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THE STRUCTURE OF THE SO-CALLED TOLUIDINE BLUE

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Toluidine Blue is a dye that has certain rather unique properties, in particular, its spectral absorption characteristics, which do not resemble those of ordinary blue dyes (Fig. 1). While it has long been available on the market, its structure has never been divulged. It is not listed in Schultz (1) or the Colour Index (2), presumably because it is not important in the dyeing of textiles.[1] In connection with other work on color and constitution being carried out in this laboratory, it became of interest to learn the structure of this dye.

From the results of elementary analyses of the purified, salt-free dye, it was possible to write an empirical formula, $C_{28}H_{20}N_2Na_2O_{10}S_2$. Qualitative reactions for classification indicated that it was an acid anthraquinone dye. Toluidine Blue gives a yellow vat on reduction of its aqueous solution in the presence of Raney nickel or by the use of alkaline hydrosulfite. The dye is regenerated by aerial oxidation. The anthraquinone nucleus was detected by a zinc dust distillation, using Clar's procedure (3), by which a small quantity of anthracene was secured. When the fusion mixture was acidified, considerable hydrogen sulfide was evolved, indicating the presence of a sulfonic acid group that had also been reduced in the fusion. From the residue, a purple solid was isolated; this contained carbon, hydrogen, nitrogen, and oxygen, but no sulfur or sodium. The empirical formula, $C_{28}H_{22}N_2O_4$, was arrived at from the analytical data; the dye is, thus, the disodium salt of the corresponding disulfonic acid. By subtraction of $C_{14}H_8O_2$ (anthraquinone), one is left with $C_{14}H_{14}N_2O_2$, which it seemed highly probable, comprised two toluidine residues (C_7H_7N) and two atoms of oxygen. Reconstructing a structure from these conclusions led to a ditoluidinodihydroxyanthraquinone. Of course, the hydroxyl groups might have been located in the toluene ring, but evidence secured later definitely excluded this possibility. The dye was entirely unlike the isomeric green dye, Alizarin Viridin (Schultz No. 1193), which conforms with these conclusions as to building units. Since there are 114 possible isomeric

[1] It is not the azine dye, Toluidine Blue O (No. 1041 in Schultz; No. 925 in Colour Index).

ditoluidinodihydroxyanthraquinones, without any consideration of the possibilities that the hydroxyl groups might have been in the toluene rings, or that the toluidine residues were not *para*, but *ortho* or *meta*, further degradation was essential.

The dye is sensitive to the action of nitric acid, hydrogen peroxide or ferric chloride in the presence of acid, and fuming sulfuric acid, but is unaffected by hydrochloric acid in the absence of ferric chloride or hydrogen peroxide, concentrated sulfuric acid, and bases. Although from the nature of the reagents that attack the dye one would suspect oxidation, the product appears to be the result of a hydrolysis.[2] The new substance resulting from the use of these reagents was a red solid which analyzed for $C_{14}H_8O_6$, or a tetrahydroxyanthraquinone. It formed a tetraacetate, $C_{22}H_{16}O_{10}$, from

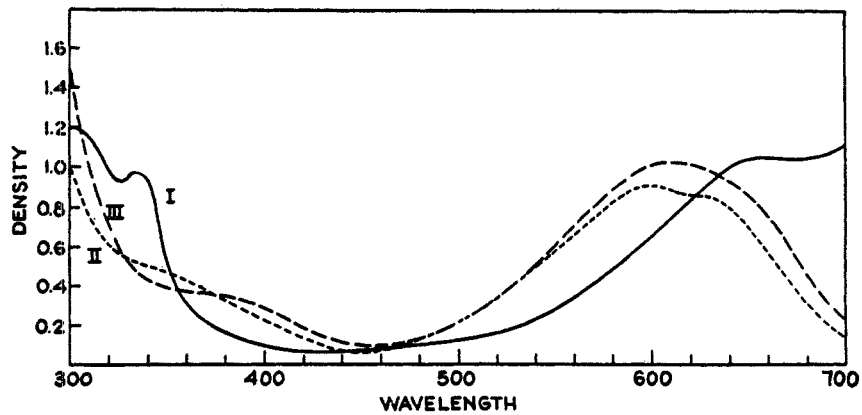


FIG. 1. I = Toluidine Blue
II = A commercial dye, Schultz No. 1199
III = A commercial dye, Schultz No. 1188

which the parent substance was regenerated on hydrolysis. The properties of the two compounds did not agree with those of any of the 14 isomers reported in the literature (there are 22 possible tetrahydroxyanthraquinones). However, the latter substances do not have sharp melting points, but tend to sublime or decompose at elevated temperatures, so that the comparisons have to be made through their color reactions or derivatives, such as the tetraacetates.

A survey of the literature on dihydroxyditoluidinoanthraquinones revealed the commercially available Alizarin Viridin, which is 7,8-dihydroxy-1,4-di-*p*-toluidinoanthraquinone, and several patents according to which other isomers may be produced. Thus, the 1,4-ditoluidino-5,8-

[2] For convenience, this type of cleavage is called oxidative hydrolysis.

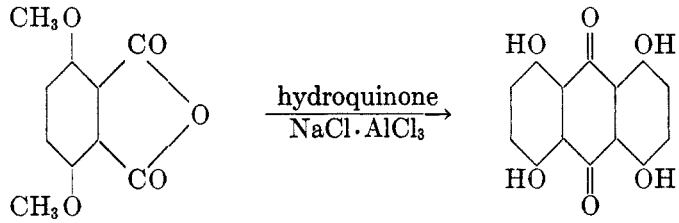
dihydroxyanthraquinone is said to result from the interaction of *p*-toluidine and 1,4,5,8,9,10-hexahydroxyanthracene (4), or 5,8-diaminoquinizarin (5), or 5,8-dichloroquinizarin (6). 1,5-Ditoluidino-4,8-dihydroxyanthraquinone is probably a product from *p*-toluidine and *p*-dinitroanthrarufin (7), or dibromoanthrarufin (8), while its disulfonic acid is probably a component of the mixture obtained when 4,8-dinitroanthrarufin-2,6-disulfonic acid is melted with *p*-toluidine (9). Further, several dyes having the sulfonic acid groups in the toluidine residue are described. These were secured by using sodium 4-aminotoluene-2-sulfonate and leuco-1,4,5,8-tetrahydroxyanthraquinone, and carrying out the reaction in a solvent (10). All of the dyes obtainable by these procedures, if attacked by the hydrolyzing (oxidizing) agents mentioned above, would be expected to yield 1,4,5,8-tetrahydroxyanthraquinone, the properties of which as given in the literature (11) were not in good agreement with those of the red solid secured by the degradation of Toluidine Blue. Hence, there seemed to be no reason to make an extensive study of such dyes.

