Bachmann and Chemerda.[6] The identity was established by analysis and by a mixed melting point with a sample kindly furnished by Dr. Bachmann.

A report concerning the physiological activity of 9,10-dimethyl-1,2-benzanthracene will be published elsewhere by Dr. M. J. Shear. In a private communication he has indicated that this hydrocarbon has marked biological activity. When 1 mg. per mouse was injected in lard solution severe ulceration was produced in most of the mice in one month, but no tumors had appeared after three months. Doses of 0.1 mg. per mouse did not produce ulceration or tumors after two months.

Experimental[7]

10-Acetoxy-9-methyl-1,2-benzanthracene. II.—To a solution of 2.76 g. of I[2,4] in 10 cc. of acetic acid and 10 cc. of acetic anhydride was added 0.1 g. of anhydrous zinc chloride.[5] After refluxing for two hours, the reaction mixture was cooled and the acetic anhydride decomposed by addition of water. The crystals which separated were collected and recrystallized from acetic acid, coming out as colorless flat needles, m. p. 192.4–193.4°, in a yield of 50%.

Anal. Calcd. for $C_{21}H_{16}O_2$: C, 83.98; H, 5.37. Found: C, 84.16; H, 5.35.

9,10-Dimethyl-1,2-benzanthracene. IV.—To a solution of 1.27 g. of II in 30 cc. of benzene and 20 cc. of ether was added 10 cc. of 0.267 *M* methylmagnesium bromide. The mixture was refluxed for six hours, allowing the ether to distil slowly. The reaction mixture was decomposed with dilute hydrochloric acid and the benzene layer containing the anthrone concentrated and diluted with dry ligroin. As no crystalline material was obtained, the ligroin was removed by distillation and the residual benzene solution treated with 3 cc. of the above methylmagnesium bromide. A vigorous reaction took place with the separation of an orange complex which soon redissolved. After two hours of refluxing the reaction mixture was decomposed with dilute hydrochloric acid and the organic matter remaining after evaporation of all solvent pyrolyzed at 240–250° for fifteen minutes and then distilled at 3 mm. The distillate crystallized immediately and the hydrocarbon was isolated as a red dipicrate melting unsharply at 103–106°.[8] The hydrocarbon was obtained by passage of the dipicrate through a tower of activated alumina. It formed almost colorless plates, m. p. 122.4–122.8°, having an intense blue-violet fluorescence in ultraviolet light. The melting point was not depressed by a sample of 9,10-dimethyl-1,2-benzanthracene supplied by Dr. Bachmann. The over-all yield from acetoxy compound to hydrocarbon was 32%.

Anal. Calcd. for $C_{20}H_{16}$: C, 93.71; H, 6.29. Found: C, 93.58; H, 6.43.

Summary

The synthesis of 9,10-dimethyl-1,2-benzanthracene is described. *o*-(α-Methyl-α-1-naphthyl)-toluic acid was converted into 10-acetoxy-9-methyl-1,2-benzanthracene by a ring closure involving zinc chloride in acetic anhydride. This acetoxy compound was hydrolyzed, and the resulting anthrone was treated with methylmagnesium bromide to yield a carbinol which was dehydrated to yield 9,10-dimethyl-1,2-benzanthracene.

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[6] Bachmann and Chemerda, THIS JOURNAL, **60**, 1023 (1938).

[7] All melting points corrected. Analyses by H. S. Clark.

[8] This picrate is the dipicrate of Bachmann and Chemerda[6] who report (in a private communication) the melting range as 102–106° after sintering as low as 95°. This difference in behavior of the dipicrate may be due to the fact that Bachmann and Chemerda used melting point tubes composed of soft glass whereas I used Pyrex melting point tubes. On storing picrates in soft glass there is usually marked decomposition of the picrate due to the alkali in the glass. A similar decomposition is not noticeable when Pyrex containers are used.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Diazo Coupling of Carcinogenic Hydrocarbons

BY LOUIS F. FIESER AND WILLIAM P. CAMPBELL[1]

The coupling of aromatic hydrocarbons with diazotized amines has been observed only in very rare instances, and heretofore only three azo compounds have been obtained in a crystalline condition by utilization of this reaction. In the first published work on the problem K. H. Meyer, Irschick and Schlösser[2] observed that mesitylene, acenaphthene, and anthracene show some indications of coupling with 2,4-dinitrobenzenediazonium chloride in acetic acid solution, but the reactions were slow and incomplete and no azo compounds could be isolated. Meyer and Tochtermann[3] later found that the highly reactive diazonium salt from picramide couples rapidly with mesitylene and gives a crystalline azo compound in good yield. With the other hydrocarbons investigated, including anthracene and α-methylnaphthalene, the reaction was slow and no coupling products could be isolated. In a

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[1] Squibb Research Fellow.

