ucts. In this way a number of hydroxyl derivatives have been prepared by new methods, in particular 3-hydroxyphthalic acid. The anhydride of this substance has been obtained in much purer condition than samples previously recorded, and several of its derivatives prepared.

(V) The anils of the new iodinated phthalic acids have been prepared and one of them found to exhibit dimorphism.

(VI) Iodine in the nucleus of phthalic acid does not react smoothly with aniline, but a small yield of 3,6-dianilino-4,5-dichlorophthalanil has been obtained from the corresponding iodinated derivative.

(VII) A spectroscopic study of the new iodinated phthalic acids will be reported in a later paper.

PITTSBURGH, PA.

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY, UNIVERSITY OF PITTSBURGH.]

PHTHALIC ACID DERIVATIVES CONSTITUTION AND COLOR. VIII.[1] TETRAIODOFLUORESCEIN AND SOME OF ITS DERIVATIVES.

BY DAVID S. PRATT AND ARTHUR B. COLEMAN.

Received October 17, 1917.

A large and important class of dyes resulting from introducing chlorine into the phthalic acid residue of fluorescein with or without halogens in the resorcin portions of the molecule is recognized at the present time. Among these might be mentioned eosin, erythrosin, phloxine and a variety of their chorine derivatives. Some are especially valuable as stains and indica tors, others for dyeing silks delicate shades of red and pink.

Tetraiodofluorescein, or fluorescein with four atoms of iodine substituted in the phthalic anhydride molecule, had never been prepared. The substitution of iodine in such a colored substance, especially iodine in large amount, might be expected to show some interesting results. As is well known the effect of substituting halogen for hydrogen in many compounds especially dyes, is frequently to increase the period of molecular vibration, or, in other words, to shift the absorption of light toward the longer wave lengths. This modifies the shade of color and might result in producing desirable new dyestuffs.

Iodine is an unsaturated atom possessing considerable residual affinity and chemical activity in excess of that shown by other halogens. This activity should result in various proximity effects modifying the tendency to produce a lactone ring and altering the equilibrium between benzenoid and quinoid condition depending upon this ring. It was also with the view of obtaining information relating color and chemical constitution that we undertook the study of these derivatives.

Fluorescein itself and its related compounds exist in either of two isomeric

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[1] THIS JOURNAL, 40, 198 (1918).

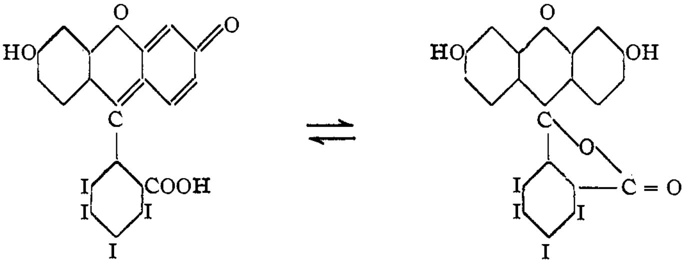
forms, the benzonoid without marked color or the quinoid with vivid color and more or less fluorescence. Fluorescein is known only in the colored condition and undoubtedly is correctly portrayed by the latter structure regardless of the question of intra-molecular interaction between quinoid and benzenoid rings which probably is responsible for its intense color.

It has already been shown that four chlorine atoms in the phthalic acid part of the molecule alter this equilibrium.[1] It was expected that iodine substitution would show even more important effects. An indication of this is evident when colorless phthalic or tetrachlorophthalic anhydride is compared with yellow tetraiodophthalic anhydride. The effect is also evident when one remembers that phthalic acid loses water with some difficulty to form its anhydride whereas tetraiodophthalic acid requires only brief heating to accomplish the same conversion. We have carried this substitution up to eight atoms in making the octaiodofluorescein.

It was also our intention to study the absorption spectra of the entire series with the most improved absorption apparatus, but war conditions made it impossible for Adam Hilger, London, to construct the necessary equipment. This portion of the work will be reported at a later date.

Tetraiodofluorescein was prepared from tetraiodophthalic anhydride[2] in a number of ways. It dissolves in alkaline solutions with a yellowish red color and vivid green fluorescence closely resembling that given by the parent substance. Acidification causes the precipitation of a golden yellow hydrate, soluble in various organic solvents and fairly stable at room temperature. The hydrate loses water when heated and at the same time changes from golden yellow to reddish brown.