The unknown 1,4,6,7-tetrahydroxyanthraquinone should be obtainable by a similar oxidative acid hydrolysis of 6,7-ditoluidino-1,4-dihydroxyanthraquinone (or the isomer in which the amine residues and hydroxyl groups are interchanged; neither of these has been described). The synthesis of this compound should be possible, starting with 6,7-dibromoquinizarin; the latter could be secured by a benzoylbenzoic acid synthesis, starting from 4,5-dibromophthalic anhydride (12) and hydroquinone. No particular difficulty was encountered in carrying out this series of reactions, and degradation of the dye obtained in the final step gave a tetrahydroxyanthraquinone identical with the red compound from Toluidine Blue. Thus, it seemed that the structure of the latter was established.

Because of the low yields of the 4,5-dibromophthalic anhydride, it seemed advisable to consider the possibility of substituting the corresponding dichloro derivative. Now, the only easily isolated dichloro substitution product from the chlorination of phthalic anhydride has the chlorine atoms in the 3 and 6 positions; this would be useless for our purpose. However, since the oxidative acid hydrolysis seemed a peculiar reaction, it was considered advisable to carry through a preparation and learn whether the same property would appear in the 1,4,5,8-series. When this was done, a green dye was obtained; to our surprise, this gave the *same* red tetrahydroxyanthraquinone upon degradation. If molecular rearrangements, which seemed extremely unlikely, were barred, this result implied that the position of the halogen atoms in one of the anhydrides, as reported in the literature, was incorrect.

It is possible to synthesize the 1,4,5,8-tetrahydroxyanthraquinone in an unambiguous manner, starting with 3,6-dimethoxyphthalic anhydride (13),

which, in turn, has been secured from hydroquinone by reactions which leave no doubt as to its structure.



This procedure gives a relatively pure product. Further, this product is identical in all respects (including its tetraacetate) with the red compound secured from Toluidine Blue. The following conclusions are inevitable: (a) the properties of 1,4,5,8-tetrahydroxyanthraquinone as given in the literature are incorrect, probably because a pure sample has never been available; (b) in Toluidine Blue, the groups are in the 1,4,5, and 8 positions; (c) considerable doubt is cast upon the location of the bromine atoms in the so-called 4,5-dibromophthalic anhydride. Accordingly, work in the 1,4,6,7-series was shelved, and a reinvestigation of the 1,4,5,8-series was undertaken.

A priori, 1,4-di-*p*-toluidino-5,8-dihydroxyanthraquinone would be excluded, because all the 1,4-ditoluidinoanthraquinone dyes so far reported are green. This conclusion was confirmed by the synthesis of a dye having the group arrangement in question from 5,8-dichloroquinizarin, followed by sulfonation. Not only was the product green, but its absorption spectrum proved to be identical with that of another little-used, unlisted dye, Toluidine Green. This substance would be expected to result on following the procedures in the patent given in reference (4). Repetition of those patent directions actually gave mixtures having dull shades and flat absorption curves.

4,8-Dichloroanthrarufin seemed the most likely starting material for the synthesis of 4,8-ditoluidino-1,5-dihydroxyanthraquinone, and was secured in reasonable amounts after some preliminary study. It was available from the interaction of sulfuryl chloride and anthrarufin in nitrobenzene (14). The fusion with *p*-toluidine offered no difficulty, and the product was readily purified; it was found much more convenient to start with a fairly crude dichloroanthrarufin and crystallize at this point than to prepare a pure dichloroanthrarufin.

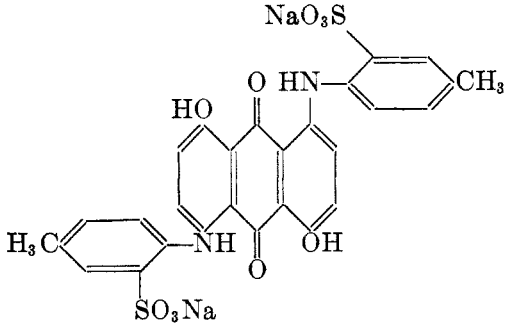
4,8-Di-*p*-toluidino-1,5-dihydroxyanthraquinone prepared from 4,8-dichloroanthrarufin is the same substance as was isolated from the zinc reduction of Toluidine Blue, and gives Toluidine Blue on sulfonation. Since oleum degrades the dye, as previously mentioned, it is essential to accom-

plish the sulfonation under milder conditions by the use of concentrated sulfuric acid at steam-bath temperature.

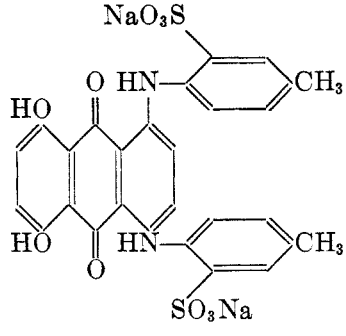
The dinitration of anthrarufin (15) is rather unsatisfactory; the yield is low and the mixtures are not readily separated. Until a carefully-purified 4,8-dinitroanthrarufin was secured, the dyes prepared by its use were unsatisfactory and the absorption curves were not identical with that of Toluidine Blue. Eventually, a pure dinitroanthrarufin was secured, and after appropriate treatment it was converted into a good specimen of Toluidine Blue. These methods of synthesis locate the toluidine and hydroxyl groups.

While the position of the sulfonic acid groups can be inferred from other dye structures given in the literature, it should be possible to locate them definitely if a reaction of cleavage of the toluidino groups were available. Toluidino groups have been split by what appears to be a simple sulfuric acid hydrolysis from 1,6,7,12-tetratoluidinopentacenequinone (16) and from some acid anthraquinone dyes *reduced* by fuming hydriodic acid (34) or by stannous chloride in the 1,4-series, but the 1,5-series was unattacked (although under the same conditions 1,5-diamino-2,4,6,8-tetrahydroxyanthraquinone was hydrolyzed) (17).

