at 90–92°. It may be purified by crystallization from alcohol, from which the chloroimide separates in the form of square prisms that melt at 93–94°. From ligroin (b. p. 70–80°), it gives crystals of the same shape, melting at the same temperature. It decomposes suddenly, with charring, when heated above 176°.

0.3443 g. subs. gave 0.6079 g. AgHal. Calc. for $C_7H_4ONCl_2Br$: Halogen, 56.12; found: 56.14.

The mother liquor, left after crystallization of the above described chloroimidoquinone, was evaporated to a small volume and allowed to cool. The solid that separated melted at 111–113°, which showed that this material could not be the chloroimide. Further crystallization from alcohol raised the m. p. to 114–115°, but the small quantity of material available made it impossible to secure a product that was entirely pure. The substance crystallized in the form of the irregular scales characteristic of 2-chloro-6-bromo-3-methylquinone described above. A mixture of a pure sample of the latter, m. p. 119°, and the product here in question, melted at 115–119°, showing no depression. When the substance to be identified was mixed with a pure sample of 2-chloro-6-bromo-3-methyl-4-chloroimidoquinone, m. p. 93–94°, both of which were produced in the same experiment, the mixture melted at 75–86°. Analysis for halogen gave too high a value, which suggests a trace of a higher halogenated product.

0.0785 g. subs. gave 0.1130 g. AgHal. Calc. for $C_7H_4O_2ClBr$: Halogen, 49.03; found: 50.06.

Summary.

1. When 4-nitro-*m*-cresol is chlorinated by passing pure or diluted chlorine into an acetic acid solution of the substance, a mixture of 2-chloro-4-nitro-*m*-cresol and 6-chloro-4-nitro-*m*-cresol, in which the first is present in largest amount, is formed.

2. When 4-nitro-*m*-cresol is chlorinated by means of a mixture giving so-called nascent chlorine, nothing but 2,6-dichloro-4-nitro-*m*-cresol is obtained.

CHICAGO, ILL.

[CONTRIBUTIONS FROM THE CHEMICAL LABORATORY OF CORNELL UNIVERSITY.]

TETRACHLOROFLUORESCEIN AND SOME OF ITS DERIVATIVES.

BY W. R. ORNDORFF AND E. F. HITCH.

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Historical.

Tetrachlorofluorescein was first prepared by Graebe[1] in the course of his investigation of tetrachlorophthalic acid. It was made by heating

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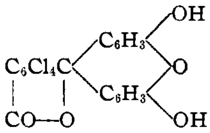
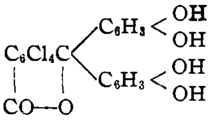
[1] *Ann. der Chemie*, **238**, 333 (1887).

to 200° thirteen parts of tetrachlorophthalic acid anhydride with ten parts of resorcinol for 2 or 3 hours. The red fused mass was thoroughly extracted with boiling water, and there was left a substance which had the same yellowish red color as fluorescein. To this compound he assigned the formula:



Graebe states that this compound, when dissolved in ammonia or sodium carbonate, is precipitated practically unchanged from these solutions by an acid, and it makes no difference whether the solution be made by warming or at ordinary temperature. When, however, the tetrachlorofluorescein was dissolved in caustic soda, either cold or hot, and then precipitated by an acid, a product was obtained containing one molecule of water more than the tetrachlorofluorescein itself, which did not lose this molecule of water even when heated for some time to 100°, and also differed in its properties from tetrachlorofluorescein. This compound, which he calls tetrachloro-orthofluorescein, or tetrachlorofluorescein hydrate, is given the following formula:



and is regarded by Graebe as derived from the unstable form of fluorescein having the composition $C_{20}H_{14}O_6$, which Baeyer[1] mentions in his work on the phthaleins. Tetrachloro-orthofluorescein is insoluble in water, only slightly soluble in alcohol but easily soluble in ether. From the last solvent it crystallizes in reddish yellow needles. It is precipitated from solution in the alkalies by an acid in the form of pure yellow microscopic needles. It dissolves in alkalies with almost the same color and the same fluorescence as fluorescein, a difference is only perceptible when the solutions are observed in very thin layers, the solution of tetrachloro-orthofluorescein then appears red, while that of fluorescein is yellow. Tetrachloro-orthofluorescein is not changed by boiling with water, hence it is obtained having the same composition, no matter whether the alkaline solution be precipitated hot or cold. The air-dried compound loses no weight at 100°, even at 150–160° it scarcely changes. When heated, however, to 180–200° it turns red and goes over into tetrachlorofluorescein. Analyses of the tetrachloro-orthofluorescein dried at 100° are given, which agree very well with the formula $C_{20}Cl_4H_{10}O_6$. When heated with

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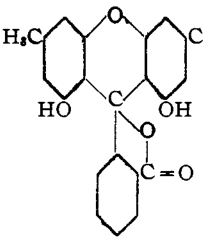
[1] *Ann.*, 183, 5 (1876).

acetic anhydride it gives the same diacetyltetrachlorofluorescein as tetrachlorofluorescein itself.

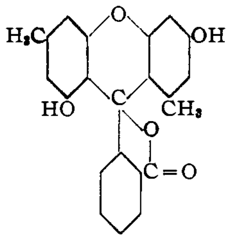
Tetrachlorofluorescein is the product which is formed on heating tetrachlorophthalic acid anhydride with resorcinol. If the fused mass be extracted with ammonia or sodium carbonate and this solution be acidified, tetrachlorofluorescein is precipitated. Longer continued action of a larger excess of ammonia or sodium carbonate results in the formation of some tetrachloro-orthofluorescein also. From this it is formed by heating to 180–200°. It is yellowish red and separates from the above mentioned solutions on the addition of acids in the form of a yellowish red precipitate, while that of the tetrachloro-orthofluorescein is yellow. In ether tetrachlorofluorescein is practically insoluble, and in this respect it differs from the compound containing a molecule of water (tetrachloro-orthofluorescein). Two chlorine analyses of the tetrachlorofluorescein dried at 100° are given and these agree very well with the formula, $C_{20}Cl_4H_8O_5$. Analyses are also given of the diacetyl derivative of tetrachlorofluorescein and of the chloride of tetrachlorofluorescein.

Since this work of Graebe, no further investigation of tetrachlorofluorescein has been made. Recently, however, C. Mettler[1] has published a paper upon dichlorodioxybenzoylbenzoic acid and its conversion into tetrachlorofluorescein and into anthraquinone derivatives. The compound which he calls tetrachlorofluorescein, while isomeric with the tetrachlorofluorescein first prepared by Graebe, and which forms the subject of this investigation, is entirely different in structure. Tetrachlorofluorescein as named by Graebe, is the anhydride of resorcinoltetrachlorophthalein, while the compound prepared by Mettler is analogous to eosin, and is tetrachlororesorcinolphthalein anhydride.

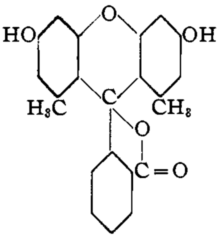
In 1896, R. Meyer and H. Meyer[2] investigated the orcinolphthaleins and found that there are three of them. They represent the structure of these compounds by the formulas:



(I)



(II)



(III)

The third formula is assigned to the isomer which fluoresces in alkaline solution on account of its great resemblance to fluorescein. It is interesting

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[1] *Ber.*, 45, 800 (1912).

[2] *Ibid.*, 29, 2627 (1896).

to note that they make the following statement regarding the possibility of the formation of isomers of fluorescein: "Die Tatsache, dass bei der Condensation von Phtalsäureanhydrid mit Orcin nicht nur ein Phtalein entsteht, sondern die drei structurell möglichen Isomeren nebeneinander, legt die Frage nahe, ob nicht die Bildung des Fluoresceins gleichfalls von der Entstehung der zwei ausser ihm noch denkbaren Resorcinphtaleine begleitet ist. Es liegen bisher, soweit uns bekannt, keine Tatsachen vor, welche einen Anhalt zu einer solchen Vermutung geben könnten. Auch haben einige Versuche, welche dahin zielten, die Fluoresceinbildung unter verschiedenen Bedingungen zu Studiren, stets nur Fluorescein geliefert."

The chlorinated eosins have been known to the manufacturers of dyes for some time, but no record of scientific investigation upon tetrachloroeosin has, up to the present time, been published. In 1885 a German patent was granted to R. Gnehm of the Gesellschaft für Chemische Industrie at Basel for the preparation of the chlorinated phthalic acids to be used exclusively for making eosin dyestuffs containing chlorine. The commercial tetrachloroeosin, known to the trade as Phloxine, is the sodium salt of tetrachloroeosin, and is made from the commercial tetrachlorofluorescein by brominating.

The fluorescence of organic substances, and especially of substances chemically related to fluorescein, has been the subject of much investigation. Most of the researches in this field have been conducted with the object of ascertaining if there exists any relation between fluorescence and chemical constitution, and to determine the exact nature of such relation, if it does exist. Many theories have been advanced by chemists and physicists to explain this phenomenon, and it is generally accepted at the present time that there is a close relation between chemical structure and fluorescence. Chemists have made attempts to formulate a theory that will explain this relationship and account for all the known facts. It is not within the scope of this work to go into a discussion of the various theories of fluorescence, but those who may be interested in this field of investigation will find an excellent review of these theories given by Samuel Smiles in his book entitled "The Relations between Chemical Constitution and Some Physical Properties." The theory of Stark and Meyer and of Kauffmann, that the benzene nucleus is the seat of fluorescence of aromatic organic compounds, is generally accepted by chemists at the present time as the one which more nearly explains the facts. Chemists and physicists are beginning to realize the great importance of studying very weak fluorescence and fluorescence in the ultraviolet portion of the spectrum. Until the complete fluorescence spectra of many more substances are studied, any theory to account for this phenomenon will be inadequate.

An excellent review of the investigations that have been undertaken to determine the relationship which exists between color and constitution of the phthaleins, and other triphenylmethane derivatives, together with a statement of some of the theories advanced to account for the color of this group of compounds, is given by Orndorff and Delbridge[1] in their first paper on "Tetrachlorgallein and Some of Its Derivatives."

This investigation was undertaken for the purpose of determining what influence the introduction of four chlorine atoms into the phthalic acid part of the fluorescein molecule would have upon the chemical conduct and general properties of this phthalein; for the purpose of determining whether any of the isomeric tetrachlorofluoresceins, theoretically possible,[2] are formed; for the purpose of extending our knowledge of colored compounds and of the relation between the color and constitution of organic compounds; and finally, to throw some light, if possible, on the phenomenon of fluorescence.

Experimental.

The resorcinol used in this investigation was obtained from Merck and Company. It was perfectly white, melted at 115–116° (uncorr.), and left no residue on ignition. It dissolved completely to a clear solution in water, in alcohol, and in ether. The tetrachlorophthalic acid was obtained from the Heller and Merz Company, and was purified by the method described by Delbridge.[3] The purity of the acid was checked by determinations of the molecular weight. A weighed portion of the acid was dissolved in a measured amount of tenth-normal sodium hydroxide solution, using about 10% excess. About 0.5 cc. excess of tenth-normal hydrochloric acid was then added, the solution boiled for one minute to expel carbon dioxide, cooled, and the excess of hydrochloric acid titrated with tenth-normal sodium hydroxide, using phenoltetrachlorophthalein as the indicator.[4] The following results were obtained with the purified tetrachlorophthalic acid:[5]

Subst. 0.3890, 0.3322; cc. 0.1 N NaOH, 24.88, 21.22.
Calculated for $C_6Cl_4(CO_2H)_2.0.5H_2O$, mol. wt. 312.9; found, 312.7, 313.1

Tetrachlorofluorescein.

Preparation.—This compound was prepared by four different methods as follows: Fusion of tetrachlorophthalic acid and resorcinol with zinc chloride; fusion of tetrachlorophthalic acid and resorcinol with phosphorus pentoxide; fusion of tetrachlorophthalic acid and resorcinol; fusion of tetrachlorophthalic anhydride and resorcinol. In the first method 313 grams of tetrachlorophthalic acid (1 mol) and 231 grams of

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[1] *Am. Chem. J.*, **42**, 183 (1909).

[2] See page 686.

[3] *Am. Chem. J.*, **41**, 414 (1909).

[4] *Ibid.*, **41**, 349, 401 (1909).

[5] International atomic weights (1914) are used in this paper.

resorcinol (2.1 mols) were thoroughly mixed. To this mixture were then added 150 g. of freshly fused and finely powdered zinc chloride. After thorough grinding in a mortar, the whole was transferred to a two-liter round-bottom flask, and the flask placed in a bath of concentrated sulfuric acid containing 40% potassium sulfate. This bath was then heated to 200–210° for two hours. The mixture softened to a pasty mass and became dark red at the end of one-half hour, but at no time did the mass become completely liquefied, nor did the temperature within the flask rise above that of the bath. At the end of two hours the mass had solidified and the heating was discontinued. After cooling, the flask was broken and the hard, solid cake pulverized and extracted with hot water containing two 2% hydrochloric acid. The crude product thus obtained was dissolved in 5% sodium hydroxide solution, the solution filtered, and an excess of hydrochloric acid added. The mixture was then boiled by blowing in steam, and the red tetrachlorofluorescein filtered off while hot. The material thus obtained was washed free from hydrochloric acid, soluble chlorides, and unchanged tetrachlorophthalic acid, with hot water and dried to constant weight at 110°. This product, contrary to the statement of Graebe (see page 681), is the anhydrous tetrachlorofluorescein. Yield, 88% of the theoretical calculated from the amount of the tetrachlorophthalic acid used.

In the second method, which was conducted in the same manner as the first, 166 g. of phosphorus pentoxide were substituted for the zinc chloride. At the end of fifteen minutes the mass had completely melted to a thin, red liquid which gradually became viscous. This change was accompanied by a considerable rise in temperature within the flask, the thermometer registering as high as 240°, or about 30° above that of the bath. At the end of an hour the mass had solidified. The heating was continued for half an hour longer to complete the reaction. The solid mass was extracted with water in the same manner as before, and washed with hot water until free from phosphoric acid. The crude material was then dissolved in 5% sodium hydroxide solution, filtered, and the filtrate acidified with 5% hydrochloric acid. The mixture was then boiled by blowing in steam, the red anhydrous tetrachlorofluorescein filtered off, washed with hot water, and dried at 110°. Yield, 90%.

In order to determine whether a condensing agent was advantageous, tetrachlorofluorescein was made by the fusion of tetrachlorophthalic acid (1 mol) and resorcinol (2 mols), without any condensing agent. It was found necessary to increase the temperature of the bath to 240°, in order that the reaction should take place readily. The mixture first became yellow, and soon changed to a thin, red liquid. This gradually became viscous, upon further heating, and at the end of an hour was of a pasty consistency. At the end of two hours the mass had completely solidified and the reaction was stopped. It was then extracted and treated in the same manner as in the fusion with phosphorus pentoxide. Yield, 85%.

