minutes, and ammonium chloride was then added until a colorless solution resulted. At this point 10 cc. of dry toluene and 1 g. of finely pulverized 10-chloromethyl-1,2-benzanthracene were added and the suspension was shaken vigorously for twenty to thirty minutes with occasional immersion of the test-tube in a carbon dioxide cooling-bath. The ammonia was allowed to evaporate and the residue was warmed on the steam-bath with 10 cc. of benzene for fifteen minutes. The solid was then collected and suspended in water, and the mixture rendered acidic to congo red with hydrochloric acid. The crude conjugate was then collected, washed and dried (2 g.) and crystallized from a hot mixture of dioxane and 2 *N* hydrochloric acid, the solution, after clarification with Norit, being allowed to cool slowly. The substance at first separated as a gel but became crystalline on standing. It was collected and washed, dried over phosphorus pentoxide, recrystallized from dioxane–hydrochloric acid, and finally digested at room temperature with dilute sodium bicarbonate solution and then with water. After thorough drying at 60°, the pale yellow, microcrystalline material decomposed at 205.7–206.7° with gas evolution when inserted in a bath at 205°.

Anal. Calcd. for $C_{22}H_{19}O_2SN$: N, 3.89. Found: N, 4.09.

A solution of 50 mg. of the conjugate in 5 cc. of a 2:1 mixture of dioxane and 2 *N* hydrochloric acid showed the rotation $[\alpha]^{28}D$ −7.5°. The conjugate is insoluble in water, in aqueous acid or alkali, and neutral organic solvents. The solutions in hot acidified alcohol or dioxane, which are strongly fluorescent in ultraviolet light, deposit the substance in an initially gelatinous condition on either dilution with water or neutralization of the acid.

Summary

On reaction with sulfur monochloride, followed by the reduction of the resulting dithiochloride with sodium sulfide, 3,4-benzpyrene gives the 5-mercaptan and 1,2-benzanthracene is converted into its 10-mercapto derivative, as shown by the synthesis of the identical compounds from known halides by reaction with potassium hydrosulfide, and through the interaction of the magnesium derivative with sulfur, respectively. 1,2-Benzanthryl-10-methylmercaptan was prepared best from the 10-chloromethyl derivate and thiourea, but the corresponding disulfide was also obtained from the tarry product of the reaction of the hydrocarbon with sulfur monochloride. From these observations and certain qualitative tests it is shown that, in a series of typical carcinogenic hydrocarbons and related compounds, the relative order of reactivity and the position of substitution is the same as in the reaction with lead tetraacetate.

Cysteine conjugates were prepared in the 3,4-benzpyrene and 1,2-benzanthracene series from the mercaptans and α-amino-β-chloropropionic acid. 1,2-Benzanthryl-10-methyl-S-*l*-cysteine was obtained by the interaction of the chloride with sodium cysteinate in liquid ammonia.

Certain inferences are presented concerning the process of hydrocarbon carcinogenesis.

CONVERSE MEMORIAL LABORATORY
CAMBRIDGE, MASSACHUSETTS RECEIVED JULY 20, 1940

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Synthesis of 4′,8′-Dihydroxy-1,2,5,6-dibenzanthracene and its Relation to Products of Metabolism of the Hydrocarbon

BY JAMES CASON[1] AND LOUIS F. FIESER

Following their very thorough and significant study of the metabolism of anthracene in rats and rabbits,[2] Boyland and Levi[3] undertook to determine the form in which the carcinogenic 1,2,5,6-dibenzanthracene is eliminated from the animal organism. From the urine of rabbits which had been fed on a diet containing 0.04% of the hydrocarbon they succeeded in isolating a phenolic substance melting at 350–360°. This was characterized by certain tests and by the preparation of several derivatives as a dihydroxydibenzanthracene with the hydroxyl groups at some positions other than 4, 8, 9 and 10. Dobriner, Rhoads and Lavin[4] have reported the isolation from the urine of rabbits injected subcutaneously or intramuscularly with the hydrocarbon of a crystalline phenolic substance (m. p. 355–358°) corresponding precisely in absorption spectrum with the metabolite of Levi and Boyland. Although the small amount of material isolated sufficed for tests in only a few animals, they found the dihydroxy derivative to be definitely less carcinogenic (no tumors in six months) than the original hydrocarbon (tumors in all controls). On this basis

(1) Research Fellow on a grant from the National Cancer Institute.

(2) Boyland and Levi, *Biochem. J.*, **29**, 2679 (1935); **30**, 728, 1225 (1936).

(3) Levi and Boyland, *Chemistry and Industry*, **15**, 446 (1937).

