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CXXVII.—Contributions to the Knowledge of Fluorescent Compounds. Part I. The Nitro-derivatives of Fluorescein.

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It is a remarkable fact that whereas the tetrabromo- and tetraiododerivatives of fluorescein show marked fluorescence in alkaline solutions, the corresponding nitro-compound exhibits no trace of this property. This fact has already been commented on by Richard Meyer (Zeit. physikal. Chem., 1897, 24, 468) and by one of the authors of the present communication (Proc., 1900, 16, 3; Zeit. physikal. Chem., 1900, 34, 1). Meyer, noticing that tetraiodofluorescein fluoresces less than tetrabromofluorescein, and this, in turn, less than fluorescein itself, would have been inclined to attribute the decrease in the fluorescence to an increase in the mass of the substituent groups, were it not that an alkaline solution of tetranitrofluorescein shows no trace of fluorescence whatever, although $NO_2 = 46$, whilst Br = 80 and I = 127. The view put forward by one of the present authors, that in the greater number of cases the fluorescence of organic compounds is caused by a doubly symmetrical tautomerism, furnishes a satisfactory explanation of the fluorescence of fluorescein, eosin, and tetraiodofluorescein where the only tautomerism possible is indicated in the following scheme:



whereas, for a nitro-derivative in which a nitro-group stands in the ortho-position relatively to a hydroxyl-group, it is not only possible, but indeed probable, that the nitrophenol group itself reacts tautometrically, producing a salt in which the metal is attached to the nitroxyl, and not to the hydroxyl grouping. The sodium salt of tetranitrofluorescein should then possess one of following constitutions:



In this case, the configuration would be stable enough to preclude tautomerism, due to the opening of the lactone ring, the oxygen of the hydroxyl groups having been previously transformed into the quinonoid form and sodium ions, thus losing their chance of migrating to the carboxyl group of the lactone ring.

If this view were correct, an exactly similar argument should apply to dinitrofluorescein, but although the so-called hydrate of dinitrofluorescein, which gives blue, non-fluorescent, alkaline solutions, has been prepared and analysed by von Baeyer (Annalen, 1876, 183, 32), the anhydrous substance of the formula $C_{20}H_{10}(NO_2)_2O_5$ does not appear to have been analysed. In purifying dinitrofluorescein, von Baeyer acetylated the crude product, recrystallised the diacetyl derivative from alcohol or ethyl acetate, and hydrolysed this by boiling with a solution of potassium or sodium hydroxide. On precipitation of the VOL. LXXVII. 4 x resulting blue solution with an acid, a yellow substance was obtained, which, even after recrystallisation, again dissolved in alkalis with a blue colour, von Baeyer's numbers for the compound agreeing with those required for the formula $C_{20}H_{12}(NO_2)_2O_6$. These results we can confirm, but by carrying out the hydrolysis of the diacetyl derivative with sulphuric acid instead of with an alkali, we have succeeded in obtaining the anhydrous dinitrofluorescein; we have also introduced various modifications in methods of purification which considerably simplify the process.

Although the anhydrous dinitro-derivative of fluorescein has been obtained and dissolves in cold alkali with the production of an orangebrown solution which evidently contains a salt of a substance of the formula $C_{20}H_{10}(NO_2)_2O_5$, the nitro-groups act in the way which might be expected from the generally accepted views as to the constitution of salts of nitrophenols, and preclude the double tautomerism required for fluorescence which would otherwise result from the opening of the The fact that a solution, even if unstable, of a sodium lactone ring. salt of dinitrofluorescein has been obtained, is of considerable importance, since we have thus been able to examine a derivative of fluorescein which, although still possessing the pyrone ring intact, nevertheless shows no fluorescence in alkaline solutions. The real significance of this result will be observed when it is stated that not only are the usual salts of dinitrofluorescein derived from a hydrate, but that in the case of tetranitrofluorescein it has not been possible to isolate any substance other than a hydrate having the formula $C_{20}H_{10}(NO_2)_4O_6$. Any arguments which might be drawn from the non-fluorescence of the salts of the so-called tetranitrofluorescein are hence invalid.

