting to decarboxylate the acid with copper carbonate in quinoline solution there was much decomposition and only a small amount of an impure neutral quinone, m. p. 121–123°, was isolated.

vic.-**Bromo-*o*-xylene.**—The preparation of this halide by sulfonating *o*-xylene,[15] brominating the resulting 4-sulfonic acid, and hydrolyzing the bromo sulfonic acid is reported by Stallard,[16] but experimental details are lacking. After some investigation, the following procedure was developed.

A mixture of 106 g. of *o*-xylene and 107 cc. of concentrated sulfuric acid was heated on the steam-bath with mechanical stirring for one and one-half hours and the clear solution was dissolved in one and one-half liters of water. The solution was rendered neutral to litmus with 473 g. of hydrated barium hydroxide dissolved in about 750 cc. of water and the solution was filtered hot, cooled to 50–60°, and treated with a solution of 192 g. of bromine and 200 g. of barium bromide in 700 cc. of water. The temperature of the solution was maintained at 50–60° for fifteen hours, during which time barium 2-bromo-1,2-dimethylbenzene-4-sulfonate separated. The solid was collected after cooling, washed well with water, and air dried; yield, 225 g. This amount of pure salt (trihydrate) would correspond to a yield of 63%, but from the results of hydrolysis it is evident that the product contained some *o*-xylene sulfonate.

The hydrolysis with superheated steam was conducted in a distillation apparatus constructed with interchangeable ground-glass joints. The joint between the copper coil superheater and the glass inlet tube was made satisfactorily with a brass compression fitting, using alternate layers of asbestos and lead wool as packing. A total of 693 g. of barium salt was hydrolyzed in three lots, each of which was mixed with about 300 cc. of sulfuric acid, sp. gr. 1.67, in the distillation flask. The inlet steam was at 200–220° and the exit vapor was held at 150–160°. With the use of an automatic separatory take-off, the aqueous portion of the distillate was discarded and the heavy layer of oil was collected. The oil was washed with water, dried and fractionated twice through a 1-meter packed column. About 40 g. of *o*-xylene was recovered and the yield of *vic.*-bromo-*o*-xylene, b. p. 210.5–212.5°, was 149 g. (42%, calculated on the basis of pure barium salt).

4 - (2′,3′ - Dimethylbenzoyl) - hydrindene (XI).—An ethereal solution of the Grignard reagent from 48 g. of *vic.*-bromo-*o*-xylene was added to an ice-cold solution of 26.1 g. of 4-cyanohydrindene in ether. Considerable heat was liberated and the precipitate first formed largely dissolved. After refluxing the mixture overnight in an atmosphere of nitrogen a clear red solution resulted. This was treated with 3 *N* hydrochloric acid, the ether was distilled, and the mixture was refluxed for four hours to effect hydrolysis. An ethereal extract of the ketone was

(15) Jacobsen, *Ber.*, **10**, 1011 (1877); **11**, 17 (1878).

(16) Stallard, *J. Chem. Soc.*, **89**, 808 (1906). See also Kelbe and Stein, *Ber.*, **19**, 2137 (1886).

washed with water, dried with saturated sodium chloride solution, and distilled, giving 38.3 g. of a yellow oil, b. p. 183–186° (4 mm.). which slowly solidified. On crystallization from petroleum ether (b. p. 20–40°), 25.6 g. (56%) of the ketone was obtained in a satisfactory condition, m. p. 74–75°. A sample recrystallized from the same solvent formed large, colorless cubes melting at 75–75.5°.

Anal. Calcd. for $C_{18}H_{18}O$: C, 86.49; H, 7.24. Found: C, 86.63; H, 7.32.

1,2 - Dimethyl - 5,10 - aceanthrene (XII) and 1-Methyl-5,6-cyclopentenoanthracene (XIII).—The ketone XI (20.5 g.) was pyrolyzed at 420–430° for two hours and the product was distilled in vacuum and taken up in petroleum ether (b. p. 20–40°). On standing at 0° a crystalline product (0.84 g.) consisting largely of the crude dimethylaceanthrene slowly separated. This was purified by passage of a solution in benzene through an adsorption tower of alumina, conversion to the picrate, regeneration with ammonia solution, and crystallization of the hydrocarbon from ligroin. **1,2-Dimethyl-5,10-aceanthrene** was obtained as pale yellow, glistening leaflets melting at 206–207°; yield 0.25 g. (1.3%).

Anal. Calcd. for $C_{18}H_{16}$: C, 93.06; H, 6.94. Found: C, 93.35; H, 6.45.

The picrate formed long, permanganate-colored needles from benzene–ligroin and melted at 169–170°.

Anal. Calcd. for $C_{15}H_{16}\cdot C_6H_3O_7N_3$: N, 9.11. Found: N, 8.96.

On refluxing a solution of 50 mg. of the hydrocarbon and 200 mg. of sodium dichromate in 5 cc. of glacial acetic acid for one hour and adding water, there was obtained a brown precipitate which partly dissolved in carbonate solution. This solution gave a strong vat test on adding sodium hydrosulfite, but the amount of product was insufficient for more definite characterization.

The second hydrocarbon, **1-methyl-5,6-cyclopentenoanthracene,** was isolated as the picrate from the initial petroleum ether mother liquors, after first removing extraneous material by purification in an adsorption tower. After repeated crystallization from benzene–ligroin, the **picrate** formed deep brownish red needles, m. p. 156–157°.

Anal. Calcd. for $C_{18}H_{16}\cdot C_6H_3O_7N_3$: N, 9.11. Found: N, 8.80.

The regenerated hydrocarbon, after crystallization from ligroin, formed pale-yellow blades melting at 131–132°; yield, 0.58 g. (3%).

Anal. Calcd. for $C_{18}H_{16}$: C, 93.06; H, 6.94. Found: C, 92.94; H, 6.87.

Oxidation to **1-methyl-5,6-cyclopentenoanthraquinone** was accomplished by refluxing a solution of 50 mg. of the hydrocarbon and 300 mg. of sodium dichromate in 5 cc. of glacial acetic acid for one hour and precipitating the product with water. The crude quinone was purified by crystallization from dilute alcohol, vacuum sublimation, and recrystallization from dilute alcohol. It formed long, slender, light yellow needles, m. p. 125–127°. Like other di-α-substituted anthraquinones, the compound does not give a vat test with hydrosulfite in aqueous alkali.

Anal. Calcd. for $C_{18}H_{14}O_2$: C, 82.49; H, 5.38. Found: C, 82.20, 82.18; H, 5.35, 5.56.

Summary

Tests with the previously described 5,10-dimethyl-1,2-benzanthracene and 10-methyl-1,2-benzanthracene indicate that these hydrocarbons are of the same order of carcinogenic potency as methylcholanthrene and cholanthrene, from which it appears that methylcholanthrene owes its high activity to the presence of the 1,2-benzanthracene ring system with a simple alkyl substituent at the 10-position. In order to determine if further simplifications of the structure can be made without loss in potency, 1,2-cyclopenteno-5,10-aceanthrene and 1,2-dimethyl-5,10-aceanthrene have been synthesized for comparison with cholanthrene (1,2-benz-5,10-aceanthrene), and biological tests are in progress.

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