In the last method of preparation, tetrachlorofluorescein was made from tetrachlorophthalic anhydride and resorcinol, following the directions of Graebe.[1] Tetrachlorophthalic anhydride (1 mol) and resorcinol (2 mols) were thoroughly mixed and heated to a temperature of 200–210°. The material changed within fifteen minutes to a light yellow pasty mass, which at the end of half an hour had become a thin, dark red liquid. This remained unchanged when the heating was continued for two hours at this temperature. The temperature of the bath was then increased to 230°. Under these conditions, the mass became solid upon heating for an hour longer. The solid cake was then extracted and treated in the same manner as before. Yield, 81%.

Purification of Tetrachlorofluorescein.—One hundred grams of the crude tetrachlorofluorescein, prepared by one of the methods previously described were dissolved in one liter of water containing 30 grams of sodium hydroxide. This solution was filtered, diluted to two liters, and poured, with constant stirring, into two liters of a solution containing 250 cc. of hydrochloric acid (sp. gr. 1.10). The orange-yellow precipitate thus obtained was extracted with ether, about two liters being required to complete the solution. A small amount of the precipitate is insoluble in the ether. This insoluble material has a very *dark red* color, and constitutes about 5% of the crude tetrachlorofluorescein. It is perhaps a mixture of the two possible isomers (see p. 723). It dissolves readily in alkalies with a deep red color and an *intense greenish fluorescence*, and is precipitated as a dark red material, probably a hydrate, upon the addition of acids to the dilute alkaline solution. This dark red material is only slightly soluble in ether, but *readily soluble in alcohol*, and hence is different from the red anhydrous tetrachlorofluorescein described below, which is practically insoluble in these solvents. The ether extract, after filtering off this insoluble material, was dried by shaking with fused calcium chloride, one-half of the ether distilled off, and an equal amount of alcohol added. The distillation was continued until orange-yellow crystals began to appear. Upon repeated recrystallization from alcohol, this substance was obtained light yellow in color with a reddish tinge. It was thought that this red color was due to a small amount of the dark red impurity remaining after the extraction with ether.[2] Since repeated crystallization from alcohol, and then from acetone, did not remove this color, the substance was converted into the diacetate by means of acetic anhydride and sodium

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[1] *Ann.*, **238**, 333 (1887).

[2] Later it was shown that the hydrate is partially converted into the red tetrachlorofluorescein by this treatment and a part of this red color was due to this substance.

acetate. This diacetate was crystallized from benzene until a *perfectly white product* was obtained, then saponified with alcoholic caustic potash. The alcoholic solution of the dipotassium salt thus formed was diluted with a large amount of water, and poured, with constant stirring, into an excess of dilute hydrochloric acid. The light yellow precipitate of the hydrate was then filtered off and thoroughly washed with water until free from hydrochloric acid and chlorides. This hydrate was found to be completely soluble in ether, thus showing that it contained none of the dark red material (possibly a mixture of the two isomeric modifications of tetrachlorofluorescein) present in the crude product.

When dried to constant weight at 170° in the electric drying oven[1] this hydrate was converted into a red compound which proved to be pure anhydrous tetrachlorofluorescein as shown by the following chlorine determinations:[2]

Subst. 0.1936, 0.2304; cc. 0.1 N $AgNO_3$, 16.43, 19.58
Calculated for $C_{20}H_8Cl_4O_5$, Cl, 30.18; found, 30.10, 30.14

The tetrachlorofluorescein thus obtained is a brick-red powder, soluble in alkalies with a reddish yellow color and greenish fluorescence. If the alkaline solution be diluted with a large amount of water, the color becomes pinkish yellow with the same greenish fluorescence. The fluorescence is much less marked than in the case of fluorescein. It is insoluble in water, acetone, ethyl acetate, ether, and chloroform, very slightly soluble in 95% alcohol with a yellow color and greenish fluorescence.

Later it was found that the above described method of purification by dissolving the hydrate in ether and recrystallizing from alcohol and from acetone is unnecessary, since the crude product can be converted directly into the diacetate, and this diacetate obtained perfectly white and pure by repeated crystallization from benzene or from acetone. This diacetate has the same melting point and other physical constants as the diacetate prepared from the previously purified tetrachlorofluorescein. When this white product is saponified with alcoholic caustic potash, and the diluted alcoholic solution acidified, a yellow hydrate is formed which, when filtered off and thoroughly washed with water, was found to be completely soluble in ether, showing that it contains none of the dark red material supposed to be a mixture of the isomeric tetrachlorofluoresceins. *All* of the tetrachlorofluorescein used in this investigation was purified by converting the more or less impure tetrachlorofluorescein into the diacetate, recrystallizing this diacetate from a number of solvents until it was perfectly white and had a constant melting point, and saponifying it with alcoholic caustic potash. In this manner a tetrachloro-

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[1] *Am. Chem. J.*, 48, 477 (1912).

[2] All the chlorine determinations in this article were made by the lime method. *Am. Chem. J.*, 41, 393.

fluorescein was obtained free from any of the possible isomeric tetrachlorofluoresceins.

Tetrachlorofluorescein Hydrate.—When a very dilute alkaline solution of pure tetrachlorofluorescein is poured into an excess of dilute mineral acid, a light yellow substance is precipitated. This material was prepared for analysis and investigation in the following manner: Ten grams of pure tetrachlorofluorescein, obtained from the saponification of the pure diacetate, (see p. 691), were dissolved in 500 cc. of water containing 3 g. of potassium hydroxide. After filtering, this solution was diluted to one liter and poured slowly and with constant stirring into a liter of water containing 3 g. of hydrochloric acid gas. The light yellow precipitate thus formed was filtered off on a Buchner funnel, transferred to a flask, washed thoroughly by shaking with water, again filtered and washed on the funnel, until the wash water gave no test for chlorides. This yellow substance was dried in the air and analyzed. When heated in the electric drying oven to a temperature of 110° the substance lost weight, moisture collected upon the cool part of the tube,[1] the material turned red and came to constant weight in *forty-five minutes*. The following results show that this substance is a hydrate of tetrachlorofluorescein:

Subst. 0.2554, 0.2356; loss at 110°, 0.0103, 0.0088
Calculated for $C_{20}H_8Cl_4O_5.H_2O$, 3.69; found, 4.03, 3.74

When this hydrate is allowed to stand in the air (temperature 23–25°) for ten days some of the combined water is lost, as shown by the following determination:

Subst. 0.5537, loss at 110°, 0.0159 = 2.87

This is also shown by the fact that the material dried for this length of time is *not completely* soluble in ether—some dark red material, anhydrous tetrachlorofluorescein, remaining undissolved—whereas the freshly prepared *dry* hydrate is *completely* soluble in ether.

The following chlorine determinations on the product, dried to constant weight at 110°, show that it is the red anhydrous tetrachlorofluorescein:

Subst. 0.2429, 0.1133; cc. 0.1 *N* $AgNO_3$, 20.68, 9.66
Calculated for $C_{20}H_8Cl_4O_5$, Cl, 30.18; found, 30.20, 30.23

Tetrachlorofluorescein hydrate is very soluble in ether, acetone, and ethyl acetate, readily soluble in methyl and in ethyl alcohol, only slightly soluble in chloroform, and insoluble in benzene. When the moist hydrate is crystallized from 95% ethyl alcohol, the excess of the solvent being distilled off below 35° (under reduced pressure), it is almost completely converted into the carbinol acid (see p. 690), which forms beautiful pale yellow needles.

The yellow hydrate also loses a molecule of water when kept in an

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[1] *Am. Chem. J.*, 41, 404 (1909) and 48, 477 (1912).

evacuated desiccator over phosphorus pentoxide, but in this case the anhydrous product has only a pale yellow color, and is probably the lactoid form of tetrachlorofluorescein, which was also obtained from the etherate and from the compound with ethyl acetate (see pp. 694 and 695). The change into the red tetrachlorofluorescein is also brought about by boiling the yellow hydrate with water, contrary to Graebe, or with benzene. Attempts to recrystallize the yellow hydrate by distilling off some of the solvent at *atmospheric pressure* always resulted in a mixture of crystals of the yellow hydrate, or carbinol acid, and the red tetrachlorofluorescein. The yellow hydrate when acetylated with acetic anhydride gives the same diacetate as is obtained from the red anhydrous tetrachlorofluorescein and from the carbinol acid. The yellow hydrate dissolves in solutions of the alkalies with the same color and fluorescence as tetrachlorofluorescein itself, and it is precipitated out again when the dilute alkaline solution is poured into an excess of acid. The yellow hydrate is also formed, contrary to the statement of Graebe, when a dilute solution of tetrachlorofluorescein in ammonia or sodium carbonate is precipitated by acid.

Acetonate of Tetrachlorofluorescein.—The hydrate dissolves in acetone with a clear yellow color, and when some of the solvent is distilled off light yellow crystals separate. These may be obtained almost white upon recrystallization from the same solvent. When this substance is heated to 160° in a current of carbon dioxide, both water and acetone are given off. The material was analyzed in the following manner: A weighed amount was placed in a platinum boat, inserted in the electric drying oven[1] and heated to a temperature of 160°. The tube was connected to two Hugershoff gas wash bottles containing distilled water and surrounded by ice. A current of dry carbon dioxide was passed over the heated substance, the acetone being absorbed in the water and subsequently determined by titration with standard iodine solution.[2] The following results were obtained from three different samples made at different times:

Subst. 0.4945, 0.6367, 0.4013; loss at 160°, 0.0773, 0.0856, 0.0578

Calculated for loss of acetone and water from $C_{20}H_8Cl_4O_5.C_3H_6O.H_2O$, 13.93; found, 15.63, 13.44, 14.40

The solution in the two Hugershoff wash bottles of Determination I was made up to 500 cc. with distilled water, and three 50 cc. portions (*a*, *b*, *c*) were titrated with standard iodine solution. Cc. 0.1 *N* iodine, *a*, 4.89; *b*, 4.85; *c*, 4.89. Acetone found. 9.58, 9.50, 9.58; mean, 9.53.

The solution in the wash bottles in Determination II was diluted and titrated in the same manner with the following results: Cc. 0.1 *N* iodine, *a*, 6.21; *b*, 6.08; *c*, 6.20. Acetone found, 9.43, 9.24, 9.42; mean,9 .36.

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[1] *Am. Chem. J.*, **48**, 477 (1912).

[2] *Ibid.*, **44**, 6 (1911).

From Determination III the following results were obtained: Cc. 0.1 *N* iodine, *a*, 3.95; *b*, 3.91. Acetone found, 9.52, 9.43; mean, 9.48. Calculated for $C_{20}H_8Cl_4O_5.C_3H_6O.H_2O$, 10.62

The compound remaining after the acetone and water are driven off is the red anhydrous tetrachlorofluorescein as shown by the following analyses:

Subst. 0.2783, 0.1738; cc. 0.1 *N* $AgNO_3$, 23.62, 14.75
Calculated for $C_{20}H_8Cl_4O_5$, Cl, 30.18; found, 30.09, 30.10

From these results it seems probable that the almost white product obtained from the acetone solution of the hydrate, is a mixture of the *di*acetonate of tetrachlorofluorescein and the hydrate itself, or it may be a mixture of the diacetonate and tetrachlorofluoresceincarbinolcarboxylic acid (see below). The fact that the compound has such a slight color indicates that it contains very little, if any, of the yellow hydrate.

Tetrachlorofluoresceincarbinolcarboxylic Acid.—This compound was made from the purified diacetate of tetrachlorofluorescein by saponifying with alcoholic sodium hydroxide, in the same manner as in the preparation of the disodium salt of tetrachlorofluorescein (see p. 698). The alcoholic solution, containing an excess of sodium hydroxide, was diluted with a large amount of water and poured into an excess of dilute hydrochloric acid. After stirring with a current of air for five minutes the precipitate was allowed to settle, the supernatant liquid poured off and the precipitate filtered off on a Buchner funnel. This precipitate was redissolved in sodium hydroxide, the solution diluted to three liters, filtered and poured slowly, and with constant stirring, into an excess of hydrochloric acid diluted to three liters. The light yellow precipitate thus formed was extracted at once with ether. The amber colored ethereal solution was separated from the aqueous layer, filtered, and dried by shaking for ten minutes with a large amount of fused calcium chloride. The solution was then filtered into a round bottom flask and one-half of the ether distilled off on the water bath. After the addition of an equal amount of absolute alcohol, the distillation was continued at 35°, under reduced pressure, until almost white crystals separated. These were filtered off, shaken with absolute alcohol, and the mixture filtered, when the material was obtained almost white, only a faint yellow color being noticeable. Some of this substance was recrystallized from absolute alcohol, under reduced pressure, but this yellow tinge remained. An attempt was made to recrystallize some of this material from methyl alcohol, under reduced pressure, but a mixture of the red anhydrous tetrachlorofluorescein and the carbinol acid was always obtained.

The air-dried material loses no weight when kept in an evacuated desiccator over phosphorus pentoxide for two weeks. When heated in a current of carbon dioxide to a temperature of 110° the carbinol acid loses water very slowly, forty-eight hours being required for all the water to be driven off. If however, the temperature be increased to 150°, the material comes to constant weight in eight hours. The loss in weight is entirely due to water, no alcohol or ether being given off. The carbinol carboxylic acid crystallizes with a half molecule of water of crystallization as shown by the following determinations of the loss in weight:

Subst. 0.6635, 0.7319; loss at 150°, 0.0360, 0.0385. Calculated for change from $C_{20}H_{10}Cl_4O_6.0.5H_2O$ to $C_{20}H_8Cl_4O_5$, 5.44; found, 5.43, 5.26

When heated to constant weight at 150°, the carbinol acid gives the red anhydrous tetrachlorofluorescein, as shown by the following chlorine determinations:

Subst. 0.2688, 0.2531; cc. 0.1 N $AgNO_3$, 22.85, 21.45
Calculated for $C_{20}H_8Cl_4O_5$, Cl, 30.18; found, 30.14, 30.05

Tetrachlorofluoresceincarbinolcarboxylic acid is also completely changed to the red anhydrous tetrachlorofluorescein by boiling for some time with water. It is partially changed to the red anhydrous tetrachlorofluorescein when recrystallized from methyl or ethyl alcohol or from ether, when the excess of the solvent is distilled off at atmospheric pressure, or if the solutions be allowed to evaporate in the air. It can, however, be recrystallized unchanged from absolute ethyl alcohol, provided the excess of the solvent be distilled off below 35°, under reduced pressure.