Tetraiodofluorescein is entirely different from ordinary fluorescein in that it lies on the border line of equilibrium between the benzenoid and the quinoid form, with the latter probably metastable at ordinary temperatures. This equilibrium is represented by:



Quinoid. Benzenoid.

Ready means of passing either way have been found. It is quite probable that the hydrate is a derivative of the second structure but it shows certain reactions which differentiate it from other similar hydrates described in the

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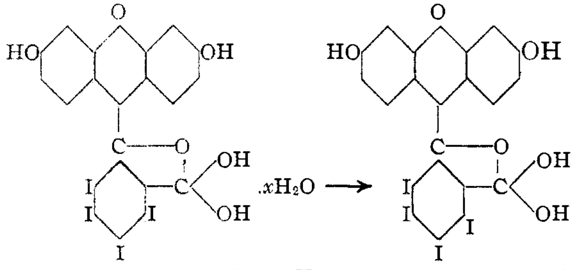
[1] Orndorff and Hitch, This Journal, 36, 680 (1914).

[2] See Pratt and Shupp, *Ibid.*, **40**, 254 (1918).

literature. When freshly precipitated by acidification of a dilute solution in alkali, the hydrate undoubtedly contains water attached to the molecule in two distinct ways. An unknown per cent. of water of constitution is lost rapidly at room temperature as the moist hydrate dries. It was not found possible to obtain analytical data on this point since the hydrate is amorphous and dries slowly. During this loss of water the color remains unchanged. The product attained constant weight within a few days and still possessed a golden yellow color. When it was heated at 120° in an oven it slowly lost weight corresponding approximately to one molecule of water, and the color darkened somewhat to a reddish brown.

We shall speak of the original hydrate as hydrous tetraiodofluorescein hydrate, the air-dried product as hydrous tetraiodofluorescein, and the final material as anhydrous tetraiodofluorescein. The three forms show distinct reactions serving to differentiate them sharply from each other. The loss of water at room temperature takes place so readily that it probably represents loss of water held loosely by molecular combination. Partial loss of this water can be brought about by boiling the hydrate suspended in water. The water still held by the hydrous tetraiodofluorescein is retained more firmly, so much so in fact that it is driven off with difficulty even at 120°. This molecule is best represented as water of constitution, and its presence in the compound explained by the action of the four negative iodine atoms in much the same way as in chloral hydrate. These forms may be represented as:

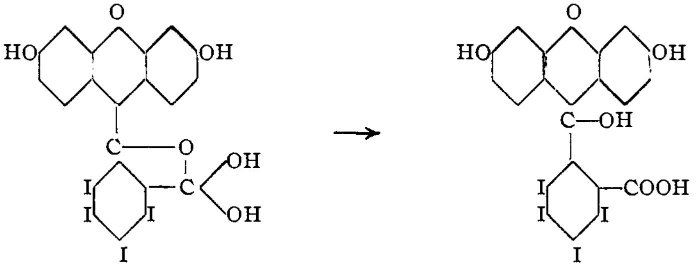


Hydrous tetraiodofluorescein hydrate (yellow).   Hydrous tetraiodofluorescein (yellow).

Heating hydrous tetraiodofluorescein at 120° for sufficient time to drive off its molecule of water *partially* converts the benzenoid form into the quinoid as is shown by the increase in color. It should be remembered that ordinary fluorescein is deep red and has never been isolated in the colorless benzenoid condition except in the form of certain derivatives such as acetate, benzoate, etc. The conversion to *quinoid* form is only partial in the case of the tetraiodofluorescein and even this change represents the formation of a metastable condition.

A drop of acetone, alcohol, or ethyl acetate applied to the reddish brown anhydrous tetraiodofluorescein causes an immediate change to clear canary-yellow, probably because some of the compound dissolves and in solution readily rearranges to the stable form. There is also a loose molecular addition product formed between tetraiodofluorescein and solvent, especially in the case of ethyl acetate, but the solvent of crystallization is readily driven off by gentle warming. This gives an excellent means of obtaining benzenoid tetraiodofluorescein free from the colored quinoid isomer. The benzenoid form is yellow instead of colorless simply because the parent tetraiodophthalic anhydride is this color.

