The sublimate melted at 215–217° (from Skellysolve C) and was identical with the mixture of dimethylanthracenes obtained previously.

A mixture of benzhydrol (25 g.), diphenylmethane (60 cc.) and aluminum chloride (56 g.) maintained for three hours at 60° yielded 40.43 g. of non-volatile product. Vacuum distillation at 180–210° (1×10^{-3} mm.) yielded 16.0% of dibenzylanthracene, m. p. 191–192°, which was spectroscopically identical with the substance isolated from the benzyl alcohol reaction (Table II).

The Reaction of Benzaldehyde with Diphenylmethane.—Benzaldehyde (25 g.), aluminum chloride (67 g.) and diphenylmethane (70 cc.) gave 55.7 g. of non-volatile product under the same conditions. Dibenzylanthracene, m. p. 190–191°, was obtained from this residue as the only distillable substance. It proved to be identical in all respects with the dibenzylanthracenes described above.

Absorption Spectra.—The ultraviolet absorption spectra of the dimethyl and dibenzylanthracenes were determined in cyclohexane in 0.00128 and 0.00073 molar solutions with a Beckmann spectrophotometer.[10]

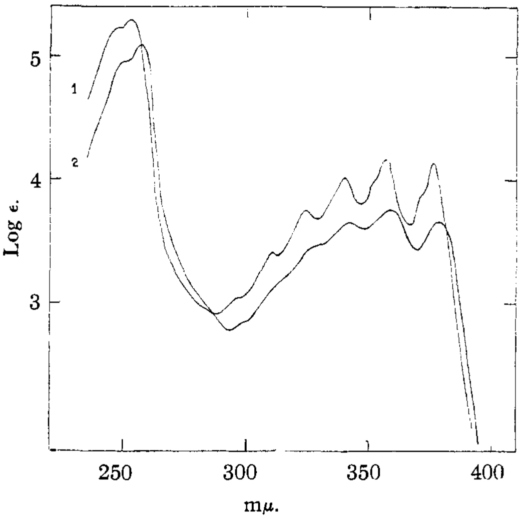

Fig. 1.—Ultraviolet absorption spectra: curve 1, dibenzylanthracenes; curve 2, dimethylanthracenes.

TABLE II

ULTRAVIOLET ABSORPTION SPECTRA

Wave length	Log ε λmax. I	II	III	Wave length	Log ε λmin. I	II	III
			Dimethylanthracenes[a]				
257.5	5.029	5.134	5.097	292.5	2.775	2.810	2.790
341.3	3.640	3.655	3.654	349	3.593	3.608	3.594
359	3.751	3.766	3.765	369	3.423	3.444	3.431
379	3.667	3.679	3.673				
			Dibenzylanthracenes[a]				
254	5.312	5.638	5.689	287.5	2.924	2.834	2.997
311	3.423	3.379	3.416	312.5	3.386	3.376	3.412
324	3.769	3.736	3.749	330	3.691	3.664	3.683
340	4.037	4.003	4.012	346	3.822	3.790	3.804
356	4.173	4.146	4.157	367.5	3.654	3.634	3.648
376	4.161	4.130	4.136	380	...	3.756	3.774
381	...[b]	3.895	3.906				

[a] I, product from benzaldehyde, II from benzhydrol, III from benzyl alcohol. [b] This small maximum, which is not present in anthracene, was probably overlooked in the first curve.

One curve each is shown in Fig. 1. Extinction values for maxima and minima of all six substances are listed in Table II. The spectral characteristics of the compounds are in good agreement with the proposed structures. The methylanthracenes exhibit a small hypsochromic effect, slight bathochromic effect and loss of fine structure as compared to anthracene[11] while the benzyl substituents cause general hyperchromic effect, no bathochromic effect and no loss in fine structure.

Summary

Evidence has been presented to show that benzyl alcohol and benzhydrol can act as carbon monoxide donors in the presence of excess aluminum chloride.

COLUMBIA, MISSOURI RECEIVED[12] APRIL 23, 1949

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[10] Absorption spectra by Dr. E. E. Pickett, University of Missouri.

[11] Jones, *Chem. Rev.*, **41**, 368 (1947); **32**, 11 (1943).

[12] The first draft of this article was received October 25, 1948.