(4) Dobriner, Rhoads and Lavin, *Proc. Soc. Exptl. Biol. Med.*, **41**, 67 (1939).

they suggested that a resistance to carcinogenesis by hydrocarbons may be dependent upon the ability of the organism to convert the carcinogen into a phenolic derivative. The hypothesis finds a certain measure of support in the results of observations concerning such synthetic hydroxy compounds as have been investigated, including 2-,[5] 3-[6] and 15[7]-hydroxy-20-methylcholanthrene, 4′-,[8] 5-[9] and 6[10]-hydroxy-3,4-benzpyrene, 10-hydroxymethyl-1,2-benzanthracene,[11,12] and impure 9-hydroxy-1,2,5,6-dibenzanthracene.[13] In tests in part completed,[14,15] and in part of a preliminary nature,[16] these substances have all been found either inactive or distinctly less active than the parent hydrocarbons. The idea that biological hydroxylation represents an avenue for the elimination of carcinogens in a detoxified form is thus an attractive one bearing highly important implications, and further information on the nature of the metabolic reaction is clearly desirable. The metabolism of 3,4-benzpyrene has been under investigation by Chalmers and Peacock,[17] who found that rats and mice injected with the hydrocarbon eliminate an as yet unidentified transformation product which is excreted largely in the feces and to a smaller extent in the urine. The metabolism of the same hydrocarbon and of methylcholanthrene is being studied by White and White.[18] Dobriner, Rhoads and Lavin[4] administered dibenzanthracene to rats[19] and mice by injection and found that in each case there is excreted in the urine and feces a phenolic substance characterized by absorption spectroscopy as a dibenzanthracene derivative. The substance from rats was spectroscopically identical with that from mice, and was similar to but definitely different from the rabbit compound of Levi and Boyland.[3]

At the request of Drs. Rhoads and Dobriner, we have undertaken to approach the problem from the synthetic side. Although no less than fifty-five isomeric dihydroxy derivatives of 1,2,-5,6-dibenzanthracene are possible, twenty-four of these structures can be eliminated, at least for the rabbit compound, by the observations of Levi and Boyland,[3] and of the remainder the small group of symmetrically disubstituted isomers seemed to us to offer the most promising field for first study. In this paper we report the synthesis of one member of the series by a method calculated to meet the latter requirement. The method is based upon observations of Sempronj,[20] who showed that the 1,2-benzanthraquinone monosulfonate[21] of previously unestablished structure can be converted by reduction and alkali fusion into a hydroxy-1,2-benzanthracene, and that alkali fusion of the quinone sulfonate gives a hydroxynaphthoic acid corresponding in melting point to the known 5,2-derivative. The phenolic product therefore is probably the 4′-hydroxy derivative. After repeating the preparation of this compound, with a few modifications, the method was applied in the dibenzanthracene series. 1,2,5,6-Dibenzanthraquinone (I) was converted successfully with 30% oleum at 35° into a disulfonic acid which was isolated conveniently in the form of the *p*-toluidine salt and which could be used as such for the further transformations. That involved in the synthesis consisted in reduction with zinc and ammonia, which proceeded smoothly and gave a very sparingly soluble product having the properties of a zinc salt (III). On fusion with alkali this yielded an alkali soluble product which at first presented difficulties because of its high sensitivity to acids when in a crude condition. Material precipitated by careful neutralization of the alkaline solution with hydrochloric or acetic acid underwent marked decomposition during the process and could not be purified by crystallization. It was found, however, that precipitation with carbon dioxide gives a stable, light tan product which can be coagulated safely at the boiling point. Although crystallization was still not feasible, this material gave analytically pure dihydroxydibenzanthracene on a single sublimation in the vacuum of the

(5) Fieser and Desreux, THIS JOURNAL, **60**, 2255 (1938).

(6) Cook and de Worms, *J. Chem. Soc.*, 1825 (1937); Fieser and Riegel, THIS JOURNAL, **59**, 2561 (1937).

(7) Fieser and Hershberg, *ibid.*, **60**, 2542 (1938).

(8) Fieser, Hershberg and Newman, *ibid.*, **57**, 1509 (1935); Fieser, Hershberg, Long and Newman, *ibid.*, **59**, 475 (1937).

(9) Fieser and Hershberg, *ibid.*, **61**, 1565 (1939).

(10) Fieser and Johnson, *ibid.*, **62**, 575 (1940).

(11) E. Kamp, Dissertation, Frankfort (1936); Badger and Cook, *J. Chem. Soc.*, 804 (1939).

(12) Results are not as yet available for 3-hydroxy-10-methyl-1,2-benzanthracene [Fieser and Hershberg, THIS JOURNAL, **59**, 1028 (1937)].

(13) Cook, *J. Chem. Soc.*, 3273 (1931).