We have found that Toluidine Blue is not hydrolyzed by hydrochloric acid in the presence of stannous chloride, but if tin and hydrochloric acid are used, the cleavage[3] takes place readily, with the formation of leuco-1,4,5,8-tetrahydroxyanthraquinone and a toluidine sulfonic acid. The latter was identified as 4-aminotoluene-3-sulfonic acid by converting it to the known 4-chlorotoluene-3-sulfonamide. Degradation by reductive hydrolysis of Toluidine Green gave the same 1,4,5,8-tetrahydroxyanthraquinone and 4-aminotoluene-3-sulfonic acid. Toluidine Blue is, thus, the disodium salt of 1,5-di-(2′-sulfo-4′-toluidino)-4,8-dihydroxyanthraquinone (I), while Toluidine Green is the 1,4-isomer (II).



I

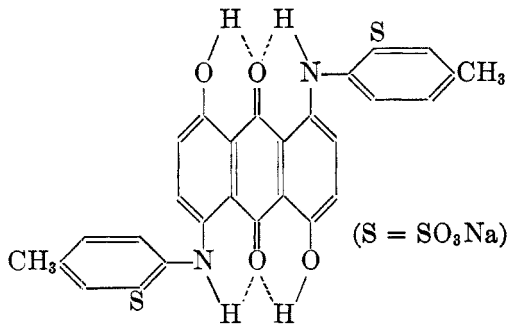


II

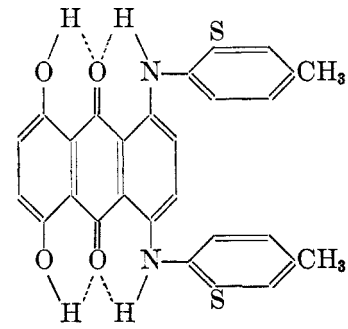
[3] For convenience this cleavage is called reductive hydrolysis.

The possibility that the blue and green dyes do not have structures of the commonly accepted types, but are 9,10-diimides, seems highly improbable, because of the method of preparation. Groups in the *alpha* positions of the anthraquinone nucleus are always replaced under milder conditions than are required to form 9,10-diimides. Further, all known 9,10-diimides are yellow to red; they are not dyes and are usually hydrolyzable by concentrated sulfuric acid (18).

Of course, it is entirely possible that Toluidine Blue is not represented by formula (I), but that it may exist to a large extent in one or more of the several possible tautomerides, each of which is a resonance hybrid. However, it may be that the unusual spectrophotometric absorption characteristics can be better accounted for by the assumption of a structure containing hydrogen bonds (III for Toluidine Blue, IV for Toluidine Green). Other properties may be reconciled with such a formulation. Since the dyes are



III



IV

not hydrolyzed except in the presence of oxidizing or reducing agents, the functions of the latter must be to destroy at least a part of the hydrogen bonding. At present the available evidence does not warrant a closer correlation between structure and absorption.

These reductive and oxidative hydrolysis procedures should be useful in reactions of degradation in the anthraquinone series. For example, Celliton Fast Blue Green B (19), which is largely 1,4-di-(β-hydroxyethylamino)-5,8-dihydroxyanthraquinone, is oxidatively hydrolyzed to give 1,4,5,8-tetrahydroxyanthraquinone. The method of reduction outlined, being applicable to the 1,5-series, is more general than that of Friedländer (17) and more useful than the use of oxidizing agents. The latter tend to destroy the amine component and may even attack the hydroxy compound; thus, quinalizarin from Alizarin Viridin is further oxidized when the procedure is applied to that dye.

A few instances of oxidative hydrolytic cleavage have been recorded in the literature. Certain aminohydroxyanthraquinones are converted to

polyhydroxyanthraquinones by the use of manganese and lead dioxides in concentrated sulfuric acid solution, or by the use of ferric salts, nitric acid, or ammonium persulfate; in some cases, additional hydroxyl groups are