When hydrous tetraiodofluorescein is treated with a little methyl or ethyl alcohol it immediately turns nearly colorless forming the carbinol carboxylic acid. This results easily from a slight rearrangement of the molecule as indicated by the structures



Hydrous tetraiodofluorescein (golden yellow).

Tetraiodofluorescein carbinol carboxylic acid (nearly colorless).

The carbinol carboxylic acid may be obtained in the form of faintly yellow, well-formed crystals by slow evaporation of a methyl alcohol solution. It differs in this respect from both the isomeric hydrous tetraiodofluorescein and tetraiodofluorescein, neither of which have ever been obtained crystalline.

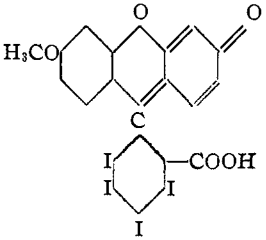
The carbinol acid loses a molecule of water on heating at 100° and gives the yellow benzenoid tetraiodofluorescein. Further heating at 180° for only five minutes carries the equilibrium toward the quinoid form with its reddish brown color but without change in weight. The carbinol acid gives the same diacetate with acetic anhydride as the hydrous or anhydrous compound.

Anhydrous tetraiodofluorescein gives a reddish orange monopotassium salt when an alcoholic solution is heated with alcoholic potassium acetate. The salt crystallizes rather well from alcohol and is difficultly soluble in water, giving, however, a fluorescent solution. The red-orange color changes to deep red when caustic alkali is added, and the more readily soluble dipotassium salt is formed. The structure of the monopotassium salt is doubtless the usual quinoid one.

Dry ammonia gas acts on anhydrous tetraiodofluorescein rapidly giving a deep red diammonium salt, difficultly soluble in cold water, readily on heating. It is quite stable when exposed to the air, but loses all its ammonia on heating at 100°, giving again the red anhydrous tetraiodofluorescein.

The monosilver salt was obtained in the form of an amorphous violet-red powder. It was hoped that this silver salt, which undoubtedly also has the structure assigned to the monopotassium salt, would react with alkyl iodides to give corresponding quinoid esters, but this could not be accomplished. Some reaction took place but no pure ester could be isolated.

The monomethyl ether of tetraiodofluorescein shows a very brilliant red color indicating a quinoid structure represented by



The ether is very slightly soluble with fluorescence in dilute sodium hydroxide, more so in concentrated solution. Its brilliant color is rather remarkable.

Tetraiodofluorescein diacetate is easily made and obtained from acetone as yellow crystals. So few of the derivatives are crystalline that the acetate offers the best means for purifying tetraiodofluorescein. The clear yellow color shows its structure to be benzenoid. The dibenzoate represents an analogous derivative, also giving pale yellow crystals.

Experimental.

Tetraiodofluorescein.—652 g. (1 mol) of tetraiodophthalic anhydride were intimately mixed with 216 g. (2.1 mols) of colorless resorcin. 216 g. of finely powdered freshly fused zinc chloride were then added, the mixture placed in a roomy casserole and heated in an oil bath to 200°. The material was frequently stirred until after about two hours it became too pasty. The temperature was maintained about four hours at 200–210°, during which time the color darkened to almost black.

The melt was finely powdered and extracted with boiling dilute hydrochloric acid until free from zinc salts. This changed the color to canary-yellow. The tetraiodofluorescein was then dissolved out with successive small portions of five per cent. sodium hydroxide, the solution diluted to three liters and precipitated by adding three per cent. hydrochloric acid while thoroughly stirring with a blast of air. The hydrate separated in a yellow amorphous form which was washed by decantation, filtered and dried at 120°. Yield of impure product, 95% theoretical.

Phosphorus pentoxide was tried as a condensing agent, but the reaction was too vigorous, the temperature rose to about 230° and the yield was very small. Some condensing agent, however, must be used as a trial fusion of anhydride and resorcin alone gave only a five per cent. yield, the greater part of the former remaining unchanged.

The purification of tetraiodofluorescein presented many difficulties. 100 g. of crude material obtained as above were finely ground and extracted with two liters of anhydrous ether leaving 26 g. of gray insoluble material. The tetraiodofluorescein was removed from the ether by shaking with five per cent. sodium hydroxide solution until no color was imparted to the alkali. The addition of dilute acid to this extract gave a copious precipitate of light pink hydrate which was washed free from chlorides, and dried to constant weight at 120°. Yield, 73 g. The method is tedious and the resulting product not quite pure as shown by analysis:

Subst. 0.3019; cc. 0.1 *N* $AgNO_3$, 12.90.
Calc. for $C_{20}H_8O_5I_4$: I, 60.49. Found: 54.23.