(14) See Fieser, *Am. J. Cancer*, **34**, 37 (1938).

(15) Shear, *ibid.*, **36**, 211 (1939).

(16) Private communications from Dr. M. J. Shear and from Drs. Shields Warren and C. E. Dunlap.

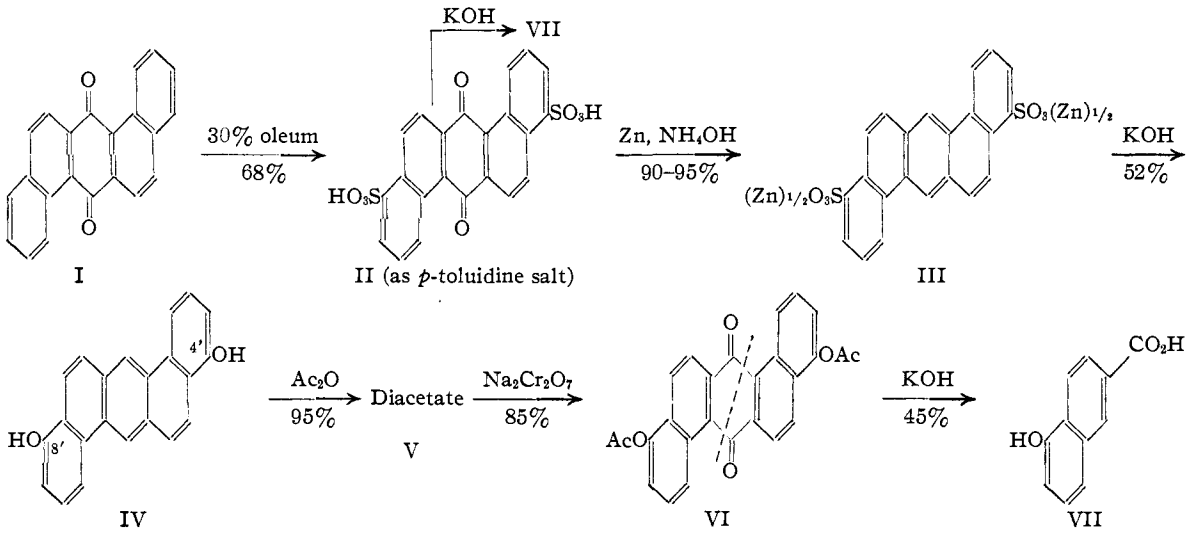
(17) Chalmers and Peacock, *Biochem. J.*, **30**, 1242 (1936); Chalmers, *ibid.*, **32**, 271 (1938); **34**, 678 (1940).

(18) White and White, *J. Biol. Chem.*, **131**, 149 (1939).

(19) Boyland and Levi[3] (1935) have made a preliminary announcement of experiments in this direction.

(20) Sempronj, *Gazz. chim. ital.*, **69**, 448 (1939).

(21) Heller and Schülke, *Ber.*, **41**, 3634 (1908).



mercury vapor pump. The sublimed material was no longer sensitive to mineral acids and was much more stable than the crude product in organic solvents, although the solubility is quite slight. The monohydroxy-1,2-benzanthracene of Sempronj does not exhibit comparable instability in the crude state. On the other hand, the difficulty encountered by Bogert and Cassaday[22] in their attempt to demethylate a tetramethoxydibenzanthracene may have been due to a similar sensitivity to acid reagents.

The structure of the dibenzanthraquinone disulfonate was established by alkali fusion of this substance in the form of the *p*-toluidine salt, which afforded 5-hydroxy-2-naphthoic acid (VII) as the sole acidic reaction product. The substance was identified by comparison with an authentic sample prepared by a known synthesis. The substituents are therefore in the 4′- and 8′-positions (II), corresponding to the position of monosulfonation indicated for 1,2-benzanthraquinone, and the end-product of the synthesis is 4′,8′-dihydroxy-1,2,5,6-dibenzanthracene (IV). In the course of preparing derivatives for further characterization, the degradation of the dihydroxy compound was investigated as a possible model for the study of phenolic products encountered in studies of the metabolism of dibenzanthracene. Acetylation proceeded very smoothly, as did oxidation with sodium dichromate to the diacetoxydibenzanthraquinone VI. On alkali fusion of as little as 50 mg. of the diacetate, pure 5-hydroxy-2-naphthoic acid was easily isolated and identified.

(22) Bogert and Cassaday, THIS JOURNAL, **61**, 3058 (1939).

Synthetic 4′,8′-dihydroxy-1,2,5,6-dibenzanthracene clearly is not identical with the rabbit-metabolism product isolated by Levi and Boyland,[3] as shown by the following comparison of melting points. The absorption spectrum of our compound (Fig. 1), kindly determined by Dr. R. N.