It is very soluble in acetone, ethyl acetate, and ether, less soluble in methyl and ethyl alcohol, practically insoluble in chloroform and benzene. It dissolves in the alkalies and alkaline carbonates with the same color and fluorescence as the yellow hydrate of tetrachlorofluorescein, and this alkaline solution when acidified gives the same yellow hydrate as the alkaline solution of tetrachlorofluorescein does. It is thus possible to convert the carbinol acid into the yellow hydrate, and this hydrate can be reconverted into the carbinol acid by the method given above. Both the yellow hydrate and the carbinol acid give the same red anhydrous tetrachlorofluorescein when heated to 150°. This product gives the yellow hydrate when dissolved in alkalies and the solution is acidified with a mineral acid. The carbinol acid also gives the same diacetate (melting point 256°) as is obtained from the anhydrous tetrachlorofluorescein and from the hydrate (see p. 688). On standing in a stoppered bottle the carbinol acid gradually takes on a yellow color.

Tetrachlorofluorescein Diacetate.—One hundred grams of anhydrous tetrachlorofluorescein, made by heating the product recrystallized from acetone to constant weight at 140°, were mixed with 100 g. of anhydrous sodium acetate and 500 g. of pure acetic anhydride, and this mixture heated to boiling for three hours. The solution was allowed to cool, when the diacetate crystallized out and most of the sodium acetate remained in the solution. The crystals of tetrachlorofluorescein diacetate were filtered off, washed thoroughly with water and dried to constant weight at 110°. Yield, 90 g. The filtrate was poured into water and 15 g. more of the diacetate were obtained. The crude material was then dissolved in benzene and crystallized twice from this solvent. The diacetate thus obtained was perfectly white and had not the slightest trace of color. The melting point of the product dried at 160° is 256° (uncorr.). Separate portions of this product were crystallized from absolute alcohol, acetone, and ethyl acetate. The material obtained from each of these solvents had the same melting point as the original, and was, therefore, pure, as shown by the following determinations on the air dried product:

Subst. 0.7958, 1.1786; loss (benzene) at 160°, 0.0935, 0.1425
Calculated for $C_{20}H_6Cl_4O_5(CH_3CO)_2.C_6H_6$, 12.35; found, 11.75, 12.09

When crystallized from benzene the diacetate, therefore, contains one molecule of benzene of crystallization. It loses a small amount of this benzene when dried in the air.

Chlorine determinations on the product heated to constant weight at 160° gave the following results:

Subst. 0.3730, 0.2805; cc. 0.1 *N* $AgNO_3$, 26.76, 20.29
Calculated for $C_{20}H_6Cl_4O_5(COCH_3)_2$, Cl, 25.60; found, 25.44, 25.65

Tetrachlorofluorescein diacetate is soluble in acetic anhydride, benzene, acetone, ethyl acetate, and slightly soluble in alcohol. When treated with alcoholic solutions of the alkalies it undergoes saponification even in the cold, quite readily when heated to boiling. Aqueous solutions of the alkalies hydrolyze the diacetate but slightly. It dissolves in concentrated sulfuric acid with a yellow color and greenish fluorescence, probably undergoing saponification. When this solution is diluted with water a light yellow compound, the hydrate, is precipitated, which is completely soluble in ether and therefore contains none of the red impurity found in the crude material (see p. 686).

Tetrachlorofluorescein Monobenzoate.—This substance was obtained in an attempt to prepare the dibenzoate of tetrachlorofluorescein by the Baumann-Schotten reaction, *i. e.*, by the action of benzoyl chloride on a cold alkaline solution of tetrachlorofluorescein. The following method was employed: 18.8 g. of tetrachlorofluorescein were dissolved in 280 cc. of water containing 20 g. of potassium hydroxide. To this solution 22.4 g. of benzoyl chloride were added slowly and with constant stirring. Within a short time after the addition of the benzoyl chloride, the benzoate separated out as a light reddish yellow, sticky mass. This substance was separated from the solution by filtration and triturated with a 2% solution of potassium hydroxide. After filtration, the material was washed well with a 2% solution of potassium hydroxide, then with hot water until all the alkali was removed. The solid residue was then extracted with 250 cc. portions of 95% alcohol until only a faint yellow color was imparted to the extract. The residue from these extractions together with the material which crystallized out from the alcoholic extract, was then dissolved in benzene and crystallized from this solvent. The crystals first obtained were orange-yellow in color but by repeated crystallization from chloroform and again from benzene, a perfectly white compound was obtained—melting point 235°. It is soluble in absolute alcohol, in benzene, and in chloroform. It dissolves with difficulty in dilute solutions of the alkalies with a yellow color and greenish fluorescence. A light yellow substance, probably a hydrate, is precipitated from these solutions on the addition of acid. The monobenzoate is slightly soluble in sodium carbonate solution with the same color and fluorescence as the dilute alkaline solution.

The air dried product loses approximately a molecule of benzene on heating to 150°, as shown by the following results:

Subst. 1.0647, 0.4052, 0.7224, loss (benzene) at 150°, 0.1152, 0.0393, 0.0588
Calculated for $C_{20}H_7Cl_4O_5(C_7H_5O).C_6H_6$, 11.97; found, 10.82, 9.70, 8.14

Determination II was made on material which had been kept in a stoppered bottle for several weeks, while Determination III was made on some of the same material which had been kept for more than a year in a stoppered bottle.

Determinations of chlorine on the product heated to constant weight at 150° show that the compound is the monobenzoate.

Subst. 0.3601, 0.2464, 0.1788[1]; cc. 0.1 *N* $AgNO_3$, 24.97, 17.06, 12.40.
Calculated for $C_{20}H_7Cl_4O_5(C_7H_5O)$, Cl, 24.71; found, 24.59, 24.55, 24.82.

When this white monobenzoate is heated in a current of carbon dioxide to 150° it becomes pale yellow in color. To determine if this change in color is due to decomposition involving a loss of chlorine, a determination of chlorine was made on the air-dried product and the results calculated for the material free from benzene, the loss in weight of some of the same substance having been previously determined by heating it to a temperature of 150°.

Subst. 0.3030; subst. at 150°, computed, 0.2736; cc. 0.1 *N* $AgNO_3$, 18.94.
Calculated for $C_{20}H_7Cl_4O_5(C_7H_5O)$, Cl, 24.71; found, 24.55.

From this result it is evident that the change in color is not due to decomposition, but is probably due to a partial molecular transformation from the lactoid to the quinoid form, as was observed in the case of the lactoid form of tetrachlorofluorescein (see p. 710).

Acetyl Tetrachlorofluorescein Benzoate.[2]—Five grams of the tetrachlorofluorescein monobenzoate, prepared and purified as previously described, were boiled for two and one-half hours with 25 cc. of acetic anhydride. The solution thus obtained was poured while still hot into one liter of water and the mixture allowed to stand over night. The white substance

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[1] This tetrachlorofluorescein monobenzoate was made and analyzed by Mr. Wm. F. Flynn.

[2] This compound was prepared and analyzed by Mr. Wm. F. Flynn.

that separated out was filtered off, washed thoroughly with water, and crystallized from a mixture of ethyl acetate and ethyl alcohol. The pure white compound thus obtained was analyzed for chlorine and the following results obtained on the product heated to constant weight at 110°:

Subst. 0.1329, 0.1577; cc. 0.1 *N* $AgNO_3$, 8.59, 10.21

Calculated for $C_{20}H_6Cl_4O_5(C_7H_5O)(C_2H_3O)$, Cl, 23.03; found, 22.92, 22.96

Acetyl tetrachlorofluorescein benzoate is very soluble in ethyl acetate and in benzene, only slightly soluble in alcohol. It is insoluble in aqueous solutions of the alkalies, and undergoes slight hydrolysis when boiled with these solutions. It is readily saponified by alcoholic solutions of the caustic alkalies.

Tetrachlorofluorescein Dibenzoate.—This compound cannot be prepared by the Baumann-Schotten reaction as shown under tetrachlorofluorescein monobenzoate but is readily made by the direct action of benzoyl chloride on the anhydrous tetrachlorofluorescein. Ten grams of anhydrous tetrachlorofluorescein were suspended in 100 cc. of benzoyl chloride and the mixture heated to the boiling point for one-half hour. At the end of this time the tetrachlorofluorescein was completely dissolved to a greenish brown liquid. When cold this solution was poured into 95% alcohol when the white dibenzoate separated out. This was filtered off and recrystallized from benzene, from which solvent it was obtained perfectly white. Melting point, 247°. When heated in a current of air to 130° this compound lost only 1.5% and no moisture condensed upon the cool part of the tube but the odor of benzene was noticeable. This compound, therefore, crystallizes without benzene of crystallization. A determination of chlorine on the product heated to constant weight at 130° shows that it is tetrachlorofluorescein dibenzoate.

Subst. 0.3025; cc. 0.1 *N* $AgNO_3$, 17.95.

Calculated for $C_{20}H_6Cl_4O_5(C_7H_5O)_2$, Cl, 21.20; found, 21.04.

Tetrachlorofluorescein dibenzoate is soluble in benzene, in acetone and in ethyl acetate, almost insoluble in alcohol. It is insoluble in aqueous solutions of the alkalies, and undergoes only slight hydrolysis when boiled with these solutions. It is slowly saponified by alcoholic solutions of the caustic alkalies.

Tetrachlorofluorescein Etherate.—The yellow hydrate dissolves completely in ether giving a light amber colored solution, and upon distilling off some of the ether an almost white compound, together with some of the dark red anhydrous tetrachlorofluorescein, crystallizes out. In order to obtain this white compound pure and free from the red, the following method was employed: Two grams of the air-dried yellow hydrate were dissolved in 200 cc. of anhydrous ether, the solution filtered from a very small amount of the red anhydrous tetrachlorofluorescein contained in the hydrate, and heated to boiling on a water bath in a flask fitted with a return condenser. The solution became lighter in color by this treatment, and upon cooling a few white crystals separated, together with some of the dark red anhydrous material. After filtering, some of the ether was distilled off below 20°, under reduced pressure, when white crystals separated. These were filtered off with the aid of suction but they turned yellow very quickly as the adherent ether evaporated, and became white again when moistened with ether or when brought into contact with ether vapor. In order to prevent this change to yellow the white crystals were filtered off on a Büchner funnel with suction, and while still moist with ether transferred quickly to a deep beaker and the remaining ether allowed to pass off slowly with a column of ether vapor above the crystals. By this means a perfectly white product was obtained. The change is probably due to a transformation of a part of the etherate to the yellow hydrate by the condensation of moisture upon the white crystals as the mechanically held ether rapidly evaporates. A weighed amount of the etherate was placed in a platinum boat and a rapid current of dry air at room temperature passed over it. At the end of twenty hours it was still losing weight and had become pale yellow in color. The substance was then gradually heated to 110° and came to constant weight at the end of seven hours. At no time did any moisture condense upon the cold part of the tube, and the material remained pale yellow in color. The tube was then heated to a temperature of 160° for fifteen minutes. Under this treatment the substance became pinkish yellow without any further loss in weight. The following results were obtained:

Subst. 0.4494, 0.2322; loss (ether) at 110°, 0.0607, 0.0308

Calculated for $C_{20}H_8Cl_4O_5.C_4H_{10}O$, 13.62; found, 13.51, 13.26

Determination I was made upon the air-dried, freshly prepared material, in II the substance had been kept in a stoppered bottle for four days.

Chlorine determinations on the material heated to constant weight at 110° gave the following results: Subst. 0.2504, 0.2012; cc. 0.1 N $AgNO_3$, 21.33, 17.03. Calculated for $C_{20}H_8Cl_4O_5$, Cl, 30.18; found, 30.21, 30.01.

Tetrachlorofluorescein etherate is a fairly stable, colorless, crystalline compound containing one molecule of ether. It loses this ether slowly in a current of dry air at room temperatures, more readily at 110°, leaving a pale yellow, anhydrous tetrachlorofluorescein in the lactoid form (see p. 710). When tetrachlorofluorescein etherate is heated with water it loses ether and the dark red (quinoid) anhydrous tetrachlorofluorescein is left. It is soluble in acetone and alcohol. Its solution in absolute alcohol gives tetrachlorofluoresceincarbinolcarboxylic acid when the alcohol is distilled off at 35°, under reduced pressure (see p. 690).

Compound of Tetrachlorofluorescein with Ethyl Acetate.—For the preparation of this compound five grams of the yellow hydrate were dissolved in 200 cc. of dry, freshly distilled ethyl acetate, the solution filtered to remove the small amount of red anhydrous tetrachlorofluorescein, and some of the solvent distilled off under reduced pressure. A white, beautifully crystalline compound separated out. This was treated in the same manner and with the same precautions as the etherate, when a perfectly white substance was obtained. This compound was placed in a tightly stoppered bottle and analyzed.

Subst. 0.4610, loss (ethyl acetate) at 130°, 0.1298.
Calculated for $C_{20}H_8Cl_4O_5.(CH_3COOC_2H_5)_2$, 27.26; found, 28.16.

When kept in a tightly stoppered bottle for two days:

Subst. 0.30034; loss, 0.0539.
Calculated for $C_{20}H_8Cl_4O_5.CH_3COOC_2H_5$, 15.78; found, 17.77

During the heating there was no condensation of moisture upon the cold part of the tube of the drying oven. The material remaining in the boat was pale yellow in color, and is the lactoid form of anhydrous tetrachlorofluorescein as shown by the following chlorine determination:

Subst. 0.2353; cc. 0.1 *N* $AgNO_3$, 19.97. Calculated for $C_{20}H_8Cl_4O_5$, Cl, 30.18; found, 30.10.

This compound with ethyl acetate resembles very closely in its properties and solubilities tetrachlorofluorescein etherate.

Monopotassium Salt.—For the preparation of this compound ten grams of the anhydrous tetrachlorofluorescein were suspended in 300 cc. of 95% alcohol and five grams of potassium acetate dissolved in 200 cc. of alcohol were added. The mixture was boiled in a flask fitted with a return condenser. At the end of half an hour the monopotassium salt crystallized out from the solution. This compound is light red in color and may be obtained beautifully crystallized from alcohol. The product thus obtained crystallizes with three molecules of water, as shown by the following analysis made on the air-dried material:

Subst. 0.1442; cc. 0.1 *N* $AgNO_3$, 10.33
Calculated for $C_{20}H_7Cl_4O_5K.3H_2O$, Cl, 25.24; found, 25.41

A potassium determination on the same material gave the following result: Subst. 0.2867; K_2SO_4, 0.0438. Calculated for $C_{20}H_7Cl_4O_5K.3H_2O$, K, 6.96; found, 6.87.

All of the water of crystallization is not removed from this salt by long heating to a temperature of 180°, one-half of a molecule remaining, as shown by the following determinations on the loss in weight:

Subst. 0.7925, 0.6270; loss (H_2O) at 180°, 0.0657, 0.0523
Calculated for $C_{20}H_7Cl_4O_5K.2.5H_2O$, 8.14; found, 8.29, 8.34

After heating to this temperature the salt was analyzed and gave the following results: Subst. 0.2664; cc. 0.1 *N* $AgNO_3$, 20.44. Calculated for $C_{20}H_7Cl_4O_5K.0.5H_2O$, Cl, 27.44; found 27.21.