This analysis is given as representing the degree of purity which may be expected from this method of purification.

The second method used was based upon obtaining pure diacetate which was then saponified to pure fluorescein. 100 g. of crude material as above were finely powdered and suspended in 300 cc. of acetic anhydride in a roomy flask. 10 g. of freshly fused sodium acetate were added, and the mixture heated 30 minutes in a water bath. The temperature was then raised sufficiently to cause gentle boiling, and maintained for one hour, at the same time taking care that no material adhered to the flask and thereby became charred.

The reaction is complete when all the dark-colored tetraiodofluorescein has changed to the yellow diacetate. The mixture was poured after cooling into hot water, and the acetate carefully washed, dried at 110°, then ground to a fine powder and rewashed with hot water. It was dried again and dissolved in acetone giving a dark red-colored solution. This was boiled with successive portions of boneblack for a period of five days when the color became amber-yellow. Concentration to one-fourth its volume and cooling gave the acetate in yellow crystals. These were recrystallized several times from acetone until pure as shown by analysis. The lime combustion method was used.

Subst. (I) 0.2847, (II) 0.4232; cc. 0.1 *N* $AgNO_3$, (I) 12.40, (II) 18.42.
Calc. for $C_{24}H_{12}O_7I_4$: I, 55.20. Found: (I) 55.28, (II) 55.23.

The crystalline diacetate is attacked very slowly by aqueous alkali but alcoholic alkali cannot be used as it removes iodine. The acetate is best saponified by first dissolving it in acetone and pouring this solution into a dilute alkaline solution. The result is a flocculent, amorphous form of the acetate which is much more readily attacked. 100 g. of pure diacetate were added in this way to a liter of 5% aqueous sodium hydroxide solution and the suspension stirred mechanically until complete solution was effected. Acidification of the red highly fluorescent alkaline solution caused the hydrated tetraiodofluorescein to precipitate as a canary-yellow jelly. This was washed six times by decantation using large amounts of water, vigorous stirring, followed by settling. The material was then filtered, washed with hot water until free from chlorides, and dried to constant weight of 120°. This gave anhydrous fluorescein.

Subst. (I) 0.3910, (II) 0.2570; cc. 0.1 N $AgNO_3$, (I) 18.78, (II) 12.21.
Calc. for $C_{20}H_8O_5I_4$: I, 60.49. Found: (I) 60.96, (II) 60.29.

The yield was about 50% pure product calculated on the basis of crude material. The red anhydride treated with acetone, ether, or alcohol changes at once into a canary-yellow form.

Hydrous Tetraiodofluorescein.—The canary-yellow material obtained as above was air-dried instead of at 120° without marked darkening in color. It reached constant weight after 30 days whereupon it was heated at 110°. The loss in weight corresponded approximately to one molecule of water and the color changed to the reddish brown of anhydrous tetraiodofluorescein.

Subst. 3.8512 g. loss 0.0526 g.
Calc. for $C_{20}H_8O_5I_4.H_2O$: H_2O, 2.11. Found: 1.36.

Hydrous tetraiodofluorescein is readily soluble in ether giving a yellow solution which deposits a light yellow product on evaporation. A drop of ether applied to the hydrate does not appreciably change its color as it does in the case of the anhydrous material. It gives no color in the cold with dimethylaniline.

Tetraiodofluorescein Carbinol Carboxylic Acid.—Hydrous tetraiodofluorescein was treated with methyl alcohol which caused the color to change at once to a very pale yellow. The resulting carboxylic acid was dissolved in methyl alcohol and obtained upon concentrating as a light yellow, amorphous substance. Very slow concentration by evaporation gave pale yellow, well-formed crystals of the same compound.

An air-dried sample lost weight on heating at 100° corresponding approximately to one molecule of water.

Subst. (I) 0.6212, loss 0.0231 g. at 100°.
Calc. for $C_{20}H_{10}O_6I_4$: H_2O, 2.06. Found: 3.71.