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF HARVARD UNIVERSITY, THE DEPARTMENT OF SURGERY OF THE BETH ISRAEL HOSPITAL, BOSTON, AND THE HARVARD MEDICAL SCHOOL]

Preparation of Azo Compounds for the Study of Inhibition of Tumor Growth*

BY ORRIE M. FRIEDMAN, RALPH M. GOFSTEIN AND ARNOLD M. SELIGMAN

Following the demonstration by Haddow[1] that certain carcinogenic hydrocarbons are able to inhibit the growth of certain tumors in animals, many compounds with a structural resemblance to carcinogenic substances were subjected to this type of bioassay.[2] Isolated instances of inhibition of tumor growth with non-carcinogenic substances were observed, for example, with the unsymmetrical azonaphthalene (I).[2] Haddow suggested that this compound bears a superficial resemblance to the carcinogen, 1,2,5,6-dibenzanthracene (II). However, the only forms in which the unsymmetrical azonaphthalene (I) has features in common with the polynuclear carcinogenic hydrocarbons are the *cis* structures (III) and (IV) which resemble benz- derivatives of both 1,2-benzanthracene and chrysene. The hydrocarbon 1,2,5,6-dibenzphenanthrene, suggested by formula (IV), was found to be an inhibitor of tumor growth.[2] In order to explore further possible relationships between structure of azo compounds and their activity as inhibitors

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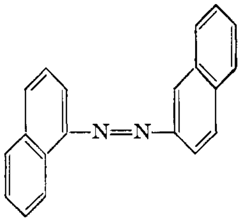
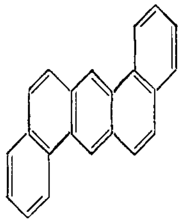
(*) This investigation was aided by a research grant from the National Cancer Institute, National Institutes of Health, Public Health Service.

[1] (a) Haddow, *Nature*, **136**, 868 (1935); (b) Haddow and Robinson, *Proc. Roy. Soc.* (*London*), **B122**, 442 (1937); (c) Haddow, Scott and Scott, *ibid.*, **B122**, 477 (1937); (d) Haddow, *J. Path. Bact.*, **47**, 567 (1938); (e) Haddow and Robinson, *Proc. Roy. Soc.* (*London*) **B127**, 277 (1939).

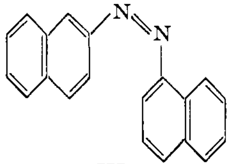
[2] Badger, Elson, Haddow, Hewett and Robinson, *Proc. Roy. Soc.* (*London*), **B130**, 255 (1941).



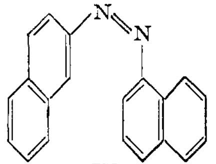
I



II

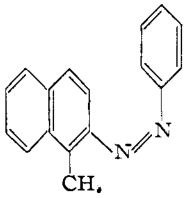


III

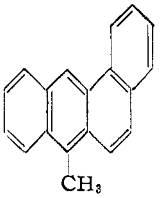


IV

of tumor growth,[3] about twenty-five azo compounds were prepared. When written in the hypothetical *cis* configuration, most of them bear a spatial resemblance to a carcinogenic polynuclear hydrocarbon. As an example the *cis* structure is written for 2-benzeneazo-1-methylnaphthalene (V) and may be compared with the potent carcinogen 10-methyl-1,2-benzanthracene (VI). This azo compound was prepared by the method previously described.[4] No presumption is made as to the actual existence of these substances in the *cis* form, although there is evidence that conversion of the *trans* to the *cis* form can be effected to a significant degree by exposure to ultraviolet light.[5]

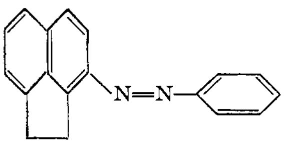


V

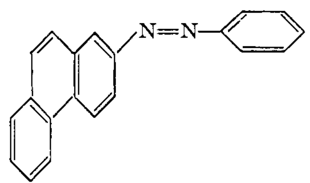


VI

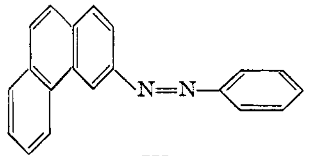
The novel method of Michaelis and Petou[6] for the preparation of 1-benzeneazonaphthalene by the action of phenylhydroxylamine on 1-thionylaminonaphthalene proved useful for the preparation of 3-benzeneazoacenaphthene (VII), 2-benzeneazophenanthrene (VIII), and 3-benzeneazophenanthrene (IX). The required 3-aminoacenaphthene was prepared by catalytic reduction of 3-nitroacenaphthene, described by Morgan,[7] and its structure was confirmed by comparison with the amine obtained from 3-acetoacenaphthene[8] by a Hoffmann rearrangement. The thionylamino compounds were prepared by the reaction of thionyl chloride with the respective amines. The products thus obtained were treated with phenylhydroxylamine in benzene. Heat was evolved and a black tarry by-product deposited as a sludge.[6] The products were obtained pure although in rather small yield after chromatographic treatment on alumina.