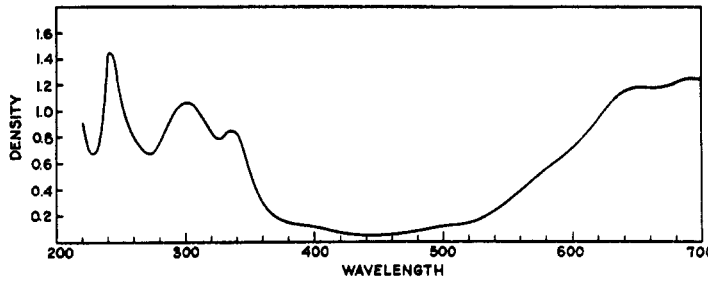


FIG. 2. TOLUIDINE BLUE

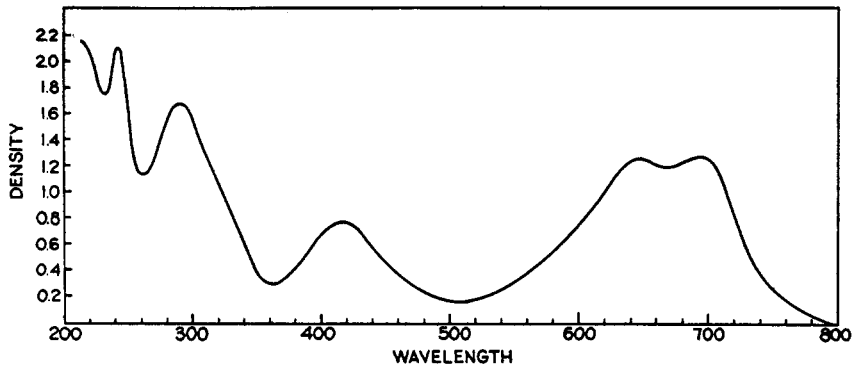


FIG. 3. TOLUIDINE GREEN

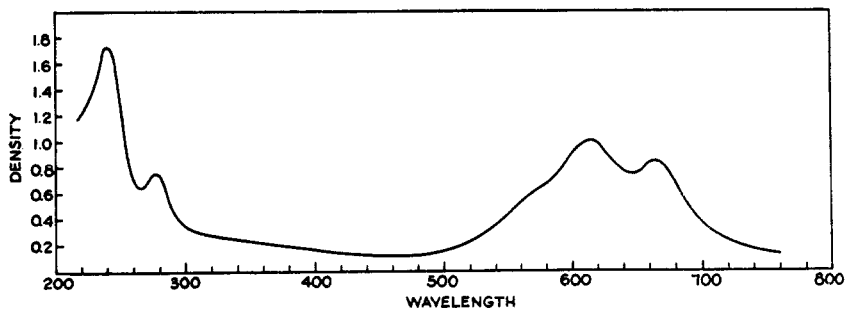


FIG. 4. SODIUM 1,4-di-β-SULFATOETHYLAMINO-5,8-DIHYDROXYANTHRAQUINONE

formed by a seeming direct oxidation of a hydrogen atom (20). According to Houben (21), it would seem that the first step is the removal of (two) hydrogen atoms to form a quinoneimide (A); this latter class of compound is known to be susceptible to acid hydrolysis, but the product is always a

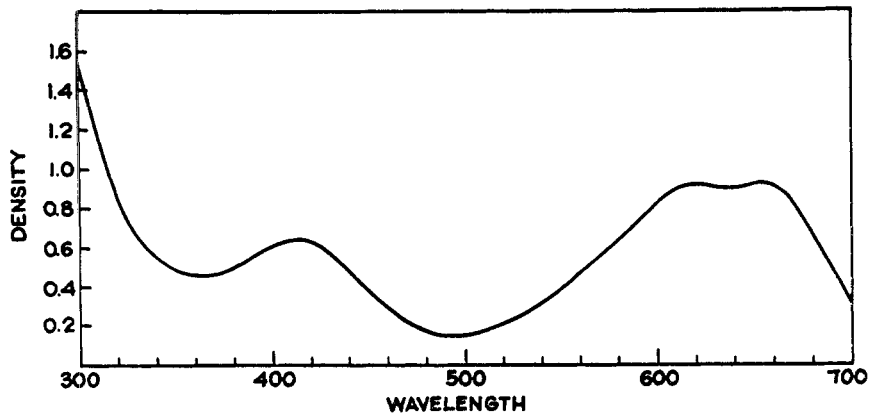


FIG. 5. ALIZARIN VIRIDIN

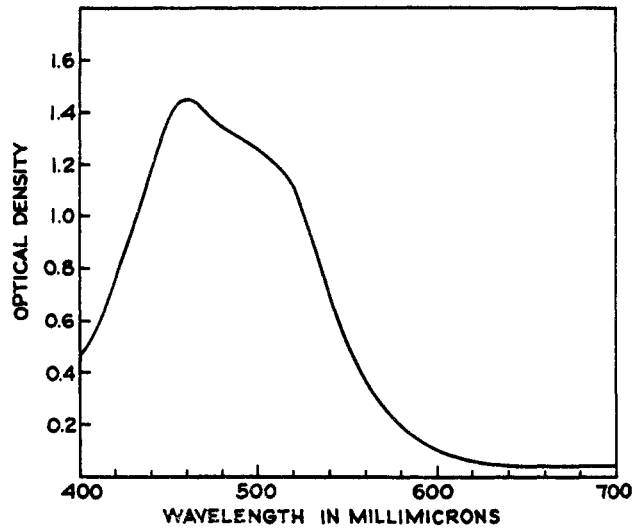
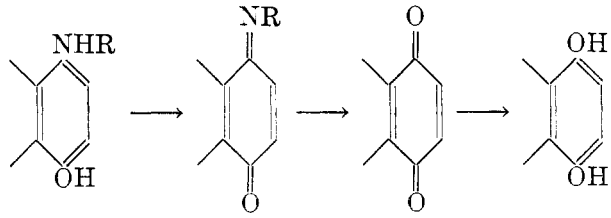


FIG. 6. VAT OF TOLUIDINE BLUE

quinone. To secure the hydroxy compound actually isolated (B) would require some sort of reduction. The high yield of 1,4,5,8-tetrahydroxyan-



thraquinone obtained from Toluidine Blue contradicts such an assumption.

The principal difference between the spectral absorption characteristics of Toluidine Blue and other blue dyes (Fig. 1) is the high flat absorption in the longer wave lengths of the visible, extending over a considerable range of the spectrum; there is also evidence of two maxima in the curve of Toluidine Blue at 646, 694 (Fig. 2). An inspection of the curves of several anthraquinone dyes reveals similar instances in which there are two absorption bands of this kind in the curve; each molecule contains two hydroxyl groups (Fig. 3), Toluidine Green; Fig. 4, sodium-1,4-di-β-sulfatoethyl-

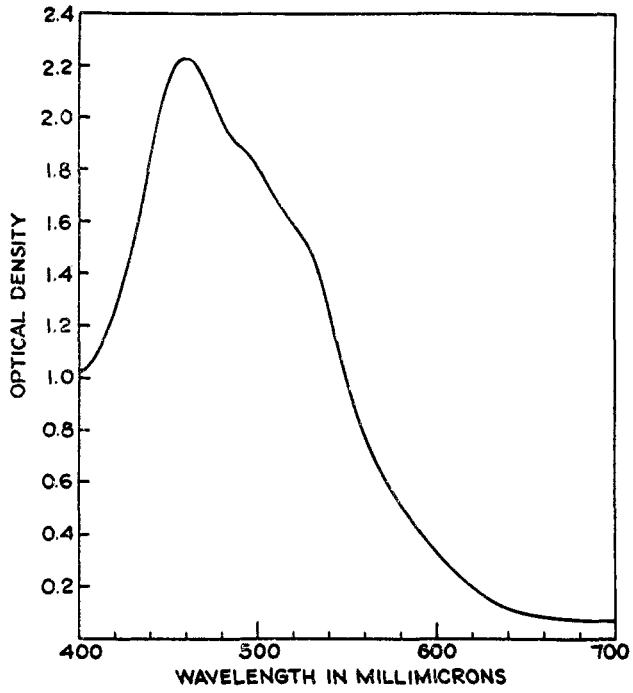


FIG. 7. VAT OF TOLUIDINE GREEN

amino-5,8-dihydroxyanthraquinone; Fig. 5, Alizarin Viridin). The inference may be drawn, then, that there is a connection between the unusual type of curve and the presence of the hydroxyl groups; it is hoped that this may be cleared up by further study.