During drying the color deepened to that of benzenoid tetraiodofluorescein. Further heating for five minutes at 170-180° caused the appearance of the reddish brown color characteristic of the quinoid product but with no change in weight. The heating at 170° was continued for 4 hours but there was no loss of weight.

Diammonium Salt.—Dry ammonia gas passed over weighed amounts of anhydrous tetraiodofluorescein gave this salt. Constant weight was reached within 12 hours.

Subst. 0.3995 g., gain in weight 0.0176 g.
Calc. for $C_{20}H_8O_5I_4.2NH_3$: NH_3, 3.91. Found: 4.21.

The red diammonium salt loses its ammonia easily at 100° giving the original reddish brown tetraiodofluorescein.

Subst. 0.7434 g., loss in weight 0.0286 g.
Calc. for $C_{20}H_8O_5I_4.2NH_3$: NH_3, 3.91. Found: 3.84.

Tetraiodofluorescein Dibenzoate.—The dibenzoate was prepared both by the Baumann-Schotten reaction and by boiling anhydrous tetraiodofluorescein in benzoyl chloride. The resulting product was the dibenzoate in each case although it was thought probable that the former method would give a monobenzoate.

10 g. of tetraiodofluorescein were dissolved in 200 cc. of 5% sodium hydroxide solution and treated with successive portions of benzoyl chloride until the red color disappeared. The crude benzoate was washed with dilute warm alkaline solution, followed by warm water, and crystallized several times from chloroform in the form of pale yellow crystals. The yield of crude dibenzoate is nearly theoretical, that of pure product about 30%.

In the second method of preparation 10 g. of tetraiodofluorescein were suspended in 75 cc. of benzoyl chloride. This was gently boiled for 30 minutes and poured into alcohol which separated the benzoate as a grayish green amorphous mass. This was filtered off, washed with hot water and dried. The solution in chloroform was boiled with boneblack until almost colorless and the benzoate finally recrystallized from acetone. It melted at 288° (corr.) and showed no depression of the melting point when mixed with the previous product. They are identical.

Subst. (I) 0.2362, (II) 0.2282; cc. 0.1 *N* $AgNO_3$, (I) 9.03, (II) 8.80.
Calc. for $C_{34}H_{16}O_7I_4$: I, 48.63. Found: (I) 48.52, (II) 48.94.

Monopotassium Salt.—In the preparation of this salt 5 g. of tetraiodofluorescein were dissolved in 150 cc. of absolute ethyl alcohol, giving an amber-colored solution. To this was added an alcoholic solution containing 2 g. of anhydrous potassium acetate, both solutions being hot when mixed. The color changed at once to red and the salt began to deposit in shining, red crystals. It was recrystallized from alcohol in which it is very sparingly soluble. Yield, about 50 %.

Subst. (I) 0.2137; cc. 0.1 *N* $AgNO_3$, 9.84.
Calc. for $C_{20}H_7O_5I_4K$: I, 58.16. Found: 58.44.

The dipotassium salt is much more soluble in water and difficult to purify.

Tetraiodofluorescein Monomethyl Ether.—Dimethyl sulfate was added in small portions with vigorous shaking to a weakly alkaline solution of tetraiodofluorescein. Dilute alkali was added from time to time to replace that used up by the dimethyl sulfate and to keep the solution from becoming acid. It was found to be advantageous to shake vigorously as this gave a more finely divided product easier to purify. The solution became less and less red, and its green fluorescence almost entirely disappeared. The resulting ether separated as an insoluble, amorphous, fiery red mass which was filtered off, washed, dried and finely powdered. It was extracted with two per cent. sodium hydroxide solution until the filtrate gave no precipitate on acidifying, and finally washed with water and dried.

The bright red powder was quite soluble in ethyl alcohol, more so in methyl alcohol and acetone, but all attempts to crystallize it failed. Purification was finally accomplished by repeatedly dissolving in methyl alcohol and partially separating by concentrating the solvent. The ether was finally treated with strong potassium hydroxide solution to saponify any methyl ester present and repurified from methyl alcohol. This procedure gave a brick red product slightly soluble with fluorescence in strong alkali. It decomposed on heating at about 251°.

Subst. (I) 0.3491, (II) 0.1700; cc. 0.1 *N* $AgNO_3$, (I) 16.26, (II) 8.05.
Calc. for $C_{21}H_{10}O_5I_4$: I, 59.74. Found: (I) 59.15, (II) 60.10.