[2] Meyer, Irschick and Schlösser, *Ber.*, **47**, 1741 (1914).

[3] Meyer and Tochtermann, *ibid.*, **54**, 2283 (1921).

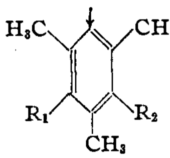
TABLE I

COLOR REACTIONS WITH *p*-NITROBENZENEDIAZONIUM CHLORIDE

Rapid (deep color in 5 min., or less)	Slow (moderate color in 15–20 hrs.)	Negative (no color, or only a trace of color)
3,4-Benzpyrene††††	Anthracene	1,2-Benzanthracene
20-Methylcholanthrene††††	Mesitylene	10-Methyl-††† (ethyl-††, *n*- or *i*-propyl, *n*-butyl-, *n*-amyl-, allyl-) 1,2-benzanthracene
Cholanthrene††††	Acenaphthene	
4,10-Ace-1,2-benzanthracene†††	Acephenanthrene	
8,9-Ace-1,2-benzanthracene††	1-Methyl-8,9-acephenanthrene	5,10-Dimethyl-1,2-benzanthracene†††?
1,2-Cyclopenteno-5,10-aceanthrene†	9-Methyl-1,2-benzanthracene††	5-Methyl-1,2-benzanthracene††
	1′,9-Methylene-1,2,5,6-dibenzanthracene†	1,2,5,6-Dibenzanthracene††
1,2-Dimethyl-5,10-aceanthrene	15,16-Benzdehydrocholanthrene†	Pyrene
		Chrysene

† Indicates carcinogenic activity in mice.

more recent investigation L. I. Smith and Paden[4] found that diazotized picramide couples readily with isodurene (I, $R_1 = H$, $R_2 = CH_3$) and with pentamethylbenzene (I, $R_1 = R_2 = CH_3$),



I

but not with durene. Isodurene and pentamethylbenzene did not couple with less active diazo components. From these observations it would appear that aromatic hydrocarbon coupling is a highly specific reaction requiring special structural features in each component. In each of the three known cases the hydrocarbon is a polymethylbenzene in which a nuclear position is activated by one para and two ortho groups, and reaction occurs only with a very potent diazo component having three activating nitro groups.

In view of the limitations of hydrocarbon coupling, it was surprising to discover that the powerfully carcinogenic hydrocarbons methylcholanthrene and 3,4-benzpyrene enter into this highly specific reaction. Solutions of these hydrocarbons in acetic acid rapidly acquire a deep red color on the addition of even such a moderately active coupling component as *p*-nitrobenzenediazonium chloride. The reaction with 3,4-benzpyrene proceeds particularly rapidly and smoothly, and a crystalline reaction product was isolated without difficulty. The substance forms purple plates with a greenish reflex and the composition is that required for a *p*-nitrobenzeneazo-3,4-benzpyrene. Methylcholanthrene reacts somewhat less readily with the same diazo component and thus far all attempts to isolate a pure azo compound have been unsuccessful; material precipitated and fractionated in various ways is highly colored, but amorphous and of variable composition.

Before attempting to prepare crystalline products from other components, or to investigate the position of substitution (probably 5, 8 or 10[5]) in the 3,4-benzpyrene derivative and the further transformations of the substance, a preliminary survey has been made to determine the value of the reaction as a color test and to explore the limits of coupling in the field of polynuclear aromatic hydrocarbons. Qualitative tests conducted with a number of hydrocarbons prepared or purified in this Laboratory or by Dr. M. S. Newman are recorded in Table I. The tests were carried out in acetic acid solution under comparable conditions and according to the procedure described in our paper on resin acids.[6] There is a distinct difference in the behavior of the compounds in the first two groups in both the speed and the extent of the reaction; the rapidly acting compounds give deeply colored solutions while with the substances which respond slowly the solutions acquire at most a light orange color. 2,4-Dinitrobenzenediazonium chloride was tried with some of the hydrocarbons and found to react at about the same rate as the *p*-nitro compound; methylcholanthrene gives a slow color reaction with the diazonium salts from aniline, sulfanilic acid, and 4-nitroaniline-2-sulfonic acid.