The other curves shown are the reduced forms (vats) of Toluidine Blue (Fig. 6) and Toluidine Green (Fig. 7). In each figure, the abscissa is in millimicrons, while the ordinate is optical density; the latter is defined as $\log_{10} \frac{1}{T}$, where T = transmission. The solution is examined in a cell 1 cm. in thickness.

EXPERIMENTAL

I. TOLUIDINE BLUE SERIES

A. *Degradation*

1. *Purification and analyses.* The commercial dye appears as a dark blue powder, completely soluble in water. Qualitative classification tests indicated that the coloring matter present was a derivative of anthraquinone. It gives a yellow "vat," which appears red except in very dilute solutions, on reduction by hydrogen in the presence of Raney nickel, or by alkaline hydrosulfite (Fig. 6), or by zinc and acetic acid; this is oxidized back to the original blue by atmospheric oxygen.

After a specimen of the commercial product was extracted with absolute alcohol in a Soxhlet apparatus for twenty-one days, the dye was completely removed, leaving 35% of a residue, mainly sodium chloride, but containing an appreciable quantity of magnesium chloride. A spectroscopic examination of an ashed specimen of dye revealed the presence of sodium, magnesium, iron, aluminum, calcium, and lead, only the first being in significantly large amounts. The alcoholic solution was concentrated, the dye being collected in fractions. The first fraction (needles) was analyzed.

Anal. Calc'd for $C_{28}H_{20}N_2Na_2O_{10}S_2$: N, 4.28; S, 9.78; Na, 7.03.
Found: N, 4.33; S, 9.80, 9.82; Na, 6.82.

2. *Zinc dust distillation.* A mixture of 17 g. of the commercial dye, 85 g. of zinc chloride, 17 g. of coarse sodium chloride, and 17 g. of zinc dust (3), in a 500-cc. flask fitted with stirrer and thermometer, was heated by a metal-bath to 230°. As the mass liquefied, the temperature was raised to 260° and there was so much frothing that stirring had to be discontinued. After five minutes the melt was allowed to cool to room temperature and was treated with warm, dilute hydrochloric acid (copious evolution of hydrogen sulfide[4]). The insoluble portion was filtered and dried. When this was heated at 200–300°/2–3 mm. for ten minutes, a small amount of a pale yellow solid sublimed, which, after resublimation, was identified as anthracene, m.p. 208–210°; a mixed melting point with an authentic specimen was not depressed. The sublimation residue was further boiled with dilute hydrochloric acid to remove most of the zinc, then washed and dried. It was successively extracted with boiling alcohol and nitrobenzene, the insoluble carbonized residue being rejected. After dilution of the nitrobenzene extract with twice its volume of ethyl alcohol, and standing overnight, purplish needles with a bronzy luster had separated. These gradually decompose when heated above 300°; the solution in dioxane or nitrobenzene is a brilliant blue.

Anal. Calc'd for $C_{28}H_{22}N_2O_4$: C, 74.7; H, 4.8; N, 6.2.
Found: C, 74.8; H, 4.9; N, 6.2.

This is the 4,8-ditoluidinoanthrarufin, the synthesis of which is described below.

3. *"Oxidative hydrolysis."* During the classification tests, it was discovered that several oxidizing agents destroyed the blue color of the dye, with production of a brownish-red solution; the full list includes dilute nitric acid, or a combination of hydrochloric acid with ferric chloride or potassium periodate or persulfate or 30% hydrogen peroxide.

In illustration, to a solution of 10 g. of (purified) Toluidine Blue in 125 cc. of water, not above 85°, was added 43 cc. of concentrated nitric acid, and the whole was heated for one and one-half hours on the steam-bath. As the color changed, a dull reddish-

[4] The production of hydrogen sulfide indicated the presence of a sulfonic acid group in the dye. This was checked by similar fusions with anthraquinone-α-sulfonic acid, and 4-aminotoluene-2-sulfonic acid.

brown solid separated. After filtering and drying, this amounted to 2.6 g. (78%, based on the assumption that the dye contained 80% of coloring matter). The aqueous solution did not contain an amine (tested by diazotizing and non-coupling). The dull red solid was purified by a semimicro sublimation; it formed very fine red rods with a greenish metallic luster. The recovery was 67%. 1,4,5,8-Tetrahydroxyanthraquinone dissolves fairly readily in xylene, acetic acid, or dioxane, but is very slightly soluble in alcohol or water.

Anal. Calc'd for $C_{14}H_8O_6$: C, 61.8; H, 2.9.
Found: C, 61.5; H, 2.9.

A more dilute nitric acid used in the above hydrolysis gave a similar result.

The colors produced by the pure substance with reagents are as follows: in concentrated sulfuric acid, blue with a strong red fluorescence (pure blue if very dilute), essentially unchanged on the addition of boric acid; a blue-violet solution with sodium or potassium hydroxide, followed by separation of the colored salt in a few minutes; yellow with zinc and acetic acid, restored to red by potassium persulfate. The identity of this pure substance with the 1,4,5,8-tetrahydroxyanthraquinone described below was shown by microscopic and crystallographic examination,[5] a comparison of its behavior on sublimation, and the identity of the tetraacetates.

4. *Synthesis of 1,4,5,8-tetrahydroxyanthraquinone and its tetraacetate.* To a melt of 40 g. of anhydrous aluminum chloride and 7 g. of sodium chloride at 180° was added an intimate mixture of 6 g. of 3,6-dimethoxyphthalic anhydride (13) and 6 g. of hydroquinone, over a period of five minutes, with hand stirring. The temperature was then raised to 200–220° and maintained for a half hour at the higher figure. After cooling, the blue solid was finely pulverized, and decomposed by slow addition to hot dilute hydrochloric acid. The reddish-brown solid was collected on a filter, washed, and dried. The yield was 4 g. The product was recrystallized from xylene. It does not show a definite melting point;[6] there is visible sintering at about 350°, with some sublimation. The analysis and other properties have already been given.

The tetrahydroxy compound can also be secured by heating 5,8-dibromoquinizarin with calcium hydroxide solution in an autoclave at 240–260° for twenty-four hours.