VII

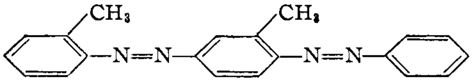


VIII

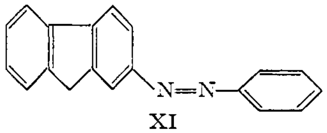


IX

Use was made of the condensation of phenylamines with nitrosobenzene in acetic acid for the preparation of 2-benzeneazo-5-(*o*-tolueneazo)-toluene (X). This method worked well for the preparation of 2-benzeneazofluorene (XI). The reaction followed a different course with amines of naphthalene, acenaphthene, anthracene and phenanthrene. Products deep purple in color were produced, and although some were obtained in crystalline form after chromatographic separation they were not characterized further.



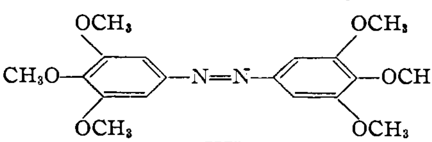
X



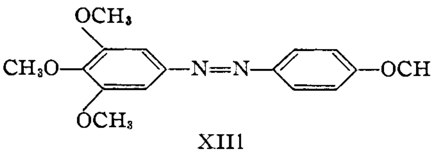
XI

Azo-3,4,5-trimethoxybenzene (XII) was prepared by reaction of 3,4,5-trimethoxybenzene diazonium chloride with an excess of cuprous chloride in aqueous solution under conditions described by Borgoslovski.[9]

The diazo coupling reaction was used for the preparation of 3,4,5,4'-tetramethoxyazobenzene (XIII) and 1-(3,4,5-trimethoxybenzeneazo)-



XII



XIII

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[3] Seligman, Milden, Sweet, Mollomo, Gofstein and Friedman, to be published.

[4] Bargellini and Silvestri, *R. A. L.*, **57**, 16 II, 261 (1907).

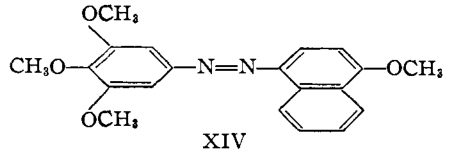
[5] Gortner and Gortner, THIS JOURNAL, **32**, 1294 (1910); Hartley, *Nature*, **140**, 281 (1937); *J. Chem. Soc.*, 633–642 (1938); Cook, *ibid.*, 876 (1938); Cook, Jones and Polya, *ibid.*, 1315 (1939); Cook and Jones, *ibid.*, 184 (1941).

[6] Michaelis and Petou, *Ber.*, **31**, 995 (1898).

[7] Morgan, *J. Soc. Chem. Ind.*, **49**, 413–21 (T) (1930).

[8] Fieser and Hershberg, THIS JOURNAL, **61**, 1275 (1939).

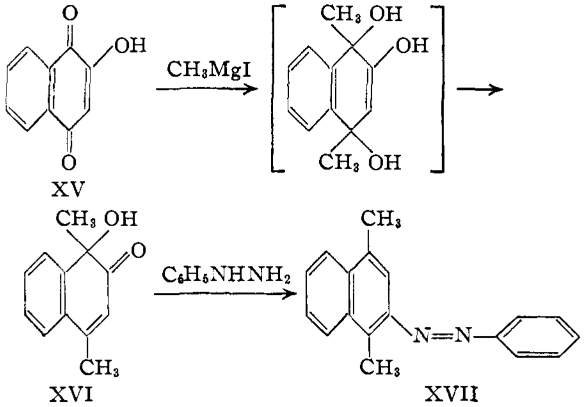
[9] Borgoslovski, *J. Gen. Chem.* (*U. S. S. R.*), **16**, 193 (1946).



XIV

4-methoxynaphthalene (XIV). 3,4,5-Trimethoxybenzenediazonium chloride was coupled with phenol and α-naphthol, respectively. The products were converted to the corresponding methyl ethers with dimethyl sulfate.