A potassium determination on the same material gave the following result: Subst. 0.1511; K_2SO_4, 0.0252. Calculated for $C_{20}H_7Cl_4O_5K.0.5H_2O$, K, 7.56; found, 7.49

The monopotassium salt, therefore, contains three molecules of water of crystallization, and retains one-half a molecule on heating in dry air to a temperature of 180°. It is soluble in water and in methyl alcohol, difficultly soluble in ethyl alcohol.

Dipotassium Salt.—One hundred grams of the pure diacetate were suspended in one liter of absolute alcohol, and one liter of absolute alcohol containing 50 g. of potassium hydroxide was added. This mixture was boiled for an hour in a flask connected with a return condenser. Some of the alcohol was then distilled off and the potassium salt crystallized out. This salt was recrystallized from absolute alcohol several times, and the air-dried product gave the following results on analysis:

Subst. 0.2597, 0.2648; cc. 0.1 *N* $AgNO_3$, 16.36, 16.97; Calculated for $C_{20}H_6Cl_4O_5K_2.4.5H_2O$, Cl, 22.62; Calculated for $C_{20}H_6Cl_4O_5K_2.5H_2O$, Cl, 22.30; found, 22.35, 22.73

The determination of potassium by fuming down with concentrated sulfuric acid gave the following results: Subst. 0.2121, 0.3067; K_2SO_4, 0.0589, 0.0857; Calculated for $C_{20}H_6Cl_4O_5K_2.4.5H_2O$, K, 12.47; Calculated for $C_{20}H_6Cl_4O_5K_2.5H_2O$, K, 12.49; found, 12.46, 12.54

It was found to be impossible to remove all of the water of crystallization by heating without decomposing the salt—one molecule being held even when the salt was heated for a long time to a temperature of 170°. The analyses of the air-dried material indicate that it contains four and one-half or five molecules of water. It crystallizes from alcohol in beautiful dark red prisms having a cantharides green luster. These crystals are very soluble in water and in methyl alcohol, less soluble in ethyl alcohol, insoluble in ether and in acetone. Solutions of the dipotassium salt are red with a greenish fluorescence, if very dilute, yellowish red with a greenish fluorescence.

When a very dilute solution (0.5%) of the dipotassium salt is added to a slight excess of very dilute (tenth normal) acid, the yellow hydrate of tetrachlorofluorescein is precipitated. This precipitate becomes crystalline on boiling, with but little change in color. If, however, a stronger solution be added to a large excess of acid, and the mixture be boiled, the hydrate first formed is changed completely to the red anhydrous tetrachlorofluorescein.

Monosodium Salt.—This salt was prepared in the same manner as the monopotassium salt of tetrachlorofluorescein. Ten grams of the anhydrous tetrachlorofluorescein were suspended in 250 cc. of 95% alcohol, and five grams of anhydrous sodium acetate dissolved in 250 cc. of alcohol were added. After boiling for half an hour the monosodium salt separated out. This was filtered off, washed thoroughly with alcohol to remove any sodium acetate and analyzed.

Subst. 0.3927; loss (H_2O) at 150°, 0.0242.
Calculated for $C_{20}H_7Cl_4O_5Na.2H_2O$, 6.82; found, 6.16.

A sodium determination on the product dried at 150° gave the following result:

Subst. 0.1444; Na_2SO_4, 0.0206
Calculated for $C_{20}H_7Cl_4O_5Na$, Na, 4.68; found, 4.62

A chlorine determination on this salt dried at 150° gave the following result:

Subst. 0.1386; cc. 0.1 N $AgNO_3$, 11.31
Calculated for $C_{20}H_7Cl_4O_5Na$, Cl, 28.84; found, 28.94

The monosodium salt of tetrachlorofluorescein is orange-yellow in color, and is only slightly soluble in alcohol. When placed in water it undergoes hydrolysis and gives a colloidal solution of the yellow hydrate.

Disodium Salt.—This compound was prepared from the diacetate in a manner analogous to the preparation of the dipotassium salt, an alcoholic solution of sodium hydroxide being substituted for the alcoholic caustic potash. The disodium salt being much less soluble in absolute alcohol is precipitated out during the preparation—more completely when the solution is cooled. The salt was filtered off from the alcoholic solution and extracted with benzene to remove any unsaponified diacetate, and recrystallized from a mixture of methyl and ethyl alcohols, and then from absolute alcohol.

The following results were obtained on the material crystallized from absolute alcohol and dried in the air at room temperatures:

Subst. 0.2961, 0.3208; cc. 0.1 N $AgNO_3$, 19.53, 21.23
Calculated for $C_{20}H_6Cl_4O_5Na_2.5H_2O$, Cl, 23.49; found, 23.39, 23.47

The sodium determinations made by fuming down with concentrated sulfuric acid gave the following results: Subst. 0.1788, 0.2779; Na_2SO_4, 0.0416, 0.0652; Calculated for $C_{20}H_6Cl_4O_5Na_2.5H_2O$, Na, 7.57; found, 7.53, 7.60

This salt crystallizes from alcohol in dark red prisms very similar to the dipotassium salt—the dipotassium salt being a little darker in color. It is very soluble in water and soluble in methyl alcohol, but less soluble in ethyl alcohol than the dipotassium salt.

Action of Dry Ammonia on the Red Anhydrous Tetrachlorofluorescein.

Diammonium Salt of Tetrachlorofluorescein.—A weighed quantity of the red anhydrous tetrachlorofluorescein obtained by heating the yellow hydrate to constant weight at 110°, was placed in a platinum boat and a current of dry ammonia gas passed over it. The ammonia was dried by passing it through soda lime and then over sodium. The substance became darker in color, and absorbed ammonia very rapidly at first, but five days were required for the material to come to constant weight. The following results were obtained:

Subst. 0.5373, 0.6380; gain in wt., 0.0390, 0.0465; wt. of salt, 0.5763, 0.6845
Calculated for $C_{20}H_6Cl_4O_5(NH_4)_2$, NH_3, 6.76; found, 6.77, 6.79

When this diammonium salt is heated to 150° in a current of dry hydrogen it loses all of its ammonia and the original weight of the red anhydrous tetrachlorofluorescein is obtained.

Action of Dry Ammonia on the Colorless Anhydrous Tetrachlorofluorescein; Triammonia Salt of Tetrachlorofluorescein.—When the almost colorless lactoid form of tetrachlorofluorescein, obtained from the etherate or from the compound with ethyl acetate (see pp. 694 and 695), is placed in dry ammonia gas it turns bright red at once and there is an increase in weight corresponding to exactly *three* molecules of ammonia. If this salt be heated to a temperature of 110° in a current of dry air or hydrogen, all of the ammonia is lost and the almost colorless lactoid form of anhydrous tetrachlorofluorescein is obtained. This will again take up *three* molecules of ammonia. If this lactoid form be heated to a temperature of 160–170° for some time, it is converted without loss into the red anhydrous tetrachlorofluorescein, and will then absorb an equivalent of only *two* molecules of ammonia. The following results were obtained:

Subst. 0.1385, 0.4180; gain in wt., 0.0151, 0.0455; wt. of salt, 0.1536, 0.4635
Calculated for $C_{20}H_8Cl_4O_5(NH_3)_3$, NH_3, 9.81; found, 9.83, 9.82

The second determination was made on the lactoid form of the anhydrous tetrachlorofluorescein obtained from the compound with ethyl acetate (see p. 695).

When left exposed to the air this triammonia salt loses one molecule of ammonia very rapidly. The remainder of the ammonia is lost very slowly at room temperature, but very rapidly if heated to 110°.

Action of Dry Ammonia on Tetrachlorofluoresceincarbinolcarboxylic Acid.—When the carbinol carboxylic acid of tertachlorofluorescein is placed in dry ammonia it turns bright red at once and there is a considerable increase in volume. The increase in weight is very rapid at first, but at the end of one hour the substance begins to lose weight. The results taken at the point of greatest increase in weight show that the substance takes up an equivalent of *three* molecules of ammonia, with but little loss of water.

Subst. 0.4708, 0.3489; gain in wt., 0.0470, 0.0359; wt. of salt, 0.5178, 0.3848.
Calculated for $C_{20}H_8Cl_4O_5(NH_3)_3.H_2O$, NH_3, 9.48; found, 9.08, 9.33.

When this ammonia salt is left in the ammonia there is a very gradual decrease in weight. Even after remaining in a slow current of dry ammonia for one week the material did not come to constant weight. When this salt is heated in a current of air to 120°, it loses both water and ammonia—the loss on the original material being the same as that obtained when the carbinol acid is heated to constant weight at 170°. When this substance, which is yellowish red in color, is placed in ammonia an equivalent of exactly *two* molecules is absorbed, as in the case of the red anhydrous tetrachlorofluorescein. This is shown by the following result:

Subst. 0.4460; gain in wt., 0.0324; wt. of salt, 0.4784
Calculated for $C_{20}H_6Cl_4O_5(NH_4)_2$, NH_3, 6.76; found, 6.77

Action of Dry Ammonia on Tetrachlorofluorescein Hydrate.—The yellow hydrate of tetrachlorofluorescein turns bright red at once when placed in dry ammonia, and absorbs an equivalent of *three* molecules of ammonia with very little loss of water, as shown by the following determination:

Subst. 0.4940; gain in wt., 0.0541; wt. of salt, 0.5481
Calculated for $C_{20}H_8Cl_4O_5(NH_3)_3.H_2O$, NH_3, 9.48; found, 9.87

When this salt is heated in a current of air to a temperature of 150°, both ammonia and water are given off and the red anhydrous tetrachlorofluorescein is obtained. The weight of this material is less than the weight of the yellow hydrate used at the beginning of the determination, and the loss is equivalent to nearly one molecule of water, as shown by the following:

Yellow hydrate, 0.4940; red anhydrous product, 0.4786; loss, 0.0154
Calculated for $C_{20}H_8Cl_4O_5.H_2O$, H_2O, 3.69; found, 3.12

Tetrachlorofluorescein Methyl Ester.—This compound was prepared in the following manner: Ten grams of pure anhydrous tetrachlorofluorescein were suspended in 500 cc. of methyl alcohol and 100 grams of concentrated sulfuric acid added. This mixture was boiled on the water bath for one hour when the tetrachlorofluorescein had completely dissolved. About one-half of the methyl alcohol was then distilled off and the remaining solution poured into water when the dark red methyl ester was precipitated. This was filtered off, washed thoroughly with water to remove sulfuric acid, dried, and recrystallized from benzene. The product thus obtained crystallizes without benzene of crystallization since the loss in weight is inconsiderable—being less than 1%—when the air-dried material is heated in a current of air to 150°.

A chlorine determination on the material dried to constant weight at 150° resulted as follows:

Subst. 0.1545; cc. 0.1 N $AgNO_3$, 12.64
Calculated for $C_{20}H_7Cl_4O_5CH_3$, Cl, 29.12; found, 29.00

The monomethyl ester of tetrachlorofluorescein is insoluble in water, difficultly soluble in methyl alcohol, soluble in ethyl alcohol, and very soluble in acetone and in benzene. It dissolves in cold aqueous solution of caustic soda and is precipitated out again unchanged upon the addition of acids. It is insoluble in *cold* aqueous solutions of the alkaline carbonates, but dissolves slowly when the solution is boiled, and separates out on cooling. The alkaline solutions of the methyl ester have the same color and fluorescence as the alkaline solutions of tetrachlorofluorescein itself. Strong hot hydrochloric acid (sp. gr. 1.10) dissolves the methyl ester with difficulty, forming the orange hydrochloride which crystallizes out on cooling.

Tetrachlorofluorescein methyl ester does not absorb ammonia even when kept in a current of dry ammonia for several days.

Action of Dry Hydrochloric Acid Gas on Tetrachlorofluorescein Methyl Ester. Tetrachlorofluorescein Methyl Ester Hydrochloride.—When the dark red methyl ester is placed in a current of dry hydrochloric acid gas, it becomes orange in color and absorbs an equivalent of nearly two molecules of hydrochloric acid, as shown by the following:

Subst. 0.1958; hydrochloride, 0.2219
Calculated for $C_{20}H_7Cl_4O_5CH_3.2HCl$, HCl, 13.14; found, 11.76

When this compound is placed in a current of dry air at room temperatures, the excess of hydrochloric acid above one molecule is lost very readily, and the hydrochloride of tetrachlorofluorescein methyl ester is obtained, as shown by the following:

Subst. 0.1958; hydrochloride, 0.2104
Calculated for $C_{20}H_7Cl_4O_5CH_3.HCl$, HCl, 7.04; found, 6.94

Action of Dry Hydrochloric Acid Gas on Fluorescein; Fluorescein Hydrochloride.—A weighed quantity of the dark red anhydrous fluorescein, obtained by saponification of the pure diacetate of fluorescein, was placed in a platinum boat and subjected to the action of dry hydrochloric acid gas. The material became light yellow in color and absorbed an equivalent of one molecule of hydrochloric acid. This yellow substance is fluorescein hydrochloride as shown by the following result:

Subst. 0.2724; hydrochloride, 0.3058
Calculated for $C_{20}H_{12}O_5.HCl$, HCl, 10.98; found, 10.92

When heated in a current of air to a temperature of 150° this hydrochloride loses all of its hydrochloric acid and the original weight of dark red fluorescein remains.

The light yellow hydrochloride of fluorescein is also formed when the dark red fluorescein is boiled in concentrated hydrochloric acid.[1] It is slightly soluble in the hydrochloric acid with a yellow color but without fluorescence.

Dry hydrochloric acid gas is not absorbed by the red anhydrous or the colorless anhydrous tetrachlorofluorescein. When the red anhydrous tetrachlorofluorescein is boiled with concentrated hydrochloric acid no color is imparted to the solution nor is there any noticeable change in the tetrachlorofluorescein.

Tetrachloroeosin.—The bromination of tetrachlorofluorescein was carried out in the following manner: Ten grams of pure anhydrous tetrachlorofluorescein were suspended in forty grams of glacial acetic acid and 75 grams of a 20% solution of bromine in glacial acetic acid were added. The greater part of the tetrachlorofluorescein dissolved at first, but at the end of five minutes the tetrachloroeosin began to crystallize out. The mixture was allowed to stand over night, then heated on a water bath for ten or fifteen minutes and allowed to cool. The crystals which separated were filtered off and washed with glacial acetic acid. Yield, ten grams. This substance, which was dirty pink in color, was dissolved in glacial acetic acid and the solution filtered while hot. Upon cooling this solution, white crystals of tetrachloroeosin separated. The material thus obtained was filtered off with suction and washed with glacial acetic acid.