The tetraacetate. A mixture of 0.3 g. of the tetrahydroxy compound, 20 cc. of acetic anhydride, and 1 g. of sodium acetate was refluxed for one and one-half hours. After being worked up in the usual way, the product was crystallized from hot acetic acid, from which it separates in bright yellow needles. It melts with decomposition, the temperature observed being very dependent on the rate of heating. If the bath is at 275° when the sample is immersed, the melting point is sharp at 281–282°; if the bath is heated fairly rapidly, the decomposition point is 273–275°.

Anal. Calc'd for $C_{22}H_{18}O_{10}$: C, 60.0; H, 3.6.
Found: C, 59.7, 59.9; H, 3.5, 3.5.

On carrying out a synthesis from 3,6-dimethoxyphthalic anhydride and catechol with a view to possibly obtaining a 1,4,6,7-tetrahydroxyanthraquinone, only quinalizarin (1,4,7,8) was obtained.

5. *"Reductive hydrolysis."* Twenty-five grams of the dye (approximately 80%)

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[5] For this and other work of a similar nature we are indebted to Dr. E. E. Jelley of the Kodak Research Laboratories.

[6] In the literature (11a, 22) the melting point 246° is given; the color with concentrated sulfuric acid is described as greenish-blue, which is changed to blue by boric acid. The tetraacetate is said to form bright yellow needles, decomposing at about 250° (11b).

was boiled with 2250 cc. of methanol and filtered from the undissolved salts. The solution was then transferred to a flask fitted with a reflux condenser, stirrer, and inlet tube for gas. After the addition of 40 g. of mossy tin, hydrogen chloride was passed in for two and one-half hours, while refluxing and stirring. The color became orange-brown. The solution was filtered hot from unused tin and 4.1 g. of the sparingly soluble leuco-1,4,5,8-tetrahydroxyanthraquinone. The filtrate was evaporated nearly to dryness, 300 cc. of hot water was added, and the solution was again filtered; the residual solid (3.23 g.) was leuco-1,4,5,8-tetrahydroxyanthraquinone. The total yield was 7.33 g. (86%). It was dissolved in sodium hydroxide and the solution aerated. The crude material was then further purified and identified as above.

The brown aqueous filtrate was decolorized with Darco, concentrated, and chilled in ice; 6.64 g. of 4-aminotoluene-3-sulfonic acid was obtained; a further 0.3 g. was isolated from the filtrate, making the total yield 6.94 g. (61%). For identification, the product was diazotized and converted into the 4-chloro derivative in the usual manner (23, 24). The sulfonamide was readily secured; it melted at 155–156°. An authentic specimen was prepared, which melted at 155–156°. There was no depression on admixture of the two, whereas the melting points of mixtures with the known 2-isomer (23) were depressed 15–20°.

This procedure, when applied to Alizarin Viridin, gave leuco-quinalizarin and 4-aminotoluene-3-sulfonic acid.

B. *Synthesis.*

1. *4,8-Dichloroanthrarufin.* This substance was secured by a modification of a patented procedure (14). A mixture of 2 l. of nitrobenzene and 250 g. of finely-ground anthrarufin was heated to the boiling point with vigorous stirring, and then cooled to 60° to produce a fine suspension of crystals. After the addition of 250 cc. of sulfuryl chloride, the mixture was refluxed and stirred on the steam-bath; at one-half hour intervals two 200-cc. portions of sulfuryl chloride were added, after which the heating and stirring were continued for eighteen hours. The excess sulfuryl chloride was then removed by heating to 205°. After cooling to 40–50°, the crude dichloroanthrarufin was filtered by suction and washed first with methanol and then with ether. The yield of crude product (varying with the source of the anthrarufin) is 160–170 g. The melting point varies with each lot, but always falls within the range 310–323°. If the melting point is below 310°, which will be the case if insufficient sulfuryl chloride is used, the product is not suitable for conversion to Toluidine Blue. The crude material may be recrystallized from nitrobenzene, trichlorobenzene or anisole, if desired. The pure substance melts at 336–337° with slight sublimation.

2. *4,8-Dinitroanthrarufin.*[7] A mixture of 50 g. of anthrarufin, 25 g. of boric acid, and 500 cc. of concentrated sulfuric acid was stirred at 50–60° for two hours, during which time most of the solid dissolved. The red solution was cooled to 10–15°, and 50 g. of potassium nitrate (10% excess) was added in small portions; stirring was then continued at 10–15° for three hours. The mixture was poured upon chipped ice, the crude nitration mixture was filtered through Vinyon fabric, washed with much water, and dried. This crude mixture was exhaustively extracted with three 500-cc. portions of boiling acetic acid, boiling each for five minutes, and filtering hot. The insoluble material (about 20 g.) was crude 4,8-dinitroanthrarufin. Upon recrystal-

[7] For the preparation of this substance we are indebted to Dr. Bell of these Laboratories.

lization from 1000 cc. of dioxane, 18 g. (26%) of product was recovered, in the form of yellow plates. This material is suitable for the next step.

3. *Toluidine Blue base; 4,8-di-p-toluidinoanthrarufin.* A mixture of 250 g. of dichloroanthrarufin and 3 kg. of *p*-toluidine was heated in an oil-bath at 160–175° for eighteen hours. After cooling to 60–65°, it was poured into dilute hydrochloric acid, the precipitated dye base being filtered and well washed with hot water. The dried product was digested for two hours with 2.5 l. of boiling chlorobenzene and filtered hot. The base so obtained is entirely satisfactory for conversion into the dye. The total yield (including working up filtrates) is 272 g. (75%). The other properties have already been described.

4. *Sulfonation; the dye, Toluidine Blue, I.* A solution of 270 g. of 4,8-di-*p*-toluidinoanthrarufin (Toluidine Blue base) in 2025 cc. of sulfuric acid (sp. gr. 1.84) was heated with stirring on the steam-bath (internal temperature 95–98°) for three hours. After cooling, the solution, the original green color of which had changed to blue, was poured upon about 12 l. of crushed ice and the precipitated dye was filtered. The dye acid was then dissolved in 12 l. of hot water to which had been added slightly more than the calculated amount of 40% sodium hydroxide, was filtered to remove a small amount of insoluble material, and was salted out in the usual manner. The yield was 355 g. of a product having the desired spectral absorption characteristics; it contains about 20% of sodium chloride. Fuming sulfuric acid gradually decomposed the dye, but the nature of the decomposition was not determined, other than to isolate a small amount of 1,4,5,8-tetrahydroxyanthraquinone.