Preparation of Dinitrofluorescein Hydrate.-In the nitration of fluorescein, von Baeyer's directions with regard to temperature need careful attention. By following his instructions in adding 10 grams of fuming nitric acid to 5 grams of fluorescein dissolved in 100 grams of concentrated sulphuric acid cooled to 0°, and pouring into water immediately after the last addition of nitric acid has been effected, a bright vellow precipitate separates containing very little unaltered fluorescein, much of the dinitro-compound, and always some of the tetranitro-On the other hand, if the solution is allowed to stand derivative. overnight, tetranitrofluorescein is practically the only product, as it also is if the temperature is allowed to rise unduly. A very convenient method of separating the tetranitro- and dinitro-derivatives from one another is either to digest the mixture with a solution of sodium acetate, or to dissolve the whole precipitate obtained by pouring into water in hot, dilute sodium hydroxide, and then to precipitate the dinitro-compound with acetic acid, the solution being allowed to cool before filtering, as the dinitrofluorescein hydrate is fairly soluble in hot water. The colour exhibited by an alkaline solution of the residue shows that it is an almost pure dinitro-compound; for most purposes, it is merely necessary to recrystallise the substance from hot alcohol, whereby small, orange, rhomboidal plates are obtained, which dissolve in cold alkali with a beautiful blue colour. The solution, however, occasionally shows a slight green fluorescence, due to the presence of unattacked fluorescein. If this is the case, it is best to acetylate the product with acetic anhydride, and to recrystallise the acetyl derivative from glacial acetic acid until a sample gives an alkaline solution from which all fluorescence is absent. We confirmed the composition of the dinitrofluorescein hydrate so obtained by analysis

Diacetyldinitrofluorescein.—On acetylating dinitrofluorescein hydrate, water is split off, and the diacetyl derivative of dinitrofluorescein produced. This acetyl derivative also results on the acetylation of the crude product formed by adding nitric acid to the sulphuric acid solution of dinitrofluorescein, as von Baeyer has previously shown, whilst naturally it may also be obtained, in better yield and of greater purity, by the action of acetic anhydride on the residue left after the crude nitration product is extracted with a dilute sodium acetate solution. It crystallises in colourless rosettes of needles from glacial acetic acid. On analysis:

0.1729 gave 0.3632 CO_2 and 0.0450 H_2O . C = 57.28; H = 2.87. $C_{24}H_{14}O_{11}N_2$ requires C = 56.91; H = 2.76 per cent.

When diacetyldinitrofluorescein is boiled continuously for 10 hours with a mixture of ethyl alcohol and water to which ethyl acetate is added to increase its solubility, a yellow solution is obtained from which, on evaporation of the ethyl acetate and cooling of the mother liquors, small, yellow crystals separate out which partially dissolve in hot sodium carbonate or cold sodium hydroxide (a residue of unattacked diacetyl derivative being left) with an orange colour. On boiling with caustic soda, a blue colour is produced, as might be The substance might be dinitrofluorescein from the colour expected. of its solutions, although this is impossible, since the continued boiling with ethyl alcohol and water would convert any such compound into the hydrate, which dissolves with a blue shade in cold sodium Since the diacetyldinitrofluorescein is insoluble in soda, hydroxide. there seems only one possibility left, namely, that water has been added on to the substance, and a hydrate of the constitution

4 x 2



produced. The orange colour of the alkaline solution is precisely what might be expected from a nitrophenolic compound of this type.

Tetrasodium Salt of Dinitrofluorescein Hydrate.—When a solution of an excess of sodium ethoxide in absolute alcohol is added to a hot saturated alcoholic solution of dinitrofluorescein, an orange colour is observed changing to blue on the addition of a few drops of water. On stirring the solution, a crystalline, purple paste rapidly separates. This, when collected and washed with alcohol, was found to contain 4 atoms of sodium per molecule.

Anhydrous Dinitrofluorescein, $C_{20}H_{10}(NO_2)_2O_5$.—This substance may be obtained by heating the diacetyl derivative with five times its weight of 80 per cent. sulphuric acid for an hour on the water-bath. At first a clear brown solution was obtained, from which on further heating a canary precipitate separated, the remainder of the substance being thrown out by water. The dinitrofluorescein thus obtained was ground up to a fine paste with water, well washed, and finally dried at 110°.