Since Meyer observed that mesitylene reacts smoothly with diazotized picramide but not with less active diazonium salts, it would appear that 3,4-benzpyrene is considerably more susceptible to the specific type of substitution reaction than mesitylene, and this is shown clearly by the results

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[4] Smith and Paden, THIS JOURNAL, **56**, 2169 (1934).

[5] Vollmann, Becker, Corell and Streeck, *Ann.*, **531**, 1 (1937).

[6] Fieser and Campbell, THIS JOURNAL, **60**, 159 (1938).

of direct comparison (Table I). Of the four aromatic hydrocarbons now known to give crystalline azo compounds, the carcinogenic constituent of coal tar is by far the most reactive coupling component. The relative carcinogenic potencies of this and other hydrocarbons tested are indicated roughly by the characters in Table I,[7] and it will be observed that a preponderant number of cancer-producing hydrocarbons fall in the group of rapidly acting coupling components. It is particularly striking that this group includes 3,4-benzpyrene, methylcholanthrene and cholanthrene, for by all available criteria these three compounds surpass all other carcinogenic hydrocarbons in general potency. 10-Methyl-1,2-benzanthracene exhibits comparable carcinogenic activity in one test which has been applied but not in another,[8] and it is interesting that the compound shows no reactivity in the coupling test. It is perhaps not a mere coincidence that both superior carcinogenic potency and high reactivity in a specific chemical test are encountered in hydrocarbons as different structurally as 3,4-benzpyrene and the cholanthrenes. Although there clearly is no rigid correlation between the results of the chemical and physiological tests, the indications of a general parallelism suggest that chemical reactivity of the specific type involved in the coupling reaction may be one factor of importance in determining the carcinogenic activity of a hydrocarbon. While methylcholanthrene displays in its addition reactions considerably less reactivity than related hydrocarbons of lower carcinogenic activity,[8,9] it is now apparent that this and other potent carcinogens are by no means inert chemically but display unusual reactivity in a specific type of substitution. The implications of these findings to the cancer problem will be developed more fully in another publication.

Pronounced reactivity in the coupling test seems to be associated with certain rather clearly defined features of the hydrocarbon structure. All of the rapidly·acting hydrocarbons except 3,4-benzpyrene contain an anthracene nucleus with a five-membered ring extending from an α-position to a *meso*-position; they are, in short, derivatives of aceanthrene. While the two hydrocarbons at the bottom of the list of slowly reacting compounds also contain the aceanthrene ring system, the presence of an additional ring fused to the ace-nucleus doubtless alters the character of the parent substance to a marked degree. 3,4-Benzpyrene may be said to bear some resemblance to aceanthrene in that there is present in the molecule an anthracene system with a six-membered ring extending between an α- and a *meso*-position, although it is possible that the comparison is invalidated by a difference in the bond structures[10] and that 3,4-benzpyrene is of a structural type distinct from the aceanthrene derivatives. The pronounced reactivity of the hydrocarbon is comprehensible in terms of an unusual quinonoid bond structure. The reactivity of the compounds of the aceanthrene series is more difficult to understand, particularly in view of the surprising contrast in the behavior of 5,10-ace-1,2-benzanthracene (cholanthrene) and 5,10-dimethyl-1,2-benzanthracene. The coupling reaction reveals a hitherto unsuspected function of the ace- ring which clearly calls for further investigation.

In order to determine whether the diazo reaction can be employed to advantage in the colorimetric determination of small amounts of certain of the carcinogenic hydrocarbons, coupling tests were made with representative compounds at successive dilutions with the results given in Table II. The tests were conducted in acetic acid solution and comparison was made in each case with a suitably diluted blank, which invariably showed no change over the period in question. No difference was observed in tests made with *p*-nitrobenzenediazonium chloride prepared as described[6] and with a solution of the pure sulfate in 92% acetic acid.

That 3,4-benzpyrene gives a positive reaction at higher dilution than the other compounds may be because the purple color of the product contrasts with the pale yellow color of the diazo component, whereas in very dilute solutions the other hydrocarbons give orange colors. The color of *p*-nitrobenzeneazo-3,4-benzpyrene is quite distinct in solutions considerably more dilute than the limiting concentration at which coupling occurs to a detectable extent. The results indicate that 3,4-benzpyrene can be detected by this method at a dilution of about 1.2 parts per million and that the limiting concentration for methylcholanthrene and

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(7) The ratings are based upon results previously published and upon the following additional observations of Dr. M. J. Shear. 1,2-Dimethyl-5,10-aceanthrene, injected subcutaneously into 20 mice, has given no tumors; 10 of the mice survived for one year and 5 were alive after fifteen months. Administered in the same way in lard solution, 4,10-ace-1,2-benzanthracene has given tumors in an average time of seven months in dosages as small as 0.1 mg. per mouse.