	Dihydroxy compound	Diacetate	Diacetoxy quinone
Rabbit-metabolism product[3]	350–360°	291°	294°
Synthetic IV (corr.)	415–418°	360–362°	340–345°

Jones, also deviates in several significant respects from that of the metabolite, the absorption curve for which was made available to us for comparison through the courtesy of Dr. K. Dobriner. On the other hand, there appears to be a correspondence with the phenolic product eliminated by dibenzanthracene-treated mice and rats.[4] The first seven absorption maxima for IV listed in the legend of Fig. 1 correspond closely, with an average deviation of less than 1 mμ, with those found for the rat and mouse metabolite, as communicated to us by Dr. Dobriner. The relative extinction coefficients are also in substantial agreement. Dr. Dobriner independently examined our synthetic material and reports that in plates taken with this and with the rat compound in absolute ethanol "the bands are in identical positions and the relative intensities of the bands in both plates seem to be similar." We are indebted to Dr. Dobriner and Dr. L. C. Craig for samples of the partially purified metabolite from mice which, they ascertained. did not melt at 370°; the purified rat metabolite also did not melt at 360° and gave a distinct depression (335°)

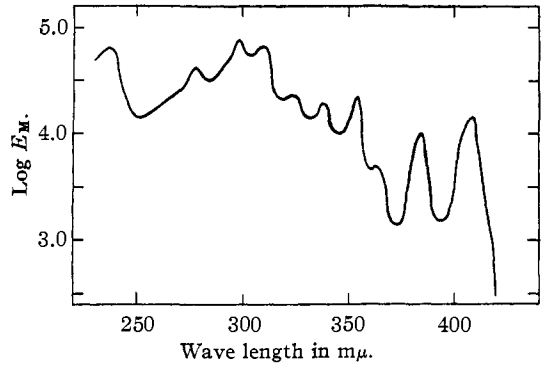
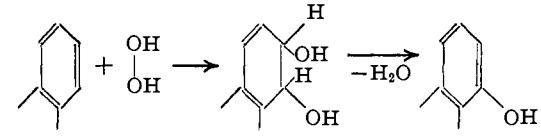


Fig. 1.—4′,8′-Dihydroxy-1,2,5,6-dibenzanthracene in absolute alcohol; absorption maxima in mμ (log E_M values in parentheses): 237.5 (4.80), 278 (4.62), 298 (4.88), 309 (4.82), 324 (4.36), 338 (4.28), 354 (4.35), 364.5 (3.69), 384 (3.99), 403 (4.15).

when mixed with the rabbit metabolite. In a test carried out in an evacuated capillary tube, as found expedient with synthetic IV, we found the best of the samples from mice to melt at 390–400°, corr., and to give no depression when mixed with IV. While further evidence is clearly desirable (and is being sought in collaboration with Drs. Rhoads and Dobriner), the preliminary indications are that dibenzanthracene is eliminated by mice and rats as the 4′,8′-dihydroxy derivative.

Although studies of the metabolism of carcinogenic hydrocarbons are still at an early stage, it is becoming apparent that the reaction of detoxification, if indeed it may be so regarded, involves an attack of the molecule at some site other than the center of special susceptibility to various forms of substitutions, including particularly diazo coupling,[23] chromic acid and lead tetraacetate oxidation,[7,9,24] and condensation with sulfur monochloride.[25] Thus dibenzanthracene is subject to oxidation or substitution at one or both *meso* positions.[18] While methylcholanthrene yields as the primary product of chemical oxidation the 15-hydroxy compound[24] which is a neutral alcohol, Drs. Dobriner and Rhoads inform us that the hydrocarbon yields a phenolic product of metabolism. Assuming the validity of the recently summarized evidence[25] indicating that the reactivity displayed in the chemical substitutions is directly associated with the process of hydrocarbon carcinogenesis, it may appear paradoxical on first consideration to suppose that a given hydrocarbon on administration to an animal can undergo biological transformation at two entirely different parts of the molecule. The situation becomes comprehensible, however, on the hypothesis that the carcinogen is subject to two independent and competing reactions proceeding by different mechanisms, the one responsible for the induction of malignant growth and the other leading to detoxification. Furthermore, there is chemical analogy for an attack of the molecule at a point different from that particularly sensitive to substitutions. It has been found that on catalytic hydrogenation typical carcinogens are attacked not at the reactive *meso* centers but at less hindered parts of the molecule.[26] Since hydrogenation represents an addition rather than a substitution, this would suggest that the metabolic reaction likewise proceeds by an addition mechanism. The work of Boyland and Levi[2] on the metabolism of anthracene provides a significant clue to the nature of the addition. These investigators found that anthracene is eliminated in part as 1,2-dihydroxy-1,2-dihydroanthracene, and commented on this remarkable attack on a side ring rather than at the *meso* position. They further showed that this diol is easily converted into α-anthrol by rather mild treatment with a dilute mineral acid. The series of reactions (1) would account for the meta-