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[1] Compare Gattermann, *Ber.*, **32**, 1135 (1899).

When washed with water or alcohol these white crystals of tetrachloroeosin became pink. This color was removed by washing again with glacial acetic acid. The compound was exposed in the air until it no longer had the odor of acetic acid and was then analyzed.

Subst. 0.4582, 0.6231; loss (acetic acid) at 150°, 0.0339, 0.0453.
Calculated for $C_{20}H_4Cl_4Br_4O_5.CH_3COOH$, 7.08; found, 7.40, 7.27.

The air-dried product crystallized from glacial acetic acid loses one molecule of acetic acid when heated to a temperature of 150°, and no water condensed on the cold part of the tube. The compound remains white unless it is heated above 150° or the heating be continued for a long time at 150°, when it becomes light pink in color.

The chlorine and bromine determinations on tetrachloroeosin and its derivatives were made in the following manner: A weighed quantity of the material was mixed with lime, placed in a Jena glass tube and heated in the same manner as in the determination of chlorine.[1] After heating the contents of the tube were dissolved in dilute nitric acid, the carbon filtered off and thoroughly washed with water. A measured excess of tenth-normal silver nitrate was then added to the filtrate and the mixture of silver halides filtered off on a weighed Gooch crucible. After washing thoroughly with 1% nitric acid, the mixture of silver halides on the Gooch was dried to constant weight at 145°, and the weight recorded. The excess of silver nitrate in the filtrate was then titrated with tenth-normal ammonium thiocyanate solution. From the weight of the silver halides and the volume of the tenth-normal silver nitrate solution required to precipitate them, the percentage of chlorine and bromine in the original compound can be indirectly determined, using the following formulas:[2]

Percentage of chlorine $= C - 53.249B/0.6676\,A$
Percentage of bromine $= 69.763\,B - C/0.38809\,A$
in which A = the weight of the substance taken
B = the weight of the silver halides
C = the volume in cc. of the tenth-normal silver nitrate solution required to exactly precipitate the chloride and bromide.

As a check upon this indirect method of determination, the mixture of silver chloride and bromide on the Gooch was treated with dry chlorine gas until there was no further loss in weight. The silver bromide was thus completely converted into silver chloride. The percentage of the two halides in the original substance can be calculated from this loss in weight and the weight of the silver halides, by means of the following formulas:

Percentage of chlorine $= 24.739\,B - 104.495\,D/A$
Percentage of bromine $= 179.76\,D/A$

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[1] *Am. Chem. J.*, 41, 393 (1909).

[2] *Ibid.*, 41, 380 (1909).

in which A = the weight of the substance taken
B = the weight of the silver halides
D = the decrease in weight of the silver halides on treatment with chlorine

Halogen determinations on tetrachloroeosin crystallized from glacial acetic acid and heated to constant weight at 150°, gave the following results:

	Substance. Gram.	Silver halides. Gram.	Tenth-normal silver nitrate. Cc.	Chlorine. Per cent.	Bromine. Per cent.
I	0.1994	0.3286	19.88	17.90	39.41
II	0.2109	0.3478	21.02	17.76	39.63
III	0.2324	0.3801	22.96	17.53	39.47
IV	0.1896	0.3147	19.02	17.86	39.96

In Determinations III and IV the mixture of silver halides was treated with chlorine and the following results obtained:

Substance. Gram.	Silver halides. Gram.	Decrease. Gram.	Chlorine. Per cent.	Bromine. Per cent.
0.2324	0.3801	0.0506	17.71	39.14
0.1896	0.3147	0.0430	17.36	40.77
Calculated for $C_{20}H_4Cl_4Br_4O_5$			18.06	40.69
Calculated for $C_{20}H_4Cl_4Br_4O_5.H_2O$			17.65	39.78

These results seem to show that the substance is tetrachloroeosin with one molecule of water, that is, that it may be the carbinol-carboxylic acid of tetrachloroeosin. It will be seen that the percentages of chlorine and bromine in the two compounds are so close together as to be within the limit of experimental error. Especially is this the case, since an error of 0.01 cc. in reading the volume of the silver nitrate solution makes a difference of 0.10% in the percentage of bromine, and an error of 0.0001 g. in the weight of silver halides makes a difference of 0.20%. It is therefore impossible to state with certainty, from the results of the halogen determinations alone, whether the compound is anhydrous tetrachloroeosin or tetrachloroeosincarbinolcarboxylic acid.

If this substance does contain a molecule of water it does not lose this water when heated for twenty-four hours at a temperature of 190–200° in a current of dry air, but becomes pink without any loss in weight. In this respect it is analogous to the lactoid form of tetrachlorofluorescein (see pp. 695 and 696). One gram of this white material was dissolved in nitrobenzene, which had been previously heated to boiling for some time to remove all the water. The dark red solution was then boiled in a distillation flask, but no moisture collected upon the cold neck of the flask. From these facts, and from the behavior of the compound when placed in dry ammonia (see p. 706), it seems highly probable that the substance is the anhydrous tetrachloroeosin.

This tetrachloroeosin dissolves in alcohol, acetone, ether, ethyl acetate, chloroform, benzene, nitrobenzene and carbon disulfide. It is difficultly soluble in glacial acetic acid. The alcohol and acetone solutions are reddish pink in color with a greenish yellow fluorescence, while the solution in ether is pink without any fluorescence. The color of these solutions is destroyed by the addition of a drop of mineral acid. The solutions in ethyl acetate, chloroform, benzene, and carbon disulfide are colorless. The solution in nitrobenzene is dark red in color and probably contains the quinoid modification. Dilute solutions of the alkalies and alkaline carbonates dissolve tetrachloroeosin with a cherry-red color and greenish fluorescence. In very dilute alcoholic solutions of the alkalies the fluorescence is brick-red (see p. 709). If strong alkali be added in large excess the color becomes purple, then blue, *without any fluorescence*. If this solution be diluted at once, it becomes red with a greenish fluorescence. The same color change is observed if an insufficient amount of acid be added at once to this strong alkaline solution. If the strong alkaline solution be allowed to stand over night, and the greenish blue solution is then diluted with water, a deep blue solution without fluorescence results, like that obtained when gallein or tetrachlorogallein is dissolved in an excess of dilute alkali. If this blue solution be heated to boiling it becomes red, with the characteristic greenish fluorescence of tetrachloroeosin in dilute alkali. When the greenish blue strong alkaline solution is poured into an excess of acid a pink precipitate forms which dissolves in dilute alkali with a *blue* color without fluorescence. This blue solution changes to red with a greenish fluorescence, when heated or on long standing. This change in strong alkaline solution is probably due to the formation of a tetrapotassium salt of tetrachloroeosin (see p. 722).

Tetrachloroeosin Hydrate.—This compound was prepared in a manner analogous to the preparation of tetrachlorofluorescein hydrate (see p. 688). Five grams of the anhydrous tetrachloroeosin were dissolved in 500 cc. of water containing two grams of potassium hydroxide. This solution was filtered, diluted to two liters and poured, with constant stirring, into water containing an excess of hydrochloric acid. The reddish pink precipitate thus obtained was washed throughly by decantation, filtered on a Buchner funnel and washed free from chlorides. The product on drying in the air becomes lighter in color and loses some of its combined water, as shown by the following results on analysis:

Subst. 0.6424, 0.5428; loss (H_2O) at 110°, 0.0079, 0.0065
Calculated for $C_{20}H_4Cl_4Br_4O_5.H_2O$, 2.24, found, 1.23, 1.20

When heated to constant weight at 110° the pink hydrate loses water very readily and becomes almost white. This faintly pink compound is anhydrous tetrachloroeosin, as shown by the following halogen determinations:

	Substance. Gram.	Silver halides. Gram.	Tenth-normal silver nitrate. Cc.	Chlorine. Per cent.	Bromine. Per cent.
I	0.1767	0.2930	17.73	18.01	39.60
II	0.2144	0.3584	21.65	17.96	40.25
			Decrease. Gram.		
II	0.2144	0.3584	0.0481	17.91	40.32
Calculated for $C_{20}H_4Cl_4Br_4O_5$				18.06	40.69

Tetrachloroeosin hydrate is soluble in alcohol, acetone, ether, and ethyl acetate, difficultly soluble in chloroform, and in benzene. When treated with solutions of the alkalies or alkaline carbonates, it behaves in the same manner as the anhydrous tetrachloroeosin (see p. 704). When treated with glacial acetic acid at ordinary temperatures, or more readily on heating, it turns white, forming the compound of tetrachloroeosin with glacial acetic acid described above (see p. 702). The hydrate is insoluble in petroleum ether and in ligroin. When boiled with these solvents it becomes white, probably being transformed into the anhydrous tetrachloroeosin.

Tetrachloroeosin Diacetate.—Five grams of pure anhydrous tetrachloroeosin were mixed with five grams of anhydrous sodium acetate and 50 g. of acetic anhydride. This mixture was boiled for four hours, and, upon cooling, almost white crystals of the diacetate separated. This material was filtered off, washed thoroughly with water, and extracted with absolute alcohol to remove any unacetylated tetrachloroeosin. When this residue was crystallized from acetone a perfectly white compound was obtained, which did not contain any acetone and melted at 298° to 300°. This product was then recrystallized from ethyl acetate and heated to 150° until all the ethyl acetate of crystallization was removed. The melting point remained unchanged. The perfectly white diacetate obtained from the ethyl acetate solution, and dried in the air, gave the following results on analysis:

Subst. 1.1649; loss (ethyl acetate) at 150°, 0.1035
Calculated for $C_{20}H_2Cl_4Br_4O_5(COCH_3)_2.CH_3COOC_2H_5$, 9.20; found, 8.88

No moisture collected upon the cold part of the tube of the electric oven, but the odor of ethyl acetate was very noticeable. The compound therefore loses some of its ethyl acetate on drying in the air.

A halogen determination on the substance heated to 150° gave the following result:

Subst. 0.1926; silver halides, 0.2915; cc. 0.1 N $AgNO_3$, 17.63
Calculated for $C_{20}H_2Cl_4Br_4O_5(COCH_3)_2$, Cl, 16.31; Br, 36.76; found, 16.37, 36.30

Tetrachloroeosin diacetate is soluble in acetic anhydride, benzene, acetone, ethyl acetate, and but slightly soluble in alcohol. When treated with alcoholic solutions of the alkalies it undergoes saponification even in the cold, quite readily when heated to boiling. Aqueous solutions of the alkalies hydrolyze this diacetate but slightly. It dissolves in concentrated sulfuric acid with a pink color and greenish fluorescence, probably undergoing hydrolysis, for when this solution is diluted with water a pink compound, the hydrate, is precipitated.

Dipotassium Salt of Tetrachloroeosin.—Five grams of anhydrous tetrachloroeosin were dissolved in 200 cc. of alcohol and 100 cc. of a solution of alcoholic caustic potash, containing three grams of potassium hydroxide, were added. This red solution was heated to boiling for half an hour, then some of the solvent was distilled off. Dark red crystals of the dipotassium salt separated. These were washed thoroughly with alcohol, dried to constant weight at 150° and analyzed. A determination of potassium showed that this substance was not pure, but contained some of the tetrapotassium salt. (See p. 704.) The material was then recrystallized twice from alcohol, dried in the air, and analyzed. The following results were obtained:

Subst. 1.1689; loss (H_2O) at 150°, 0.1009; Calculated for $C_{20}H_2Cl_4Br_4O_5K_2.4.5H_2O$, 8.60; Calculated for $C_{20}H_2Cl_4Br_4O_5K_2.5H_2O$, 9.46; found, 8.83

A determination of potassium in the salt dried to constant weight at 150° gave the following result:

Subst. 0.1248; K_2SO_4, 0.0254; Calculated for $C_{20}H_2Cl_4Br_4O_5K_2$, K, 9.07; found, 9.13

Attempts to prepare the monosodium salt of tetrachloroeosin in a manner similar to the preparation of the monosodium salt of tetrachlorofluorescein, *i. e.*, by the action of anhydrous sodium acetate upon the anhydrous tetrachloroeosin in alcoholic solution, resulted in the formation of a mixture of the mono- and disodium salts. These two salts could not be separated by repeated crystallization from alcohol.

Action of Dry Ammonia on Tetrachloroeosin.—When the white compound, obtained by heating the tetrachloroeosin crystallized from glacial acetic acid (see p. 702) to constant weight at 150°, is placed in a current of dry ammonia it turns red at once, and absorbs ammonia very rapidly with a large increase in volume and evolution of heat. The following result was obtained:

Subst. 0.2256; gain in wt., 0.0237; wt. of salt, 0.2493
Calculated for $C_{20}H_4Cl_4Br_4O_5.5NH_3$, NH_3, 9.78; found, 9.51.

When the red ammonium salt thus formed is heated in a current of air to a temperature of 150°, all of the ammonia is lost and the original weight of white material is obtained. During this heating, no moisture collected upon the cold part of the tube, the loss in weight being due entirely to loss of ammonia. In the case of the compound formed by the action of ammonia on tetrachlorofluoresceincarbinolcarboxylic acid (see p. 699), however, both ammonia and water are given off, and hence the white substance obtained by heating the compound crystallized from glacial acetic acid to constant weight at 150°, must be the lactoid form of tetrachloroeosin and not the colorless carbinol carboxylic acid (see p. 721).

When the light pink tetrachloroeosin obtained by heating the pink hydrate to constant weight at 110° is placed in dry ammonia it behaves in the same manner as the white tetrachloroeosin described above. This is shown by the following result:

Subst. 0.5363; gain in wt., 0.0561; wt. of salt, 0.5924
Calculated for $C_{20}H_4Cl_4Br_4O_5.5NH_3$. NH_3, 9.78; found, 9.47

When this red salt is heated in a current of air to a temperature of 150°, all the ammonia is lost, no moisture collected upon the cold part of the tube, and the original weight of the light pink tetrachloroeosin was obtained.