0.1347 gave 0.2832 CO_2 and 0.0325 H_2O . C = 57.33; H = 2.68. $C_{20}H_{10}O_9N_2$ requires C = 56.80; H = 2.39 per cent.

The substance is sparingly soluble in strong alcohol, in which it dissolves with a reddish-yellow colour showing no fluorescence; the colour becomes somewhat green in shade on addition of a drop of hydrochloric acid. On long continued boiling with methylated spirit, it dissolves with a red colour and shows no fluorescence, addition of water precipi-It is somewhat soluble in acetic acid, and fairly tating the hydrate. so in ethyl acetate, from which it separates in small, radiate aggregates It dissolves in cold, dilute sodium hydroxide or in sodium of prisms. carbonate solution with an orange-brown colour; the solution exhibits no fluorescence. On warming a caustic soda solution, the colour soon changes to a deep blue, a salt of the hydrate being produced, but the orange or red solution in sodium carbonate does not become blue on boiling.

The transformations experienced by dinitrofluorescein and its derivatives may be expressed by the following table :



In this scheme, the dotted lines represent changes which have not been confirmed by analyses of the products, diacetyldinitrofluorescein hydrate not having been isolated in a sufficiently pure condition for the purpose.

Dibenzoyldinitrofluorescein, $C_{20}H_8(NO_2)_2(CO \cdot C_6H_5)_2O_6$.—This substance was prepared by boiling 3 grams of dinitrofluorescein with 10 grams of benzoyl chloride in a reflux apparatus until the evolution of hydrogen chloride ceased. The product was then poured into alcohol, and stirred until the pasty substance first precipitated became a brown, crystalline powder. This was collected, washed with alcohol, dried, and dissolved in boiling ethyl acetate. The solution was decolorised as far as possible with animal charcoal, filtered, and absolute alcohol added until the liquid began to show turbidity. On standing overnight, a glistening, colourless, crystalline powder had separated, which, under the microscope, proved to consist of rhomboidal plates, the smaller angles of which were about 75°.

0.1626 gave 6.5 c.c. moist nitrogen at 18° and 767 mm. N = 4.63. $C_{34}H_{18}O_{10}N_2$ requires N = 4.57 per cent.

The substance dissolves fairly easily in acetone, ethyl acetate, or pyridine; it is only taken up sparingly by benzene and ethyl alcohol. Its behaviour towards caustic soda solution corresponds with that of the diacetyl derivative.

Tetranitrofluorescein.—In preparing this compound von Baeyer acted on fluorescein with a considerable excess of fuming nitric acid (Annalen, 1876, 183, 33). We have found that by using corresponding quantities for dinitrofluorescein, but allowing the temperature to rise, or, preferably, the mixture to stand overnight, a very good yield of the tetranitro-derivative is obtained. This is isolated by pouring into water, when a pinkish paste separates, this is filtered, washed, and dissolved in a hot solution of sodium acetate. The solution is of a beautiful red colour, and, on cooling, deposits crystals of a sodium salt which, on recrystallisation, separates in small, brilliant red tetrahedra strongly resembling potassium dichromate in appearance. On precipitating the hot solution of the above sodium salt with a mineral acid, a pinkishwhite precipitate is obtained, which, after drying, has the composition of a hydrate of tetranitrofluorescein.

Von Baeyer does not mention the existence of a hydrate, but certainly our hydrate shows exactly the colour changes in alcoholic solution which he describes, namely, a yellowish-red colour with a yellowishgreen fluorescence, the colour becoming reddish-violet on the addition of a drop of concentrated hydrochloric acid. Noteworthy is the fact that in alkaline solution no fluorescence is observed, which agrees well with the hypothesis of the conversion of the nitrophenol group into the quinone-isonitro-group on addition of alkali.

$$\frac{\mathrm{HO} \cdot \mathrm{C}}{\mathrm{NO}_2 \cdot \mathrm{C}} + \mathrm{NaOH} = \mathrm{H}_2\mathrm{O} + \frac{\mathrm{O} \cdot \mathrm{C}}{\mathrm{NaNO}_2 \cdot \mathrm{C}}.$$