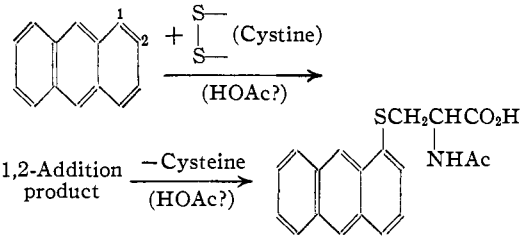
(1) 

bolic production of 4′,8′-dihydroxydibenzanthracene. As far as known, the dehydration may occur either in the organism or during the processing of the excreted material. The hypothesis lends itself to certain predictions offering a means of testing its validity, in addition to the obvious course of searching for the postulated intermediates. The dihydroxydibenzanthracene isolated as a product of rabbit metabolism would be expected to have the two hydroxyl groups distributed in different rings and not located in the same ring, for otherwise the stepwise dehydration of a tetra-ol would be interrupted by ketonization; the most probable structure is that of the 3′,7′-isomer. A phenolic metabolite of benzpyrene would not be expected to carry a hydroxyl group at position 5, for although this position is highly reactive to substitutions it is not amenable

(23) Fieser and Campbell, THIS JOURNAL, **60**, 1142 (1938).
(24) Fieser and Hershberg, *ibid.*, **60**, 1893 (1938).
(25) Wood and Fieser, *ibid.*, **62**, 2674 (1940).
(26) Fieser and Hershberg, *ibid.*, **59**, 2502 (1937); **60**, 940 (1938).

to additions. Contrary to the preliminary inferences of Boyland and Levi,[2] the labile anthracene metabolite which yields the hydrocarbon on acid hydrolysis would not now be expected to have the character of a hydroxydihydroanthracene.

As a corollary speculation, which however is not a necessary part of the above argument, it is suggested that mercapturic acid formation may follow an additive course similar to the "perhydroxylation." Boyland and Levi[2] found that anthracene is detoxified by rats and rabbits in part by the formation of 1-anthrylmercapturic acid, and yet the fixation of sulfur by means of chemical substitution occurs at the 9-position.[25] This may find an explanation in the reaction sequence (2).

(2) 

On the basis of the tentative views presented it would seem reasonable to attempt to inhibit hydrocarbon carcinogenesis by taking measures calculated to promote addition reactions of the types (1) and (2). The perhydroxylation may be subject to influence by oxido-reduction enzymes or vitamins, perhaps with preference for those possessing lipoid solubility[27]; an amino acid, protein, or proteinoid hormone rich in disulfide links might promote reaction (2). In the light of recent observations by Bernheim and Bernheim,[28] it would appear possible that the maintenance of prothrombin in the active —S·S— condition by administration of vitamin K might be effective in providing protection against carcinogenesis.

Experimental Part[29]

1,2-Benzanthraquinone-4′-sulfonic Acid *p*-Toluidine Salt.—Sulfonation of 1,2-benzanthraquinone proceeded unsatisfactorily with 20% oleum, as specified by Heller and Schülke,[21] and stronger oleum seemed required. The product can be isolated very readily as the amine salt as follows. Pure 1,2-benzanthraquinone (10.2 g.) was added in portions during fifteen minutes to 20 cc. of 30% oleum, swirling the mixture after each addition and keeping the temperature down to about 35°. After thirty minutes at room temperature, the opaque red solution was poured into 400 cc. of water, and after treatment with a small amount of Norit and filtering the solution was treated at the boiling point with 6 g. of *p*-toluidine. The salt separated, largely from the still hot solution, as yellow crystals; yield 16.5 g. (94%). A sample recrystallized twice from alcohol formed large, dark yellow, flat needles which decomposed above 300° without melting.

Anal. Calcd. for $C_{25}H_{19}O_6NS$: C, 67.40; H, 4.31. Found: C, 67.34; H, 4.39.

On isolating material prepared in the same way as the potassium salt (H. and S.[21]), this was obtained (as fine, light yellow needles) in only 67% yield.