C. *Isomeric ditoluidino dihydroxyanthraquinones* are obtainable from 4,8-dichloroanthrarufin and *meta*- and *ortho*-toluidines. While the *meta* isomer reacts as easily as the *para* in the procedure described above, dye base formation is incomplete with *ortho*-toluidine after twenty-four hours at 170°. The addition of boric acid to the melt in the latter case promotes the reaction so that it becomes practical. The products were recrystallized from xylene. They resemble the *para* isomer in solubilities and behavior on heating.

Anal. Calc'd for $C_{28}H_{22}N_2O_4$: C, 74.7; H, 4.9; N, 6.2.

Found: (*meta*) C, 74.7; H, 5.0; N, 6.4; (*ortho*) C, 74.9; H, 4.8; N, 6.2.

In concentrated sulfuric acid, the *para* derivative gives a yellow-green color, the *meta* a bluish-green, and the *ortho* a greenish-blue. All change to blue in a few seconds on the addition of boric acid.

II. TOLUIDINE GREEN SERIES

A. *Degradation.* This dye was secured by synthesis before it was examined otherwise. However, it is readily degraded by the processes of "reductive" and "oxidative" hydrolysis, as described above under Toluidine Blue; the products are the same and were identified in the same way. On reduction it gives an orange vat. (Fig. 7.)

B. *Synthesis.*

1. *5,8-Dibromoquinizarin.* Waldmann's procedure (25) for securing halogenated quinizarins was followed. While a melt of 535 g. of anhydrous aluminum chloride and 107 g. of sodium chloride was mechanically stirred at 200–220°, an intimate mixture of 136.5 g. of 3,6-dibromophthalic anhydride, m.p. 208–210° (section IV below) and 64 g. of hydroquinone was slowly added over a period of twenty-five minutes; after ten minutes a red color was noticed. When the addition had been completed, stirring was continued for twenty minutes. On cooling, the reddish-violet solid was pulverized, and decomposed by adding to 1500 cc. of dilute hydrochloric acid. The orange-red solid was filtered, well washed with warm water, and dried. It was then

extracted with warm alcohol. The yield of air-dried product was 149 g. (84%); it melts at 230–231° with preliminary softening at 221°. For purification it was recrystallized from acetic acid, from which it separated as red needles, m.p. 245°.

Anal. Calc'd for $C_{14}H_6Br_2O_4$: Br, 40.2. Found: Br, 39.9

2. *Formation of the dye base.* This was readily effected in three ways: heating the components in the presence of sodium acetate, or in the presence of boric acid, or refluxing the components in pyridine. Though each method gives a good product, the second is considered preferable.

A mixture of 640 g. of *p*-toluidine, 125 g. of boric acid, and 125 g. of the dibromoquinizarin was heated, with stirring, at 130–140° for three hours; a green color was noticed after a half hour. After cooling to about 75°, the melt was poured into 4 l. of dilute hydrochloric acid. The product was worked up in the usual manner. The yield was 135 g. (95%); m.p. 284–287°. The same substance, in essentially the same yield, was also secured by the use of 5,8-dichloroquinizarin (25). The product is easily recrystallized from aniline or chlorobenzene; the analytical sample melted at 311°.

Anal. Calc'd for $C_{28}H_{22}N_2O_4$: C, 74.7; H, 4.9.
Found: C, 74.9; H, 5.1.

Substances of identical appearance were equally easily secured by substituting *ortho-* and *meta*-toluidines in this procedure.

3. *Sulfonation; the dye, Toluidine Green.* To a solution of 2.7 g. of the above dye base in 27 g. of concentrated sulfuric acid, 17 g. of 60% oleum was added with cooling and stirring. After stirring for three hours at room temperature, the free acid was isolated by pouring upon ice. The sodium salt was then prepared by the same procedure as described under Toluidine Blue.

Toluidine Green could be secured equally well by the use of concentrated sulfuric acid alone, on the steam-bath, essentially as outlined under Toluidine Blue.

III. 1,4-DI-β-SULFATOETHYLAMINO-5,8-DIHYDROXYANTHRAQUINONE SERIES

A. *Degradation by oxidative hydrolysis.* When 5,8-di-β-hydroxyethylaminoquinizarin in a large volume of hot 50% acetic acid was treated with nitric acid, the solution rapidly became red. The flocculent precipitate that separated was collected and found to be 1,4,5,8-tetrahydroxyanthraquinone by a comparison of its properties (including the tetraacetate) with those of the synthetic material.

B. *Synthesis.*

1. *5,8-Di(β-hydroxyethylamino)quinizarin.* A solution of 4.5 g. of 5,8-dibromoquinizarin (m.p. 230–231°), 4.5 g. of ethanolamine, and 18 cc. of pyridine was refluxed with stirring for one hour; the solution became deep blue after a short time. After cooling to about 60°, the solution was poured into dilute hydrochloric acid. The precipitated rather gummy solid was filtered, washed with water, and dried by heating on the steam-bath. It was recrystallized from 20 cc. of boiling aniline, from which it separated in dark blue shining needles, m.p. 215–220° (with sublimation). The recrystallized material is quite soluble, with a blue-green color, in dioxane, methanol, ethanol, and acetone. 5,8-Di-(β-hydroxyethylamino)quinizarin has been made previously from leuco-1,4,5,8-tetrahydroxyanthraquinone (19).

Anal. Calc'd for $C_{18}H_{18}N_2O_6$: C 60.3; H, 5.0; N, 7.8.
Found: C, 60.6; H, 5.3; N, 8.1.

2. *Sulfation.* A solution of 10 g. of 5,8-di-(β-hydroxyethylamino)quinizarin in 10 cc. of concentrated sulfuric acid was warmed with stirring at 50–60° for thirty-five minutes. On cooling to room temperature, the viscous solution was mixed with ice

and was diluted to 300 cc. with cold water. Then, quite slowly, a solution of 14.1 g. of sodium hydroxide in 100 cc. of water was added to neutralize the sulfuric acid. The solution was evaporated to dryness on the steam-bath. The dye was then extracted in a Soxhlet apparatus with methyl alcohol for a hundred and forty-two hours. The yield was 6.2 g. (52.3%).