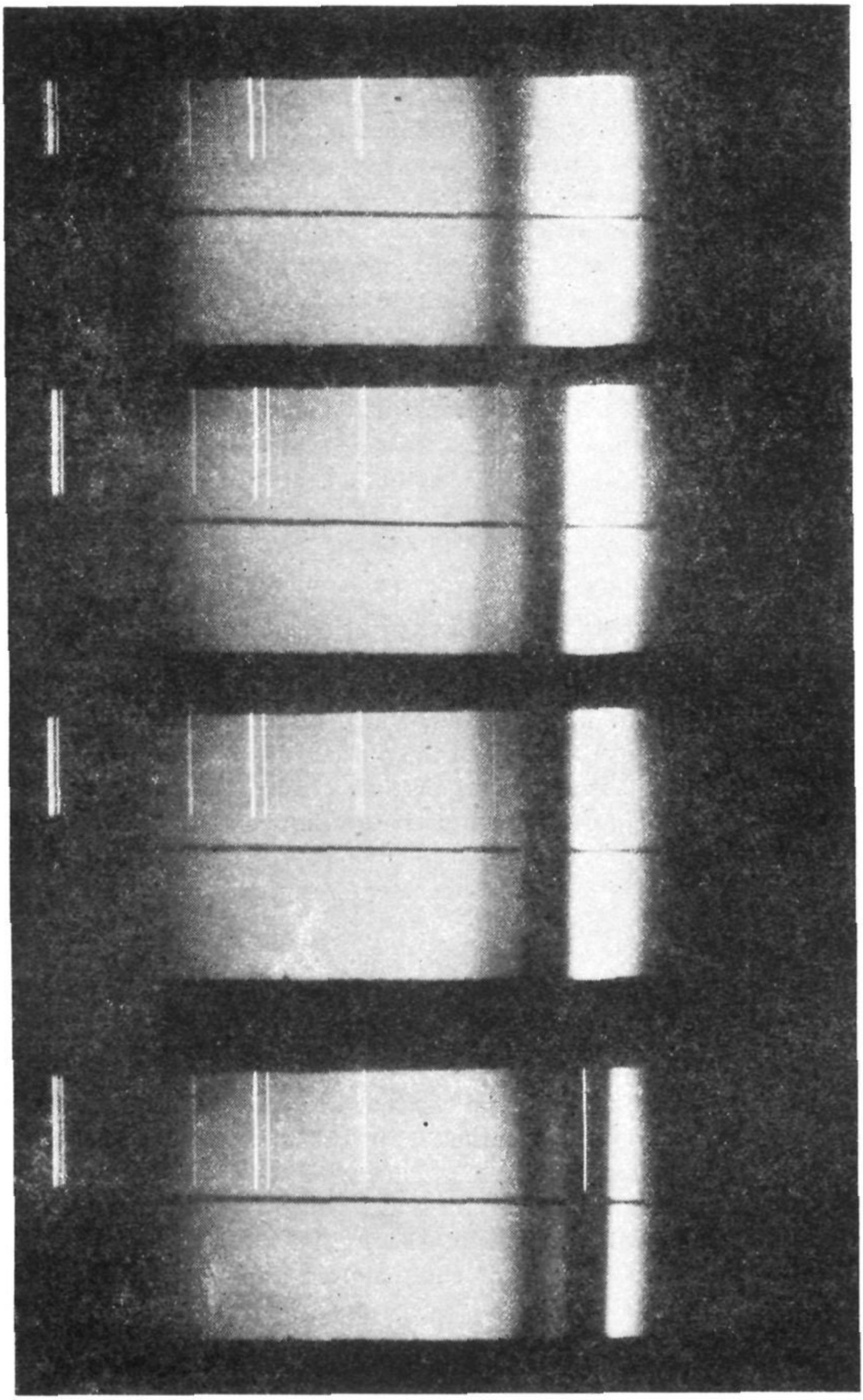
Absorption and Fluorescence Spectra of Solutions of Fluorescein, Tetrachlorofluorescein, Eosin and Tetrachloroeosin.

Professors E. L. Nichols and Ernest Merritt have examined solutions of fluorescein, tetrachlorofluorescein, eosin and tetrachloroeosin, and have furnished the following description of the absorption and fluorescence spectra of these solutions:

"Photographs of the absorption spectra of fluorescein (I), tetrachlorofluorescein (II), eosin (III), and tetrachloroeosin (IV), were made with different thicknesses of solution ranging from 2 mm. to 15 mm. The solutions were prepared as follows: The phthalein was suspended in neutral absolute alcohol and an amount of alcoholic caustic potash equivalent to four molecules of the alkali to one of the phthalein was added. The solutions were then diluted with neutral absolute alcohol until the absorption bands could be clearly seen through a layer of liquid 10 mm. thick. The following concentrations were used: Fluorescein, 4.37 mg. per liter; tetrachlorofluorescein, 12.42 mg. per liter; eosin, 19.25 mg. per liter; tetrachloroeosin, 26.00 mg. per liter.

"As a source of light the acetylene flame was used in all cases. For showing the general character of the absorption spectrum a thickness of approximately 10 mm. was found to be best and this was the thickness used in making the photographs here reproduced.

"It will be noticed that with the concentration and thickness used the absorption of fluorescein is less marked than that of either of the other substances. The chief band lies in the blue with its crest approximately at 0.501μ. There is a second fainter band, which does not show on the photograph, lying toward the red and having its crest approximately at 0.528μ. The bands are so broad that the crests cannot be located with accuracy and the wave lengths given are approximations only. The absorption of tetrachlorofluorescein is more marked and is very nearly identical with that of eosin. In each case two bands are clearly seen, the denser one lying toward the red. The crests of these bands are approximately


I
II
III
IV

0.523μ and 0.501μ for the tetrachlorofluorescein, and 0.523μ and 0.490μ for the eosin.

"The absorption bands of tetrachloroeosin are sharper than with any of the other substances. The two bands which appear in the photograph have their crests at approximately 0.553μ and 0.510μ.

"Upon some of the negatives taken there was evidence that the more intense band in the case of tetrachlorofluorescein, eosin, and tetrachloroeosin was in each case double; but we have found it impossible to obtain negatives in which the division is sufficiently sharp to show in the reproduction. The wave lengths given for the denser bands of these three substances are therefore to be regarded as the average for the two bands that are in all likelihood present.

"Perhaps the most striking point that is noticed in comparing the absorption spectra of the four substances is the increasing wave length of the absorption bands as we pass from fluorescein to tetrachloroeosin in the order shown in the photograph. The wave length of the absorption bands increases constantly with the number of the halogen atoms introduced into the molecules of fluorescein.

"A few preliminary tests at the temperature of liquid air indicate that the absorption at that temperature is considerably increased and that the existence of three bands in the absorption spectrum of tetrachloroeosin is more readily demonstrated at that temperature than at ordinary temperatures. There is no evidence, however, that the absorption bands become extremely narrow as is the case in some fluorescent substances, for example, the uranyl compounds.

"All of the substances show strong fluorescence, the color changing from greenish yellow with fluorescein to brick-red with tetrachloroeosin. This change is to be expected, since in fluorescent substances of this type the fluorescence band always lies immediately toward the red from the absorption bands.

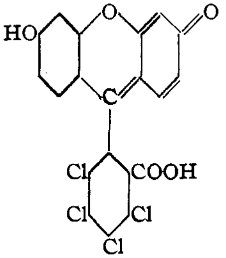
"The crests of the fluorescence bands are at approximately the following wave lengths:

Fluorescein	0.530μ
Tetrachlorofluorescein	0.554μ
Eosin	0.558μ
Tetrachloroeosin	0.585μ

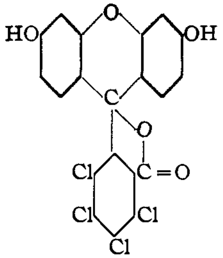
"There is some indication that the fluorescence bands are complex—as is to be expected on account of the complexity of the absorption spectrum—but a careful spectrophotometric study of the spectra would be required to establish this fact."

Theoretical.

The results of this investigation show that tetrachlorofluorescein exists in two forms. One is dark red and the other, colorless. The dark red modification is the more stable of the two. It is obtained by heating the yellow hydrate to constant weight at 110°, and from tetrachlorofluoresceincarbinolcarboxylic acid and the acetonate of tetrachlorofluorescein by heating to a temperature of 150–160°. The colorless form of tetrachlorofluorescein may be obtained from the etherate and also from the compound with ethyl acetate, by heating in a current of *dry* air to 110°. This colorless modification goes over without loss of weight to the dark red form on heating for some time to 150–160°. Because of its color, the quinoid formula I is assigned to the dark red tetrachlorofluorescein, while the lactoid formula II best represents the colorless modification.



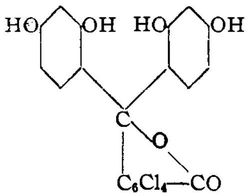
I. Tetrachlorofluorescein (colored).



II. Tetrachlorofluorescein (colorless).

As derivatives of the quinoid formula (I) there have been prepared tetrachlorofluorescein hydrate (yellow), tetrachlorofluorescein methyl ester (dark red), the dipotassium, disodium and diammonium salts (dark red), the monopotassium salt (light red), and the monosodium salt (orange). The following colorless compounds must be regarded as derived from the lactoid Formula (II): The diacetate, dibenzoate and monobenzoate of tetrachlorofluorescein, the acetate of the monobenzoate, tetrachlorofluorescein etherate, and the compound of tetrachlorofluorescein with ethyl acetate. Another colorless modification of tetrachlorofluorescein, the carbinol carboxylic acid (see p. 690), has also been prepared. The colorless acetonate described on p. 689 is probably a mixture of this carbinol acid and the diacetonate (see p. 714).

To the yellow hydrate, obtained by precipitating the caustic alkaline solution of tetrachlorofluorescein with acid, Graebe[1] assigns the lactoid formula



Tetrachlorofluorescein hydrate, according to Graebe.

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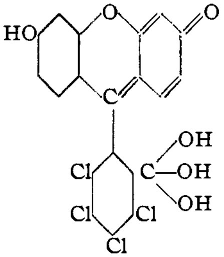
[1] *Ann. der Chemie*, **238**, 334 (1887).

Since this compound is *colored,* and loses its molecule of water *very readily* (see p. 688), it probably has the *quinoid* structure. It must therefore be represented by the following formula:



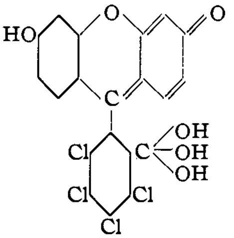
Tetrachlorofluorescein hydrate.

This is in accord with the fact that the hydrate acts like a strong acid, decomposing the alkaline carbonates and acetates with the formation of salts, and reacting with methyl alcohol to form an ester.

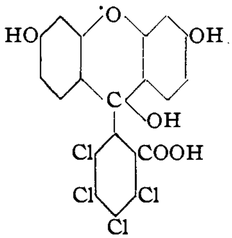
This hydrate is also formed, contrary to the statement of Graebe, when a dilute ammonia or sodium carbonate solution of tetrachlorofluorescein is precipitated in the cold by acid (see p. 689). When any dilute alkaline solution of tetrachlorofluorescein is precipitated *at the boiling temperature* by acid, a mixture of the yellow hydrate and the red anhydrous compound results. This is also contrary to Graebe's statement.

The above structure for the hydrate is confirmed by the fact that on treatment with acetic anhydride it yields the same diacetate as the anhydrous tetrachlorofluorescein, and not a *tetracetate,* as might be expected if this compound has the structure assigned to it by Graebe. The yellow hydrate loses water very slowly when left exposed to the air at room temperature, more readily in a vacuum desiccator over phosphorus pentoxide, and very rapidly when heated to 110°. This is contrary to the result obtained by Graebe, who states that tetrachlorofluorescein hydrate (tetrachloro-orthofluorescein) loses no weight at 110°, and undergoes but little change at 150–160°. From these results it seems probable that Graebe's tetrachloro-orthofluorescein was analyzed after crystallization of the precipitated hydrate from alcohol. If such was the case, it was a mixture of tetrachlorofluoresceincarbinolcarboxylic acid and a little of the hydrate, since it has been shown that the yellow hydrate when crystallized from alcohol is almost completely converted into the carbinol acid (see p. 690). This carbinol acid loses water only *very* slowly at 110°, and slowly at 150–160°. Such an explanation would account for the results obtained by Graebe. It is interesting to note that this yellow hydrate yields the colorless lactoid form of tetrachlorofluorescein when dehydrated at ordinary temperatures over phosphorus pentoxide, while at 110° it gives the deep red modification.

When prepared from the yellow hydrate as described on p. 690 the carbinol acid is colorless. This change must be accompanied by a molecular rearrangement and may be represented as follows:



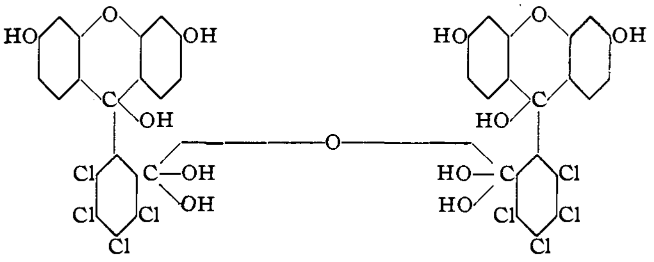
Tetrachlorofluorescein hydrate (colored).



Tetrachlorofluoresceincarbinolcarboxylic acid (colorless).

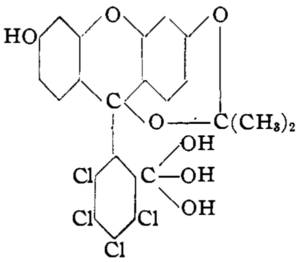
When this carbinol acid is dissolved in sodium hydroxide, the sodium salt of tetrachlorofluorescein is formed, and when the solution is acidified the yellow hydrate is precipitated. It is thus possible to reverse the above process, and convert the carbinol acid back into the yellow hydrate. When heated to 180° for an hour it is converted entirely into the dark red anhydrous tetrachlorofluorescein, and this change also takes place when the substance is boiled for some time with water.

From the fact that the carbinol acid loses almost exactly one and one-half molecules of water when heated to 180°, and loses no weight when kept for a long time in an evacuated desiccator over phosphorus pentoxide, it must be represented by the formula $C_{20}H_{10}Cl_4O_6 + 0.5H_2O$. The acid, therefore, crystallizes with half a molecule of water, and the structural formula is probably

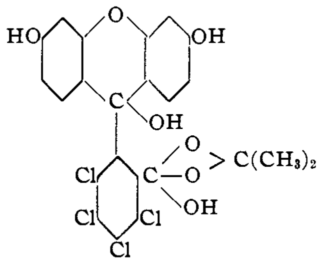


Tetrachlorofluoresceincarbinolcarboxylic acid.

When the hydrate is dissolved in dry acetone and some of the solvent distilled off, a white compound separates. This substance contains both acetone and water, but the percentage of acetone is less than and the percentage of loss greater than that calculated for either of the two possible chemical individuals containing both water and acetone—an acetonate of the hydrate (Formula I) or an acetonate of the carbinol acid (Formula II).

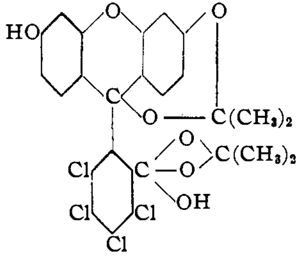


Acetonate of the hydrate.
(I)



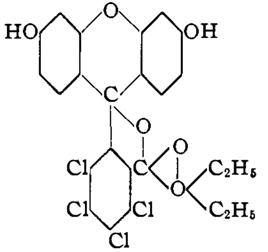
Acetonate of the carbinol acid.
(II)

It therefore seems probable that this white substance is a mixture of the diacetonate[1] of tetrachlorofluorescein (Formula III) and the hydrate, or a mixture of the diacetonate and the carbinol acid. The fact that this substance is so nearly white, and that it has to be heated for a long time at 150° before it ceases to lose weight, leads to the belief that it is a mixture of the diacetonate and the carbinol acid. It will be remembered that the carbinol acid is obtained from the alcoholic solution of the yellow hydrate in a similar manner (see p. 690).