4′-Hydroxy-1,2-benzanthracene.[20]—Reduction of the above *p*-toluidine salt was carried out as described by Sempronj,[20] except that the reaction was continued for twenty hours. The light gray, sparingly soluble product remaining after leaching out the zinc with acid proved to be the zinc salt, for on ignition it left a white ash turning yellow on heating; the yield calculated on this basis was 85–90%. Sempronj assumed the reaction product to be the free sulfonic acid and reported a sulfur analysis on a supposed potassium derivative. This, however, corresponded in sparing solubility (0.5 g. per liter of water) to our zinc salt, and since the latter is not affected by aqueous alkali it is probably the substance which Sempronj had in hand. The fusion was carried out as described by Sempronj (5 g. zinc salt, 35 g. potassium hydroxide), the pasty melt turning from gray to a brindle yellow. The cooled melt was digested with 1.5 l. of boiling water, and after filtering with Super-cel the clear yellow extract was acidified with hydrochloric acid and the brownish-yellow precipitate was coagulated at the boiling point and collected after cooling. The crude hydroxybenzanthracene was treated with Norit in 225 cc. of toluene and allowed to crystallize after concentration to about 75 cc., giving 2.5 g. (69.5%) of tan needles, m. p. 230–231.5°. Two further crystallizations from toluene gave small, light yellow needles of the constant m. p. 231.5–232.5° (Sempronj, 230°). The solutions show a brilliant violet fluorescence in daylight. The solubility in boiling 3% potassium hydroxide is approximately 3.3 g. per liter.

The **acetyl derivative** (96% yield) after one crystallization from toluene formed fine white needles, m. p. 195–195.5° (S., 193–194°). Attempts to condense this substance with chloromethyl ether gave either unchanged starting material or a polymeric product. The methylformanilide reaction also failed; after two hours at 100° about two-thirds of the acetate was recovered, and after fifteen hours only tarry products were obtained.

1,2,5,6-Dibenzanthraquinone.—1-(β-Naphthoyl)-2-methylnaphthalene was prepared by the Friedel and Crafts reaction[30] and the once-distilled crude ketone (88% yield) was used for pyrolysis. This was conducted by heating 150 g. of the ketone at 430 ± 5° for three hours and the product was distilled at 1 mm. and a bath temperature of 300–400°. One crystallization of the distillate from benzene gave 43.5 g. (31%) of yellow dibenzanthracene, m. p. 260–262°. The yield was the same in several smaller runs, including one in which the pyrolysis was carried out in the

(27) Lettré and Fernholz, *Ber.*, **73**, 436 (1940).

(28) Bernheim and Bernheim, *J. Biol. Chem.*, **134**, 459 (1940).

(29) Microanalyses by Lyon Southworth. All melting points are corrected; those above 300° were taken in evacuated capillaries using an aluminum block and are accurate to ±1%.

(30) Clar, *Ber.*, **62**, 350 (1929); Fieser and Dietz, *ibid.*, **62**, 1827 (1929).

presence of zinc dust.[31] Possibly the higher yield obtained by Bachmann was associated more with the use of pure ketone obtained by the Grignard reaction than with the presence of zinc dust.

Chromic anhydride[29] was found less satisfactory for the preparation of the quinone than sodium dichromate. A mixture of 25 g. of yellow dibenzanthracene, 42.5 g. of anhydrous sodium dichromate and 1.3 liters of glacial acetic acid was heated under reflux for one and one-half hours. The quinone collected after cooling was crystallized from toluene, a scarlet residue being removed by filtering the hot solution. The yield of material melting at 244–249° was 22 g. (79.5%). No difference was detected in a sample of quinone prepared from colorless dibenzanthracene.

1,2,5,6-Dibenzanthraquinone-4',8'-disulfonic Acid *p*-Toluidine Salt (II).—Seven grams of dibenzanthraquinone was added in 1-g. portions in five minutes to 20 cc. of 30% oleum, keeping the temperature from rising above 35°. The tough lumps which formed were worked into solution with a stirring rod, and after standing for two hours at room temperature the opaque red solution was poured into 400 cc. of water. A voluminous yellow precipitate dissolved on heating, and after filtration with Super-cel the clear red filtrate was treated at the boiling point with 10 g. of *p*-toluidine. The amine salt separated as a light yellow crystallizate; yield 10.6 g. (68.5%). A sample for analysis, after three crystallizations from water containing a little *p*-toluidine hydrochloride, formed golden yellow needles often branching into feathery forms.

Anal. Calcd. for $C_{36}H_{30}O_8N_2S_2$: C, 63.35; H, 4.42. Found: C, 63.07; H, 4.35.

The dipotassium salt, which crystallizes in flat yellow needles, may be isolated in good yield by addition of potassium chloride to the aqueous solution of the reaction mixture. This salt, however, is highly hygroscopic, and after drying a repeatedly crystallized sample at 150° in high vacuum the potassium content was only 89% of that calculated for the anhydrous salt. In reduction experiments the yields were much lower than obtained from the *p*-toluidine salt. Fusion of the potassium salt with alkali gave 5-hydroxy-2-naphthoic acid, identified as described below.