2-Benzeneazo-1,4-dimethylnaphthalene (XVII) was prepared according to Bargellini[10] from



XV XVI XVII

1,4-dimethyl-1-hydroxy-2-ketodihydronaphthalene (XVI) by condensation with phenylhydrazine. The dihydronaphthalene derivative (XVI) which had been prepared previously[11] by oxidation of 1,4-dimethylnaphthol-2 obtained by degradation of santonous acid, was prepared from the 2-hydroxy-1,4-naphthoquinone (XV) by reaction with excess methylmagnesium iodide.

The authors are grateful to Professor Louis F. Fieser for his helpful interest.

Experimental[12]

3-Aminoacenaphthene (a) From 3-Nitroacenaphthene.—The required 3-nitroacenaphthene was prepared according to Morgan[4] by nitration of acenaphthene in acetic anhydride. The nitro compound (10 g.) suspended in 100 cc. of pure alcohol with 0.5 g. of Adams catalyst was shaken under 30 pounds pressure of hydrogen for four hours. The yellow nitro compound dissolved leaving a nearly colorless solution with a strong blue fluorescence. When the solution was filtered and concentrated to small volume the amine separated in nearly pure form, 8.1 g. (95%), m.p. 82–83°.

(b) **From 3-Acetoacenaphthene.**[12a]—This preparation was carried out to obtain a reference sample of 3-aminoacenaphthene. The oxime of 3-acetoacenaphthene was rearranged in ether with phosphorus pentachloride to give a 50% yield of the acetylamino derivative, m. p. 192–193°. On acid hydrolysis the acetyl amine gave a product m. p. 82–83° which did not depress the melting point of the material obtained in (a).

3-Benzeneazoacenaphthene (VII).—3-Aminoacenaphthene (8.5 g.) in 100 cc. of benzene was treated with 3.6 g. of thionyl chloride. After five hours under reflux the solution was filtered and the benzene removed at reduced pressure. The residue, washed with petroleum ether, was treated with 11 g. of phenylhydroxylamine in 100 cc. of dry benzene, heat being evolved as a tarry sludge deposited. After being allowed to stand for twelve hours the mixture was filtered. The filtrate was chromatographed on alumina. The orange band eluted with benzene gave 1.7 g. (13%) of red crystals obtained as red needles after several recrystallizations from 70% alcohol, m. p. 99–100°.

Anal. Calcd. for $C_{18}H_{14}N_2$: C, 83.70; H, 5.46. Found: C, 83.55; H, 5.42.

2-Benzeneazophenanthrene (VIII).—The required 2-aminophenanthrene was prepared according to Bachmann and Boatner[13] from 2-acetylphenanthrene. A mixture of 12 g. of the amine and 10 cc. of thionyl chloride in 120 cc. of dry benzene was refluxed at slightly reduced pressure for twelve hours. At this time the heavy precipitate initially formed appeared to have gone into solution. The excess thionyl chloride and solvent were removed by distillation at reduced pressure and the dark residue thoroughly extracted with a mixture of equal volumes of chloroform and petroleum ether. When distilled to dryness the extract left 8.5 g. of the thionylamino compound as an orange-colored crystalline material. This product in 100 cc. of dry benzene was treated with 12 g. of phenylhydroxylamine in 150 cc. of dry benzene. The mixture became warm immediately and deposited a gray amorphous powder. After twelve hours the reaction mixture was filtered. When concentrated and cooled the filtrate deposited 3 g. of crude crystalline product. This material after three recrystallizations from benzene was obtained as lustrous golden orange platelets, 2.5 g. (15%), m. p. 143–144°.

Anal. Calcd. for $C_{20}H_{14}N_2$: C, 85.07; H, 5.00. Found: C, 84.98; H, 5.11.

3-Benzeneazophenanthrene (IX).—The required 3-aminophenanthrene was prepared according to the method of Bachmann and Boatner[13] from 3-acetylphenanthrene. The amine (6 g.) was dissolved in 100 cc. of dry benzene and 2.2 g. of thionyl chloride added. After heating for twelve hours under slightly reduced pressure, the dark solution was filtered and the benzene removed under reduced pressure. The residue was dissolved in petroleum ether and filtered. The petroleum ether was removed at reduced pressure leaving an orange crust of material. This was then treated with a solution of 6.4 g. of phenylhydroxylamine in 100 cc. of benzene. The solution became very warm and turned a bright red depositing a black tarry sludge. After filtration the benzene solution was chromatographed on an alumina column 20 × 2 cm. The orange band was eluted with benzene and the eluate rechromatographed. After removal of the benzene a red crystalline residue remained. This was taken up in alcohol and water added at the boiling point. On cooling and scratching a crystalline material deposited, m. p. 139–140°. Recrystallization is best from ethanol–acetone to give long orange needles.