Methoxyl was determined by the Zeissel method.

Subst. (I) 0.3254, (II) 0.2327; g. AgI, (I) 0.0945, (II) 0.0740.
Calc. for $C_{21}H_{10}O_5I_4$: CH_3O, 3.65. Found: (I) 3.84, (II) 4.20.

Silver Salt.—5 g. of pure tetraiodofluorescein were dissolved in 10 cc. of concentrated ammonium hydroxide and the solution diluted to 400 cc. A 10% silver nitrate solution containing a slight excess of silver was then added. The color changed at once to a deeper red but no precipitation occurred until the excess of ammonia was boiled off. The silver salt gradually deposited during the boiling as a violet-red powder which was filtered off, washed with water, finally boiled with water for 30 minutes, and washed until the filtrate gave only a slight test for silver. It was not possible to remove all adsorbed silver as the salt could not be obtained crystalline. The product dried at 120° was analyzed as follows:

A weighed sample was suspended in 25 cc. of six normal nitric acid and allowed to stand about 12 hours at room temperature. The solution was diluted to 400 cc., filtered, and the silver nitrate titrated with standard ammonium thiocyanate using ferric alum as indicator.

Subst. (I) 0.1431, (II) 0.3918; cc. 0.1 *N* NH_4SCN, (I) 1.21, (II) 4.23.
Calc. for $C_{20}H_7O_5I_4Ag$: Ag, 11.44. Found: (I) 11.48, (II) 11.65.

Summary.

1. Tetraiodofluorescein has been prepared. It exists at ordinary temperatures as an unstable mixture of benzenoid and quinoid modifications.

2. The yellow hydrate loses a part of its water readily to give a yellow hydrous derivative. This then loses a molecule of water on heating to give anhydrous tetraiodofluorescein.

3. Alcohol acting on the hydrate converts it into the pale yellow carbinol-carboxylic acid.
4. Acetone on the anhydrous material gives a lemon yellow acetonate which loses acetone to give benzenoid tetraiodofluorescein.
5. Benzenoid tetraiodofluorescein rapidly rearranges into the red quinoid form at 180°.
6. The monopotassium salt was prepared in reddish orange crystals.
7. Dry ammonia on tetraiodofluorescein gives a deep red salt with gain in weight corresponding to a diammonium salt.
8. The red silver salt and monomethyl ether are characteristic of the quinoid form, while the yellow diacetate and dibenzoate, the yellow tetraiodofluorescein and carbinol acid characterize the benzenoid type.
9. Absorption spectra of the entire series will be reported in a later paper.

PITTSBURGH, PA.

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY, UNIVERSITY OF PITTSBURGH.]

PHTHALIC ACID DERIVATIVES; CONSTITUTION AND COLOR, IX.[1] TETRAIODOEOSIN AND SOME OF ITS DERIVATIVES.

BY DAVID S. PRATT AND ARTHUR B. COLEMAN.

Received October 17, 1917.

Tetraiodofluorescein[2] in the anhydrous condition readily takes up four atoms of bromine, replacing hydrogen in the two resorcin residues. For this purpose 10 g. were suspended in 500 cc. of glacial acetic acid containing 10 cc. of bromine and the mixture boiled gently for an hour. The reddish brown tetraiodofluorescein gradually changed to a yellow crystalline substance. After cooling this was filtered off and washed with 40% alcohol until the filtrate gave no test for halogen. The product was heated to constant weight at 105° and found to be pure tetraiodoeosin. Yield, theoretical.

The material was analyzed by the lime combustion method, filtering off all acid-insoluble material before adding silver nitrate. Titration values and weight of combined silver halides gave the necessary data:

Subst. (I) 0.3946, (II) 0.4656; cc. 0.1 *N* $AgNO_3$, (I) 26.86, (II) 29.10. Weight AgI + AgBr, (I) 0.5674, (II) 0.6468.

Calc. for $C_{20}H_4O_5Br_4I_4$: Br, 27.76; I, 44.09. Found: (I) Br, 27.19, I, 43.19, (II) Br, 27.63; I, 43.88.

Tetraiodoeosin forms canary-yellow crystals which show no tendency whatever to go over into the highly colored quinoid form. It should be represented, therefore, by the structure

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[1] THIS JOURNAL, 40, 198 (1918).

[2] *Ibid.*, 40, 236 (1918).