IV. DIBROMINATION OF PHTHALIC ANHYDRIDE

The treatment of phthalic anhydride with two equivalents of bromine leads to a mixture, as would be anticipated (12, 26, 27, 28, 29, 30). The principal product is said to be the 4,5-dibromophthalic anhydride (26, 27, 28, 29, 32), which is isolated by recrystallizing the reaction product from water. In one instance (30) it is recorded that, *once only*, 3,4-dibromophthalic acid was secured. The 3,6-isomer has never been so obtained, but only by oxidation of a ring compound (31).

When an attempt was made to prepare 4,5-dibromophthalic anhydride according to the literature, the product did not crystallize from water. A solid anhydride was readily secured, however, by the use of acetic acid; this proved to be the 3,6-isomer, never before isolated from the bromination mixture. Confirmatory evidence that the compound was 3,6-dibromophthalic anhydride was secured by the synthesis of Toluidine Green, described above, and by finding that the compound would not condense with hydroquinone in the presence of concentrated sulfuric acid. According to the patent literature (33), the last reaction is characteristic of phthalic anhydrides substituted in the 3 and 6 positions; all others condense readily. Since the anhydride has been obtained many times and by five different operators by the use of acetic acid, this result is not an accident.

After the isolation of the 3,6-anhydride, the acetic acid filtrate was concentrated nearly to dryness, and a portion dissolved in hot water. A considerable amount of 3,4-dibromophthalic acid separated from the cold solution. Another portion was taken up in acetic anhydride; some of the 4,5-anhydride crystallized.

Since this work was completed, it was reported (32) that a similar inability to isolate the 4,5-dibromophthalic acid had been encountered. These authors devised a procedure in which the desired acid was separated as a double salt, after partial neutralization, and isolated as the ester. On following their procedure, it was found that a mixture of 3,4- and 4,5-esters was secured; they were not readily separated, although some of the latter isomer crystallized out first.

The method of bromination outlined in the patent literature (12) was followed. The quantitative separation of the isomers has not been worked out, and the yields given only represent the amount of products readily isolated.

A. *3,6-Dibromophthalic anhydride series.* Starting with 400 g. of phthalic anhydride, 1200 g. of 60% oleum, 520 g. of bromine, and 2 g. of iodine, at about 60°, the

yield of 3,6-dibromophthalic anhydride, after recrystallizing three times from acetic acid, was 130–150 g. (m.p. 208–210°). Since the 4,5-isomer has a melting point in the same range, mixed melting points are essential for distinguishing between the two. The analytical sample was recrystallized to constant melting point (212–214°) from acetic acid.

Anal. Calc'd for $C_8H_2Br_2O_3$: Br, 52.3. Found: Br, 52.3.

This anhydride was also isolated during the various fractional crystallizations of mixtures.

B. *3,4-Dibromophthalic acid series.* When water was used, as suggested in the patent, sixteen days elapsed before the first solid (17 g.) crude 3,4-dibromophthalic acid separated. After four recrystallizations from water, it melted at 197° with decomposition.

Anal. Calc'd for $C_8H_4Br_2O_4$: Br, 49.4. Found: Br, 49.1.

It was more readily secured, (50 g.) from the filtrate obtained in A, by removing as much of the acetic acid as possible on the steam-bath and dissolving the residue in the minimum of hot water.

The acid was condensed with hydroquinone in a sodium-aluminum chloride melt to give 5,6-dibromoquinizarin. The new substance separated from chlorobenzene in reddish-brown needles, m.p. 227°.

Anal. Calc'd for $C_{14}H_6Br_2O_4$: Br, 40.2. Found: Br, 40.1.

The methyl ester was secured by the customary procedure. It separated from methanol in short rods, m.p. 79°. A mixed melting point with the isomeric 4,5-dibromo ester (m.p. 81°) was 55–65°.

Anal. Calc'd for $C_{10}H_8Br_2O_4$: C, 34.1; H, 2.3.
Found: C, 34.0; H, 2.2.

The acid is regenerated without difficulty on hydrolysis.

C. *4,5-Dibromophthalic acid series.* When the acetic acid filtrate from A was concentrated nearly to dryness on the steam-bath, and the residue dissolved in 500 cc. of hot acetic anhydride, a brown solution resulted. On cooling, the anhydride separated; after one recrystallization from acetic acid, it amounted to 40 g. The melting point was 212–214°; a mixture of this and the 3,6-isomer melted over the range 175–190°.

When the acid double salt procedure (32) was followed, the yield of methyl 4,5-dibromophthalate was 27 g. from 400 g. of phthalic anhydride. The double salt is a mixture, containing both the 4,5- and 3,4-dibromophthalic acids for, on esterification, both the esters result. The mixture is not readily separated, though some of the 4,5-ester crystallizes first. The residue of mixed crystals (m.p. 55–60°) is unaffected by repeated recrystallizations from methanol or petroleum ether.

6,7-Dibromoquinizarin was prepared in the same way as the other homologs. It also separated as red needles from xylene, m.p. 296–298°.

Anal. Calc'd for $C_{14}H_6Br_2O_4$: Br, 40.2. Found: Br, 40.0

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SUMMARY

1. The structures of the isomeric dyes Toluidine Blue and Toluidine Green have been determined by degradation and synthesis. Toluidine Blue is the disodium salt of the 2′-disulfonic acid of 1,5-di-4′-toluidino-

4,8-dihydroxyanthraquinone, while in Toluidine Green the groups are in the 1,4 and 5,8 positions, respectively.

2. The usefulness of oxidative and reductive hydrolysis has been pointed out.

3. A new, unambiguous method of synthesis of 1,4,5,8-tetrahydroxyanthraquinone has been given.

4. The dibromination of phthalic anhydride has been described. Three of the four possible isomeric dibromophthalic acids have been secured.

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(7) German Patent 89,090, *Frdl.*, **4**, 314 (1894–1897), describes a blue substance without orienting the groups; German Patent 136,778, *Frdl.*, **6**, 381 (1900–1902), uses a 1,5-di-*p*-toluidino-4,8-dihydroxyanthraquinone which, they say, is obtainable from German Patent 89,090.
(8) German Patent 101,806; *Frdl.*, **5**, 296 (1897–1900); orientation not given.
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