Anal. Calcd. for $C_{20}H_{14}N_2$: C, 85.07; H, 5.00. Found: C, 84.96; H, 5.04.

2-Benzeneazo-5-(*o*-tolueneazo)-toluene (X).—Nitrosobenzene[14] (1.4 g.) in 30 cc. of glacial acetic acid was added to a cooled solution of 3.0 g. of 2-(3-tolylazo)-5-aminotoluene in 60 cc. of glacial acetic acid. On standing at room temperature for three days the mixture deposited 2.4 g. (60%) of crystalline product, m. p. 104–106°. The material was crystallized from chloroform–alcohol as fine orange-brown needles, m. p. 105–106°. It is soluble in hot chloroform; insoluble in methanol, ethanol or acetone.

Anal. Calcd. for $C_{20}H_{18}N_4$: N, 17.81. Found: N, 17.99.

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(10) Bargellini, *Gazz. chim. ital.*, **37^{2}**, 1407 (1907).

(11) Cannizzaro, *ibid.*, **76^{1}**, 26 (1896).

(12) Microanalyses by Shirley R. Katz, all melting points are corrected.

(12a) Provided through the courtesy of Professor Louis F. Fieser.

(13) Bachmann and Boatner, THIS JOURNAL, **58**, 2097 (1936).

(14) "Organic Syntheses," Coll. Vol. I, p. 455.

2-Benzeneazofluorene (XI).—The required 2-aminofluorene was prepared by reduction of 2-nitrofluorene with calcium chloride and zinc dust according to Diels.[15] The amine (5 g.) in 25 cc. of acetic acid was treated with 3 g. of nitrosobenzene[13] in 25 cc. of glacial acetic acid. The mixture was kept at 0° for twelve hours during which a crystalline material deposited. After recrystallization from 95% alcohol the product formed yellow leaflets, 4 g. (54%), m. p. 173–174°.

Anal. Calcd. for $C_{19}H_{14}N_2$: C, 84.41; H, 5.18. Found: C, 84.39; H, 5.22.

3,4,5-Trimethoxy-4′-hydroxyazobenzene.—The required 3,4,5-trimethoxyaniline was prepared in 65% yield from 3,4,5-trimethoxybenzoic acid amide[16] according to "Organic Syntheses"[17] by treatment with sodium hypochlorite. The amine (7.0 g.) in 47 cc. of 13% hydrochloric acid was diazotized at 0° by the addition of 2.3 g. of sodium nitrite in a small amount of water. The clear diazonium salt solution diluted to 50 cc. with ice-water was used as a stock solution. An aliquot (43 cc.) of the stock solution was mixed with an ice-cooled solution of 2.0 g. of phenol and 3.0 g. of sodium hydroxide. After fifteen minutes the mixture was acidified. The product was separated on a filter and after recrystallization from methanol–water was obtained as golden-orange needles, 2.5 g. (79%), m. p. 165–166°.

Anal. Calcd. for $C_{15}H_{16}N_2O_4$: C, 62.47; H, 5.59. Found: C, 62.70; H, 5.78.

3,4,5,4′-Tetramethoxyazobenzene (XIII).—An aqueous solution of 2.5 g. of the above azo-phenol and 0.4 g. of sodium hydroxide heated on the steam-bath and vigorously shaken, was treated with small portions alternately of dimethyl sulfate and aqueous alkali so that the solution was kept as nearly as possible slightly alkaline. The treatment was carried on for about one hour when methylation appeared complete. The product, which separated during the course of the reaction, was collected on a filter and washed with alkali and water. After a few recrystallizations from methanol–water the substance was obtained as glistening orange platelets, 1.5 g., m. p. 106–107°.

Anal. Calcd. for $C_{16}H_{18}N_2O_4$: C, 63.57; H, 6.00. Found: C, 63.80; H, 6.06.

1-(3,4,5-Trimethoxybenzeneazo)-4-methoxynaphthalene (XIV).—An aliquot (65 cc.) of stock solution of trimethoxybenzenediazonium chloride, corresponding to 3.0 g. of 3,4,5-trimethoxyaniline, was mixed in the cold with an aqueous solution of 4.5 g. of α-naphthol and 3.0 g. of sodium hydroxide. The dark red mixture that resulted was acidified with hydrochloric acid. The gummy precipitate, separated on a filter, was dissolved without purification in a minimum of aqueous alkali and treated with methyl sulfate as described in the preparation of (XIII). The methylated product dissolved in benzene was purified by passage over activated alumina in a tower 12 cm. × 1 cm. The orange band eluted with benzene gave a product obtained as orange platelets after two recrystallizations from chloroform–methanol, 2.5 g. (45%), m. p. 152–153°. The substance is soluble in chloroform, acetone and benzene; almost insoluble in methanol.