(8) Fieser and Hershberg, THIS JOURNAL, **59**, 2502 (1937).

(9) Bachmann and Kloetzel, *ibid.*, **60**, 481 (1938).

(10) Fieser and Seligman, *ibid.*, **60**, 170 (1938).

TABLE II

COLOR TESTS IN DILUTE SOLUTION WITH *p*-NITROBENZENEDIAZONIUM CHLORIDE

Series:	A[a]	B[b,c]	C[b,d]	D[b,d]	E[b,d]
Molar concentration:	1×10^{-3}	1×10^{-4}	1×10^{-5}	5×10^{-6}	1×10^{-6}
Parts per million (approx.):	240	24	2.4	1.2	0.24
3,4-Benzpyrene	Dark		Light	Faint	None
20-Methylcholanthrene	Dark	Light	Faint	None	
Cholanthrene	Dark	Light	Faint	None	
4,10-Ace-1,2-benzanthracene	Dark	Light	Faint	None	
8,9-Ace-1,2-benzanthracene	Dark	Light	Faint	None	
1,2-Cyclopenteno-5,10-aceanthrene	Dark	Light	Faint	None	
1,2-Dimethyl-5,10-aceanthrene	Dark[e]				
10-Methyl-1,2-benzanthracene	None				
10-*n*-Propyl-1,2-benzanthracene	None				
5,10-Dimethyl-1,2-benzanthracene	None				

[a] Using 1 cc. of solution and two drops of diazo solution prepared as for the tests of Table I; color was noticeable in a few minutes and the reaction reached completion in four to five hours. [b] Tests were made in 50-cc. Nessler tubes (8-cm. column of liquid) using 2 cc. of the diazo solution. [c] The color was first noticeable in fifteen to twenty minutes and became constant in about twelve hours. [d] The reaction was very slow and the maximum color was reached only in twenty-four to forty-eight hours. [e] The solution of the hydrocarbon in glacial acetic acid became orange after standing for several hours, but in the case of the most concentrated solution the occurrence of coupling was evident from a distinct darkening on the addition of the diazo solution. The tests at higher dilutions were not satisfactory.

the other aceanthrene derivatives is about 2.4 parts per million.

Experimental Part

***p*-Nitrobenzeneazo-3,4-benzpyrene.**—A solution of 0.42 g. of *p*-nitroaniline in 2.1 cc. of concentrated hydrochloric acid and 5 cc. of glacial acetic acid, diazotized at 5° with 0.21 g. of sodium nitrite in 1 cc. of water, diluted with 20 cc. of glacial acetic acid, was added to a solution of 0.36 g. of 3,4-benzpyrene in 75 cc. of the same solvent. The solution rapidly became purple-red and after standing for four hours the product was precipitated with water. The washed and dried product (0.44 g.) was crystallized once from acetone and once from acetone–benzene, giving 50 mg. of pure material in the form of lustrous purple plates with a greenish reflex. On drying at 110° in vacuum the plates changed to a very dark greenish black powder, m. p. 245–246°, corr. The mother liquors yielded a total of 110 mg. of satisfactory crystalline product.

Anal.[11] Calcd. for $C_{26}H_{15}O_2N_3$: C, 77.80; H, 3.76; N, 10.46. Found: C, 77.97; H, 4.05; N, 10.95.

The azo compound is readily soluble in benzene, moderately soluble in acetone or alcohol, and very sparingly soluble in hexane. The solutions are intensely purple, resembling solutions of potassium permanganate.

Summary

3,4-Benzpyrene reacts with *p*-nitrobenzenediazonium chloride in acetic acid solution to give a crystalline azo compound. Applied as a qualitative test, the coupling reaction reveals in 3,4-benzpyrene, methylcholanthrene, and certain derivatives of aceanthrene, a strikingly pronounced and highly specific chemical reactivity. Indications of a rough parallelism between reactivity in the coupling reaction and the carcinogenic activity suggest a possible association of this type of chemical response and hydrocarbon carcinogenesis.

3,4-Benzpyrene and methylcholanthrene can be detected by the color reaction with a diazo component at dilutions of about 1.2 p. p. m. and 2.4 p. p. m., respectively.

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[11] Microanalyses by the Arlington Laboratories, Arlington, Virginia.