Reduction of the dibenzanthraquinone disulfonate was accomplished using 5.3 g. of the *p*-toluidine salt II, 22 g. of zinc dust, 60 cc. of concentrated ammonium hydroxide, and 22 cc. of water. The mixture was stirred mechanically under reflux and in an oil-bath maintained at 85–90°. The intermediate color, red at first and then yellow, largely faded during the first day, but it was found necessary to extend the reaction period to forty-eight hours in order to secure the maximum yield. Thus in a run which was stopped after twenty-two hours the yield was only 60%. The product appears at the end of the reaction as a very sparingly soluble zinc salt. This was collected and washed and digested with dilute hydrochloric acid at the boiling point for about eight hours, when zinc particles were no longer discernible. The washed and dried salt (III), which was practically colorless, was used for the fusion; the yield was consistently 3.4–3.6 g. (90–95%). No more than a trace of the salt dissolves in boiling water, and the substance was not characterized other than by determining that it contained zinc (ignition test).

(31) Bachmann, *J. Org. Chem.*, **1**, 350 (1936).

4',8'-Dihydroxy-1,2,5,6-dibenzanthracene (IV).—Fusion of III was carried out with 1.6 g. of the zinc salt and 16 g. of potassium hydroxide at 300–310° for one hour. The initially maroon colored mixture had to be stirred vigorously during the first fifteen minutes to bring the zinc salt into solution; at the end of the reaction the melt had become bright yellow. The cooled melt was extracted with 300 cc. of boiling water and the dark red solution was filtered with Super-cel, heated at the boiling point, and treated with a stream of carbon dioxide. The hydroxy compound was slowly precipitated and at first came out in a somewhat colloidal condition; on continuing the digestion at the boiling point under carbon dioxide for about fifteen minutes it coagulated to an easily filterable form. The dried material was yellow-brown and weighed 0.62 g. When the alkaline solution was acidified with mineral acid or even with acetic acid the product rapidly decomposed to a brown-black, alkali-insoluble tar. The crude material precipitated by carbon dioxide decomposed badly in all attempts to effect purification by crystallization, but a single sublimation in high vacuum gave analytically pure material. Thus sublimation of 0.62 g. of the precipitate at $2–3 \times 10^{-4}$ mm. and 300° gave 0.51 g. (51.7%) of light orange crystalline product.

Anal. Calcd. for $C_{22}H_{14}O_2$: C, 85.14; H, 4.54. Found: C, 85.07; H, 4.76.

The pure substance is not sensitive to mineral acids even at the boiling point. The solubility in boiling ether is 6–7 mg. per 100 cc. and in boiling absolute alcohol 10–20 mg. per 100 cc. The substance is moderately soluble in ethyl benzoate at the boiling point, but the hot solution soon becomes cloudy and only amorphous material separates on cooling. An alcoholic solution shows brilliant violet fluorescence in daylight and the addition of ferric chloride produces a yellow-orange color.

When coupled with diazotized dianisidine as in Talbot's colorimetric method of determining oestrogens,[32] the substance gave a good reddish-brown color in the toluene layer. When heated in an open capillary the substance decomposed without melting, but in an evacuated capillary it melts at 415–418° to an orange liquid which immediately evolves gas and resolidifies to an orange solid which does not melt at 460°. Samples of the metabolite obtained from Dr. Dobriner were quite dark but melted at 387–397° and at 390–400° and gave no depression when mixed with IV; the mixture with the better sample melted at 400–410°.

4',8'-Diacetoxy-1,2,5,6-dibenzanthracene.—A suspension of 350 mg. of IV in 35 cc. of acetic anhydride, with a trace of sodium acetate added, was refluxed for one hour, during which time the orange starting material had given place to yellow crystals of the diacetate. After decomposition of the acetic anhydride with water, the product was collected. It consisted of 425 mg. (95.5%) of lustrous, light yellow plates, m. p. 357–361° with decomposition and evolution of gas. A sample for analysis was sublimed in high vacuum at 250°; m. p. 360–362°, dec.

Anal. Calcd. for $C_{26}H_{18}O_4$: C, 79.18; H, 4.59. Found: C, 79.10; H, 4.71.

The substance is quite insoluble in chloroform, acetone

(32) Talbot, Wolfe, MacLachlan, Karush and Butler, *J. Biol. Chem.*, **134**, 319 (1940).

or acetic acid, sparingly soluble in xylene, and moderately soluble in ethyl benzoate (boiling). It crystallizes from either of the last two solvents in golden-yellow plates, m. p. 360–362°, dec.