Tetrachlorofluorescein diacetonate.
(III)

When the yellow hydrate is dissolved in dry ether, and the excess of the solvent distilled off below 20°, under reduced pressure, the white tetrachlorofluorescein etherate crystallizes out. Since this compound is colorless and loses all of its ether readily at 100°, to give the colorless anhydrous tetrachlorofluorescein, it probably has the lactoid structure and may be represented by the following formula:



Tetrachlorofluorescein etherate (colorless).

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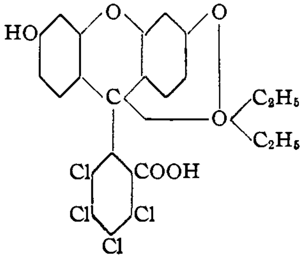
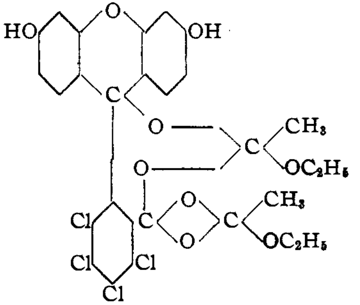
[1] *Am. Chem. J.*, 46, 13 (1911).

That the ether is attached to the carbonyl group of the phthalic acid residue and not to the quinoid grouping seems very likely since the compound loses no water, although made from the hydrate, and since, on treatment with water, the yellow hydrate results. This assumption seems all the more probable since the colorless etherate loses all of its ether in a current of *dry* air *at room temperatures*, and the *colorless* anhydrous tetrachlorofluorescein remains. If this compound had the structure,



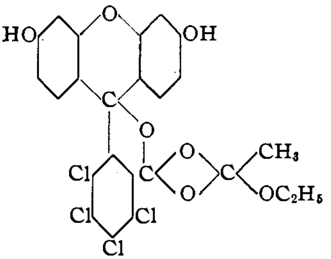
the dark red anhydrous tetrachlorofluorescein would probably result upon the loss of ether.

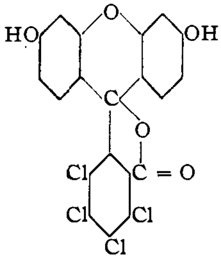
The compound of tetrachlorofluorescein with ethyl acetate, which is made in a manner similar to the preparation of the etherate, is also colorless. When first prepared the dry substance contains two molecules of ethyl acetate, and no water. When this compound is allowed to stand for some time in a stoppered bottle, one molecule of ethyl acetate is lost. The remaining molecule of ethyl acetate is driven off when the compound is heated in a current of dry air to a temperature of 130°, and the lactoid form of anhydrous tetrachlorofluorescein results. At no time during the loss of ethyl acetate does the substance become colored. In order to account for these facts the following structural formula is suggested:



Compound of tetrachlorofluorescein with ethyl acetate.

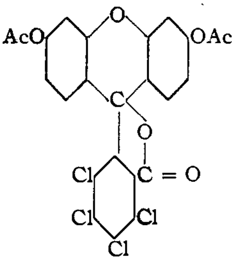
Both the ethyl acetate molecules are represented as attached to the phthalic acid residue, because of the fact stated above, that the compound loses one molecule of ethyl acetate very readily and does not become colored. The products formed by the loss of ethyl acetate may be represented by the following structural formulas:





A different condition obtains in the case of the diacetonate of tetrachlorogallein[1] where one of the acetone molecules is represented as attached to the quinoid grouping. This structure is given the compound because of the fact that a *colored* monacetonate is formed by the loss of one molecule of acetone.

That tetrachlorofluorescein contains two phenolic hydroxyl groups is shown by the fact that it forms a colorless diacetate and a colorless dibenzoate. Since both of these compounds are white and are insoluble in alkalies, they probably have the lactoid structure and may be represented by the formula in which Ac represents an acetyl or a benzoyl group:



Tetrachlorofluorescein diacetate and dibenzoate.

The colorless monobenzoate of tetrachlorofluorescein dissolves in dilute solutions of the alkalies and alkaline carbonates with a yellow color and a greenish fluorescence. When the colorless monobenzoate is heated to a temperature of 150° for some time it becomes yellow without loss of weight. This change in color is probably accompanied by a change of structure. The colorless compound is therefore represented by the lactoid Formula I, while the colored modification and its sodium salt must have the quinoid structure as shown by Formula II.

It is interesting to note that while fluorescein gives a *di*benzoate by the Baumann-Schotten reaction, tetrachlorofluorescein gives only a *mono*-benzoate. This is another indication of the fact that tetrachlorofluorescein is more acid than fluorescein (see p. 719).

The acetate of the monobenzoate (see p. 693) also shows the presence

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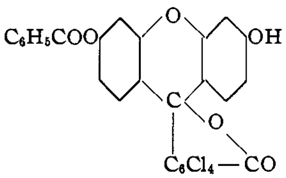
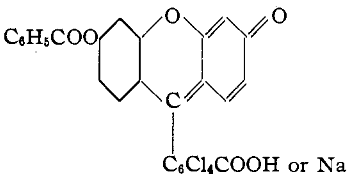
[1] *Am. Chem. J.*, 46, 10 (1911).



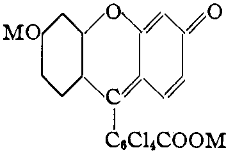
I. Tetrachlorofluorescein monobenzoate (colorless).



II. Tetrachlorofluorescein monobenzoate and its sodium salt (colored).

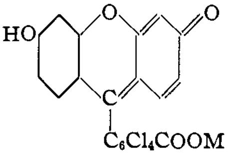
of two phenolic hydroxyl groups in tetrachlorofluorescein and as the compound is colorless and insoluble in alkalies it must have the lactoid structure and be represented by a formula similar to that on p. 715.

The dipotassium, disodium, and diammonium salts of tetrachlorofluorescein, because of their color, are considered to be derivatives of the quinoid form of tetrachlorofluorescein, and may be represented by the following general formula, where M represents the metal or the NH_4 group:



Di-metallic salts of tetrachlorofluorescein.

The colored monopotassium and monosodium salts and the red methyl ester are also derivatives of the quinoid form and may be represented by the general formula, where M represents the metal or the CH_3 group:

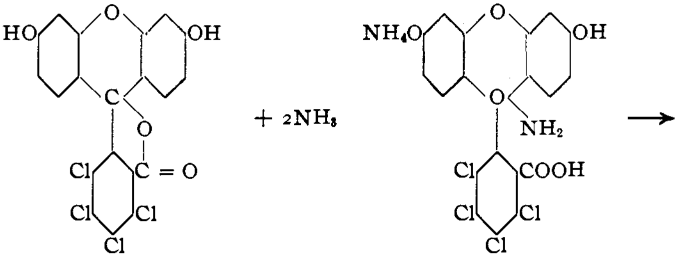


Mono-metallic salts and esters of tetrachlorofluorescein.

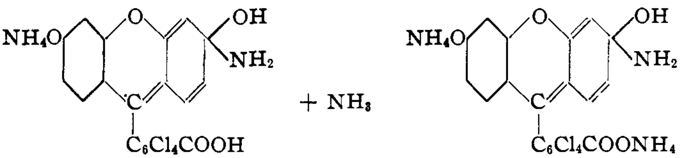
When dry ammonia acts upon the red anhydrous form of tetrachlorofluorescein, a diammonium salt is formed, which must be represented by the formula previously given for the di-metallic salts. When, however, the colorless anhydrous tetrachlorofluorescein is placed in a current of *dry* ammonia, an equivalent of *three* molecules of ammonia is absorbed, and a colored triammonia salt results. The diammonium salt loses all of its ammonia when heated to a temperature of 150°, and the *dark red*, anhydrous tetrachlorofluorescein remains, but when the colored triammonia salt is heated to the same temperature, all of the ammonia is lost and the *colorless* anhydrous tetrachlorofluorescein is obtained.

The formation of the triammonia salt may be represented in either of the following ways:

First method:



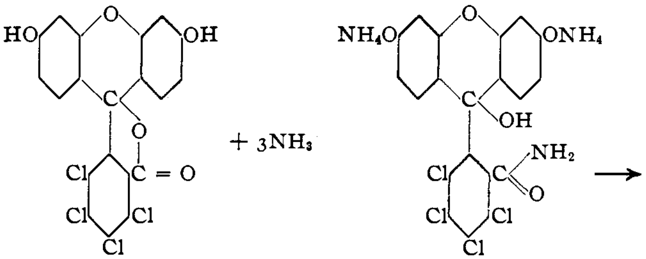
Tetrachlorofluorescein (colorless).



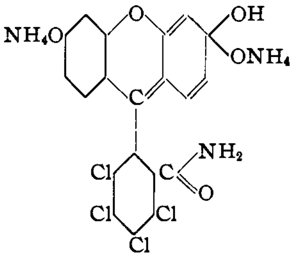
Triammonia salt of tetrachlorofluorescein.

(I)

Second method:



Tetrachlorofluorescein (colorless).



Triammonia salt of tetrachlorofluorescein.

(II)

The second method of formation is more in accord with the action of ammonia on certain lactones. Neugebauer[1] has found that the lactone

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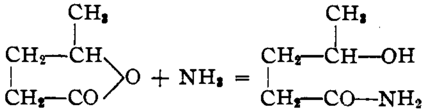
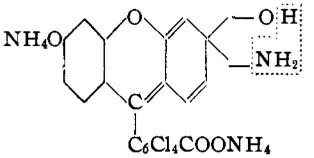
[1] *Ann.*, **227**, 103 (1885); also **256**, 147 (1889).

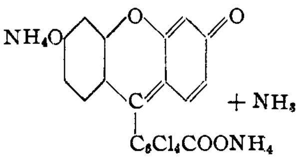
of γ-hydroxyvaleric acid is converted into the amide according to the following reaction:



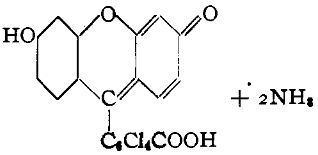
The second method of formation also seems the more probable from the fact that a triammonia salt having a structure represented by Formula II could lose three molecules of ammonia and form the lactoid modification of tetrachlorofluorescein—the steps of the formation being reversed. While a triammonia salt having a structure represented by Formula I would, upon the loss of one molecule of ammonia, probably be converted into the red diammonium salt of tetrachlorofluorescein. This diammonium salt would, as already shown, lose all of its ammonia upon heating and be converted into the red anhydrous tetrachlorofluorescein. This transformation may be represented as follows:



Triammonia salt of Tetrachloro-fluorescein. (I)

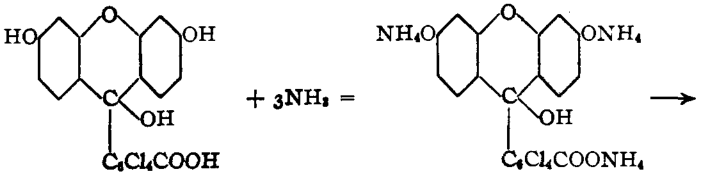


Diammonium salt of Tetrachlorofluorescein.

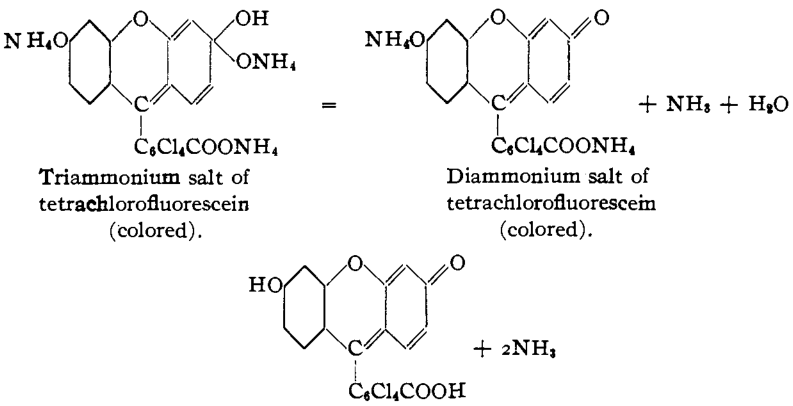


Tetrachlorofluorescein (colored).

This theory is further confirmed by the behavior of tetrachlorofluoresceincarbinolcarboxylic acid toward dry ammonia. This compound takes up an equivalent of *three* molecules of ammonia without loss of water, and when the salt thus formed is heated both water and ammonia are given off, and the red anhydrous tetrachlorofluorescein is obtained. These changes may be represented as follows:



Tetrachlorofluoresceincarbinolcarboxylic acid.

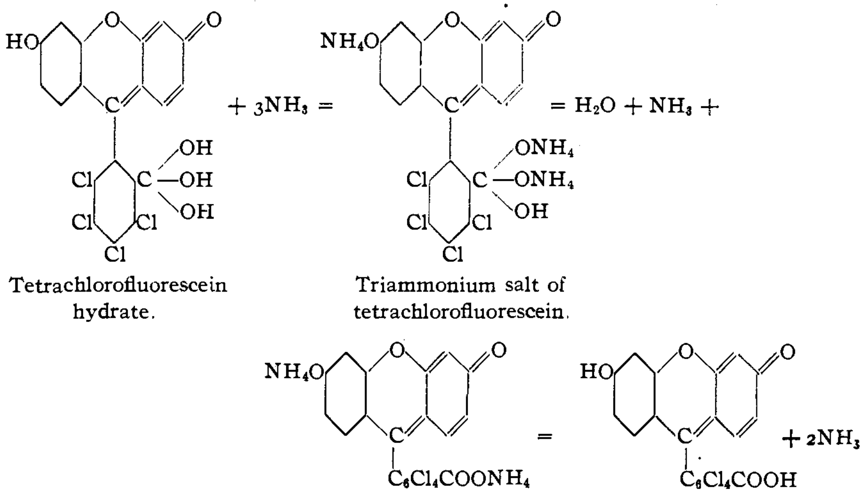


Triammonium salt of tetrachlorofluorescein (colored).

Diammonium salt of tetrachlorofluorescein (colored).

Tetrachlorofluorescein (colored).