Anal. Calcd. for $C_{20}H_{20}N_2O_4$: C, 68.17; H, 5.72. Found: C, 68.23; H, 5.94.

Azo-3,4,5-trimethoxybenzene (XII).—An aliquot (65 cc.) of the stock solution of 3,4,5-trimethoxybenzenediazonium chloride was added over a period of five minutes to a vigorously stirred ice-cooled solution of cuprous chloride prepared according to Borgoslovski[9] from 12 g. of copper sulfate in 100 cc. of hot water by addition of a mixture of 5 g. of hydroxylamine hydrochloride and 4 g. of potassium hydroxide in aqueous solution. Finally just sufficient ammonium hydroxide was added so that a clear cuprous chloride solution resulted. A precipitate formed with the addition of the diazonium salt. Stirring was continued for one hour after which the product was separated on a filter and washed with methanol. The yellow powder remaining crystallized nicely as orange needles from chloroform–methanol, 1.5 g. (50%), m. p. 217–218°.

Anal. Calcd. for $C_{18}H_{22}O_6N_2$: C, 59.65; H, 6.12. Found: C, 59.56; H, 6.30.

1,4-Dimethyl-1-hydroxy-2-ketodihydronaphthalene (XVI).[11]—A solution of 10 g. of 2-hydroxy-1,4-naphthoquinone[18] in 450 cc. of warm dry benzene was slowly added over a period of one hour to a refluxing solution of methyl Grignard prepared from 12 cc. of methyl iodide in 500 cc. of dry ether and 250 cc. of dry benzene. The addition precipitated an orange solid which rapidly turned gray. After heating under reflux for an additional twenty-four hours the mixture was decomposed with excess saturated ammonium chloride solution. The benzene–ether layer, extracted three times with 10% sodium carbonate and washed with water, was distilled to remove the solvent. From the red oily residue, by distillation at reduced pressure, there was obtained 2.2 g. of crude product as gummy crystals, b. p. 130–160°, 2 mm. A small sample crystallized from ethyl acetate–benzene as almost colorless granules, m. p. 103–105° (m. p. 104–105° reported by Cannizzaro).[11]

2-Benzeneazo-1,4-dimethylnaphthalene (XVII).[10]—The azo compound was prepared according to Bargellini[10] from the crude hydroxy ketone (XVI) in solution in 80 cc. of methanol by addition of 1.8 g. of phenylhydrazine hydrochloride in 40 cc. of water to which a few drops of hydrochloric acid was added. The mixture turned orange in color and soon began to deposit clusters of orange crystals. After forty-eight hours the reaction mixture was filtered. The product crystallized as beautiful large orange-red needles from methanol, 1.8 g. (48%), m. p. 82.5–83° (reported by Bargellini,[10] 83–84°).

Anal. Calcd. for $C_{18}H_{16}N_2$: C, 83.05; H, 6.16. Found: C, 82.99; H, 6.33.

Summary

The synthesis of eight new azo compounds is described: 3-benzeneazoacenaphthene, 2-benzeneazophenanthrene, and 3-benzeneazophenanthrene by reaction of the appropriate thionylamino compounds with hydroxylamine; 2-benzeneazo-5-(*o*-tolueneazo)-toluene and 2-benzeneazofluorene by the use of nitrosobenzene; azo-3,4,5-trimethoxybenzene by the action of cuprous chloride on the diazonium salt; and 3,4,5,4′-tetramethoxyazobenzene and 1-(3,4,5-trimethoxybenzene)-azo-4-methoxynaphthalene by the diazo coupling reaction. There is also described a new method of preparation of 1,4-dimethyl-1-hydroxy-2-ketodihydronaphthalene, an intermediate in the preparation of 2-benzeneazo-1,4-dimethylnaphthalene.

Cambridge, Massachusetts Received March 29, 1949

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(15) Diels, "Organic Syntheses," Coll. Vol. II, p. 447.

(16) Graebe and Suter, *Ann.*, **340**, 227 (1905).

(17) "Organic Syntheses," Coll. Vol. II, p. 45.

(18) Kindly supplied by Dr. Russell H. Brown.