4′,8′-Diacetoxy-1,2,5,6-dibenzanthraquinone (VI).—A suspension of 350 mg. of the above diacetate in 35 cc. of glacial acetic acid was refluxed for three hours with 420 mg. of anhydrous sodium dichromate and, after cooling, the orange quinone was collected and recrystallized from ethyl benzoate. It formed delicate orange needles, m. p. 340–345°, dec.; yield 320 mg. (85%). A sample for analysis was recrystallized from ethyl benzoate and then sublimed in high vacuum; the melting point was unchanged.

Anal. Calcd. for $C_{26}H_{16}O_6$: C, 73.58; H, 3.79. Found: C, 74.02; H, 4.04.

This quinone gives no color with aqueous alkali but forms a pale orange vat after boiling for a few minutes. On reoxidation by air the violet color characteristic of the free dihydroxy compound is produced.

Degradation of VI.—A mixture of 100 mg. of the diacetoxy dibenzanthraquinone and 1 g. of potassium hydroxide was placed in a platinum crucible and heated in a nitrite-bath at 260° for ten minutes and then at 280° for ten minutes. Other conditions of time and temperature were less satisfactory. The cooled melt was dissolved in 4 cc. of warm water and heated in a boiling water-bath for ten minutes. After filtering with Super-cel the acidic product was precipitated and collected, and it was then reprecipitated from a similarly treated bicarbonate solution. The brown product so obtained was sublimed at 1 mm. and 180–190° and crystallized once from water. This gave 40 mg. (45%) of nearly colorless needles of 5-hydroxy-2-naphthoic acid, m. p. 213–214.5°. This gave no depression with a sample of pure synthetic acid (known), m. p. 214.5–215.5,° prepared by a modified procedure which will be described in a future paper, and the color reaction with ferric chloride was the same (dirty red precipitate). The fusion was carried out successfully with 50 mg. of the quinone and 0.5 g. of potassium hydroxide, the yield being 10 mg. (22.5%).

4′,8′-Dihydroxy-1,2,5,6-dibenzanthraquinone.—The diacetoxy quinone (50 mg.) was boiled with 5 cc. of 1 *N* alcoholic potassium hydroxide for five minutes and after dilution with 50 cc. of water the opaque purple solution was neutralized at the boiling point with carbon dioxide as described for IV. The orange-red precipitate on sublimation in high vacuum afforded 35 mg. (87%) of crystalline, deep maroon colored dihydroxy quinone. This decomposes without melting at 370–375°.

Anal. Calcd. for $C_{22}H_{12}O_4$: C, 77.64; H, 3.55. Found: C, 77.35; H, 3.81.

The quinone gives a brilliant violet color in dilute alkali and a pale orange vat, reoxidation restoring the violet color. In common with the dihydroxydibenzanthracene, it dissolves with decomposition in boiling ethyl benzoate and only amorphous material separates on cooling.

Summary

4′,8′-Dihydroxy-1,2,5,6-dibenzanthracene has been synthesized by disulfonation of dibenzanthraquinone, reduction, and fusion with alkali. The structure was established by a degradative method applicable on a micro scale.

The synthetic dihydroxydibenzanthracene differs from the isomer isolated by Levi and Boyland as a product of the metabolism of the hydrocarbon by rabbits but very probably is identical with the substance excreted by hydrocarbon-injected mice and rats (Dobriner, Rhoads and Lavin).

Certain theoretical considerations are presented concerning the nature and significance of the reactions involved in the metabolism of carcinogenic hydrocarbons.

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The Synthesis of 2-, 4- and 9-Fluoreneacetic Acid

BY W. E. BACHMANN AND JOHN CLARK SHEEHAN[1]

Of the five possible isomeric fluoreneacetic acids only the 2- and 9-isomers have been prepared. What appears to be another fluoreneacetic acid has been prepared by heating fluorene and chloroacetic acid[2] but its structure does not seem to have been established. Its melting point (148°) does not correspond with either the 2- or 9-isomer. This fluoreneacetic acid has been found to possess plant hormone properties.[3] It appeared of interest to prepare the entire series of fluoreneacetic acids, and in this paper is reported the synthesis of the new 4-fluoreneacetic acid, as well as new procedures for obtaining the 2- and 9-isomers.

Von Braun and Engel[4] prepared 2-fluoreneacetic acid from fluorene by an eight-step process. We

(1) From the Ph.D. dissertation of John Clark Sheehan.

(2) Wolfram, Schörnig, and Hausdörfer, U. S. Patent 1,951,686 (1934).

(3) Zimmerman and Wilcoxon, *Contrib. Boyce Thompson Inst.*, **7**, 209 (1935); *C. A.*, **30**, 1431 (1936); Killeffer, *Ind. Eng. Chem., News Ed.*, **18**, 395 (1940).

(4) Von Braun and Engel, *Ber.*, **57B**, 191 (1924).