The yellow hydrate when placed in dry ammonia also absorbs three molecules of ammonia without the loss of water. When this salt is heated both ammonia and water are lost, and the red anhydrous tetrachlorofluorescein is formed. These steps in the formation of this triammonium salt and its transformation when heated may be represented as follows:



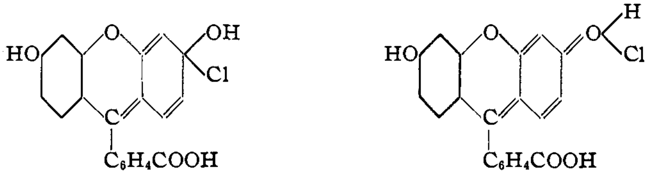
Tetrachlorofluorescein hydrate.

Triammonium salt of tetrachlorofluorescein.

Diammonium salt of tetrachlorofluorescein.

Tetrachlorofluorescein (colored).

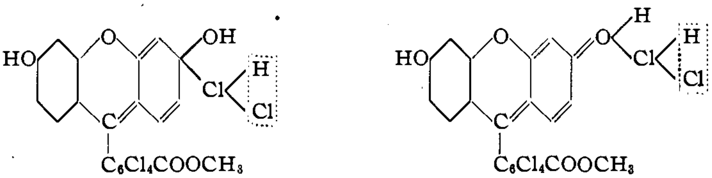
The behavior of this hydrate toward ammonia shows that the introduction of the four chlorine atoms into the phthalic acid residue of fluorescein increases its acid and decreases its basic properties. This is also shown by the fact that tetrachlorofluorescein is unchanged by boiling with concentrated hydrochloric acid, while fluorescein is converted into the hydrochloride by this treatment. When placed in dry hydrochloric acid gas, *fluorescein* also absorbs one molecule of hydrochloric acid and is converted into the hydrochloride (see p. 701), while neither the quinoid nor the lactoid form of tetrachlorofluorescein will absorb hydrochloric acid under these conditions. The hydrochloride of *fluorescein* may be regarded as either a carbonium or oxonium salt (Formulas I and II).



I. Carbonium salt. II. Oxonium salt.

Fluorescein hydrochloride.

The methyl ester of tetrachlorofluorescein, like the esters of tetrachlorogallein,[1] dissolves in hydrochloric acid (sp. gr. 1.10) with the formation of a hydrochloride. When this ester is treated with dry hydrochloric acid gas an equivalent of almost two molecules is absorbed. This dihydrochloride may be regarded as a carbonium or an oxonium salt as shown in Formulas I and II.

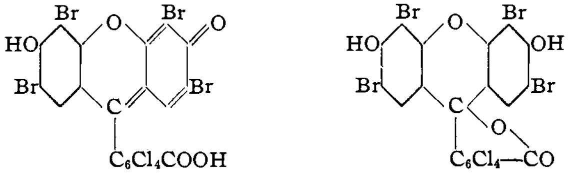


I. Carbonium salt. II. Oxonium salt.

Tetrachlorofluorescein methyl ester dihydrochloride.

Such a compound would lose one molecule of hydrochloric acid quite readily to form the monohydrochloride as shown above (see p. 700).

Tetrachloroeosin is similar, in many respects, to eosin. It forms two classes of derivatives, one colored, the other colorless. The colored compounds are regarded as derivatives from the quinoid form I, while the colorless compound must have the lactoid structure and be derivatives of the lactoid form II.



I. Quinoid form (colored). II. Lactoid form (colorless).

Tetrachloroeosin.

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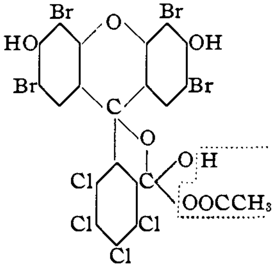
[1] *Am. Chem. J.*, 42, 258 (1909).

As examples of the first class there have been prepared, tetrachloroeosin hydrate (pink), and the dipotassium salt of tetrachloroeosin (dark red). The white diacetate and the compound with acetic acid are examples of the colorless lactoid derivatives of tetrachloroeosin.

When tetrachloroeosin is crystallized from glacial acetic acid a compound of tetrachloroeosin with acetic acid is formed. Since this substance is colorless and loses one molecule of acetic acid when heated, and gives the colorless tetrachloroeosin, it may be represented by the lactoid formula,



Compound of tetrachloroeosin with acetic acid.

It is interesting to note the effect of the introduction of substituents into the benzene nuclei of fluorescein upon the relative stability of the two possible forms of the free phthalein. Fluorescein in the free state exists only in the *colored* quinoid form. No colorless free fluorescein has ever been prepared. Tetrachlorofluorescein, on the other hand, has been obtained in two modifications, *colored* and *colorless*, but the colored form is the more stable. Eosin in the free state is flesh-colored, and may be a mixture of the two forms, while the free tetrachloroeosin is *colorless*, or only very slightly colored. The colored modification has not yet been prepared.[1]

Tetrachloroeosin, like tetrachlorofluorescein, does not absorb hydrochloric acid gas, nor is it changed by boiling with concentrated hydrochloric acid. That it is a much stronger acid than tetrachlorofluorescein is shown by its conduct toward ammonia. When placed in dry ammonia, the colorless tetrachloroeosin absorbs an equivalent of five molecules of ammonia, while the colorless tetrachlorofluorescein absorbs only three. When the red salt formed by the action of ammonia upon tetrachloroeosin is heated to 150°, all of the ammonia is lost and the colorless tetrachloroeosin remains. The formation of this compound from the white tetrachloroeosin and its transformation back into the white tetrachloroeosin may be represented as follows:

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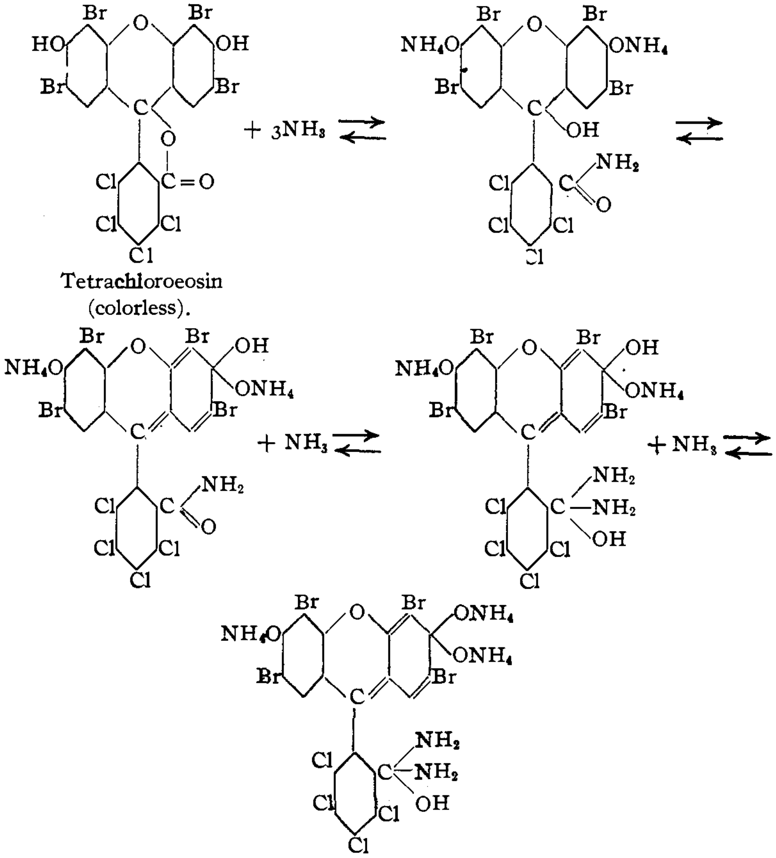
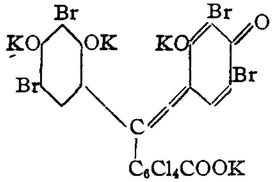
[1] The dark-red solution of tetrachloroeosin in nitrobenzene may contain the quinoid modification.



Tetrachloroeosin (colorless).

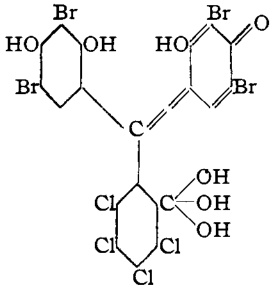
That the ammonia does not add to the pyrone oxygen to form a salt analogous to the tetrapotassium salt is shown by the fact that this ammonium salt dissolves in water with the pink color and greenish fluorescence so characteristic of tetrachloroeosin. The pink compound which precipitates when the aqueous solution of the *blue* tetrapotassium salt is acidified, dissolves in ammonia with a *blue* color and no fluorescence.

The increased acidity of tetrachloroeosin over tetrachlorofluorescein is also shown by the indications of the formation of a tetrapotassium salt when tetrachloroeosin is treated with a strong solution of potassium hydroxide (see p. 704). This salt would probably have the structure,



Tetrapotassium salt of tetrachloroeosin.

The pink compound formed when this strong alkaline solution is poured into an excess of acid would probably contain three phenolic hydroxyl groups and be represented by the formula:



This is a tetrabromo derivative of the true resorcinoltetrachlorophthalein hydrate.

That tetrachloroeosin is a stronger acid than tetrachlorofluorescein is also shown by the fact, stated on p. 706, that some of the disodium salt is always formed when tetrachloroeosin is treated with an alcoholic solution of sodium acetate. When tetrachlorofluorescein is treated with this reagent only the monosodium salt results.

The two isomers of tetrachlorofluorescein, Formulas I and II, have not yet been isolated. The very *dark red* material which was obtained from the crude product, and which dissolves in alkalies with a deep red color and an intense greenish fluorescence (see p. 686) may be a mixture of both of these isomers.

(I) (II)

Isomeric tetrachlorofluoresceins.

The absorption spectra of solutions of fluorescein, tetrachlorofluorescein, eosin, and tetrachloroeosin show in a marked degree the influence of the introduction of halogen substituents into the benzene nuclei of fluorescein. It will be noted that, in passing from fluorescein to tetrachloroeosin in the order named above, the absorption bands are gradually shifted towards the red end of the spectrum, and that this shifting is approximately proportional to the number of halogen atoms introduced rather than to the increase in the molecular weight of the compounds. For example, the absorption bands of tetrachlorofluorescein and eosin are practically identical while the molecular weights of these substances are 470 and 648, respectively.

The wave lengths of the crests of the fluorescence bands show a very interesting relation to the number and atomic weights of the halogens introduced. The introduction of *four chlorine* atoms into *one* benzene ring increases the wave length approximately the same amount as when *four bromine* atoms are introduced into *two* benzene rings. It is interesting to note that these increases in wave lengths are almost exactly in the ratio 4 Cl : 2 Br. For example, the difference between the wave length of the band for fluorescein and that for tetrachlorofluorescein is 24, and the difference for fluorescein and eosin is 28. This ratio, 24 : 28, or 6 : 7, is very nearly the same as that of 4 Cl : 2 Br, which is 6 : 6.76.

These results seem to confirm the theory that the seat of the fluorescence lies in the benzene ring, and that the introduction of substituents and the position these occupy in the molecule, brings the fluorescence within the visible part of the spectrum. The fact noted by Professors Nichols and Merritt that the more intense absorption band in the case of tetrachlorofluorescein, eosin and tetrachloroeosin is *double* would also seem to show that the absorption was due to the condition existing in the benzene ring for there are three absorption bands and three benzene rings.

Summary.

The results of this investigation may briefly be stated as follows:

1. Tetrachlorofluorescein has been prepared in two modifications, a *colored quinoid*, and a *colorless lactoid* form.

2. A colorless diacetate, a colorless dibenzoate, a colorless monobenzoate of tetrachlorofluorescein and a colorless acetate of this benzoate and colorless compounds of tetrachlorofluorescein with ether and with ethyl acetate have been prepared. These are all derivatives of the *lactoid* form of tetrachlorofluorescein.

3. The dipotassium, disodium, diammonium, monopotassium and monosodium salts, the hydrate and the methyl ester of tetrachlorofluorescein have been prepared. All of these compounds are colored and are therefore derivatives of the *quinoid* form of tetrachlorofluorescein.

4. A colorless carbinol carboxylic acid of tetrachlorofluorescein has been prepared. This substance is isomeric with the *yellow* hydrate, but forms the same *colored* salts with alkalies, and the same *colorless* diacetate as is obtained from this hydrate and from the anhydrous modifications of tetrachlorofluorescein.

5. The introduction of the four chlorine atoms into the fluorescein molecule increases its acid and decreases its basic properties. This is shown by the conduct of tetrachlorofluorescein toward hydrochloric acid and toward gaseous ammonia, as compared with that of fluorescein itself.

6. Tetrachloroeosin, which is the tetrabromo derivative of tetrachlorofluorescein, its compound with acetic acid, and its diacetate have been

made. These are all colorless compounds and therefore have the lactoid structure. Tetrachloroeosin itself has only been obtained in the *colorless lactoid* modification.

7. As examples of the derivatives of the colored quinoid form of tetrachloroeosin, the hydrate and the dipotassium salt have been prepared.

8. The introduction of four bromine atoms into the molecule of tetrachlorofluorescein increases the acidity of the compound, as is shown by the behavior of tetrachloroeosin toward gaseous ammonia and toward strong solutions of the alkalies.

9. Indications of the existence of the two possible isomeric tetrachlorofluoresceins have been obtained.

10. The absorption and fluorescence spectra of solutions of fluorescein tetrachlorofluorescein, eosin, and tetrachloroeosin have been studied. The theory of Stark and Meyer and of Kauffmann that the benzene nucleus is the seat of fluorescence, is apparently confirmed.

ITHACA, N. Y.

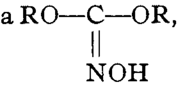
[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF CINCINNATI.]

OXIMIDOCARBONIC ESTERS AND RELATED COMPOUNDS.

BY LAUDER WILLIAM JONES AND RALPH E. OESPER.

Received February 20, 1914.

The experiments described in this paper were begun several years ago, but were interrupted before any definite conclusions were arrived at. Some time ago, the work was resumed; and, in view of the fact that, recently, Houben and Schmidt[1] have published an account of experiments in which they have duplicated some of our unpublished results, it seemed desirable that a preliminary announcement of our experiments should appear at this time.

The oximidocarbonic esters, *i. e.*, compounds of the formula  $\mathrm{RO{-}\underset{\underset{NOH}{\|}}{C}{-}OR}$, may be of two types: simple esters in which the two radicals are alike; and mixed esters in which they are different. The latter type is especially interesting, since it offers the possibility of obtaining geometrical stereoisomers in *syn-* and *anti*-modifications. This work was undertaken in the hope of isolating these stereoisomers, and, also, with the intention of investigating their behavior when they are subjected to the action of reagents which bring about the Beckmann rearrangement of oximes, etc.

We found that the preparation of the simple oximido esters offered no special difficulties, since they could be obtained from imidocarbonic esters by the action of hydroxylammonium chloride.

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[1] Houben and Schmidt, *Ber.*, 46, 2458 (1913).