The analytical numbers obtained by von Baeyer agreed with those required for the formula $C_{20}H_8(NO_2)_4O_5$, and not with those for $C_{20}H_{10}(NO_2)_4O_6$; his product was, however, recrystallised from glacial acetic acid. It seemed very probable, therefore, that the compound $C_{20}H_{10}(NO_2)_4O_6$ had become dehydrated by continued boiling with glacial acetic acid, especially since we had found that dinitrofluorescein hydrate, under similar conditions, yields a product soluble in alkali with an orange colour. We repeated the recrystallisation from acetic acid, and on analysing the recrystallised product were astonished to find that we were still dealing with the hydrate; a repetition of the experiment gave the same result, although the tetranitrofluorescein hydrate was boiled with 50 parts of glacial acetic acid for at least 10 hours. The analyses refer to the two different recrystallised proparations.

0.1855 gave 0.3096 CO₂ and 0.0480 H₂O. C = 45.52; H = 2.87. 0.1378 , 0.2280 CO₂ , 0.0306 H₂O. C = 45.12; H = 2.47.

The sodium salt above mentioned must be looked upon as a derivative of this compound, $C_{20}H_{10}(NO_2)_4O_6$.

0.4474 air-dried salt lost 0.0870 H₂O at 110° . H₂O = 19.44. C₂₀H₈O₁₄N₄Na₂,8H₂O requires H₂O = 20.06 per cent.

0.3181 anhydrous salt gave 0.0900 Na₂SO₄. Na = 8.12.

 $C_{20}H_8O_{14}N_4Na_2$ requires Na = 8.04 per cent. $C_{20}H_6O_{13}N_4Na_2$, Na = 8.45,

A barium salt was obtained by boiling tetranitrofluorescein hydrate with barium carbonate and water so long as carbon dioxide was evolved. The filtered solution deposited small, red crystals on cooling; these contained 4 mols. of water of crystallisation.

 $0.3263 \text{ gave } 0.0308 \text{ H}_2\text{O} \text{ and } 0.0979 \text{ BaSO}_4.* \text{ H}_2\text{O} = 9.45 \text{ ; Ba} = 17.59.$ $C_{20}H_8O_{14}N_4Ba, 4H_2O \text{ requires } H_2O = 9.77 \text{ ; Ba} = 18.62 \text{ per cent.}$

Acetyl Derivative.—Von Baeyer mentions the preparation of an acetyl derivative, but gives no analytical data. We have also had such a compound in our hands, but also give no analyses, since every attempt at a combustion ended with an explosion, even when the substance was mixed with copper oxide in a bayonet tube. It is hoped that analytical data will eventually be obtained, as it would be of interest to see if closure of the pyrone ring takes place during acetylation.

Constitution of the Nitro-derivatives of Fluorescein.—It will be seen from the experimental details communicated in this paper that the entry of nitro-groups into the fluorescein molecule in the ortho-positions relatively to the hydroxyl groups destroys the fluorescence, dinitrofluorescein giving an orange, non-fluorescent solution with caustic soda, although this solution readily turns blue on standing or heating, the elements of water being added on. This result is of considerable importance, since any arguments deduced from the nonfluorescent character of salts of tetranitrofluorescein are invalidated by the fact that this compound has only been obtained in the form of a hydrate.⁺ Although there is no probability of dinitrofluorescein being other than an o-nitrophenol derivative, it nevertheless seemed advisable to determine the orientation of the nitro-groups if this were possible.

Dinitrofluorescein might equally well have either of the two formulæ

^{*} We were unable to obtain more satisfactory numbers for the percentage of barium, on account of the explosive nature of the salt.

[†] The term *hydrate* has been used throughout this paper for the derivatives obtained by the addition of water to the fluorescein compounds. These substances, which really result by the opening of the pyrone-ring, are analogues of phenolphthalein and it would be more systematic to give analogous names to them, thus dinitrofluorescein hydrate should be termed dinitroresorcinol-phthalein, &c.



although the latter is, perhaps, slightly more probable, as it avoids so much crowding of substituent groups in the ortho-position. We endeavoured to settle the point by fusion with alkali, a reaction very incompletely studied by von Baeyer (*loc. cit.*, 32). One of the products should be a nitroresorcinol, of which two have been described, the 1:3:4-derivative melting at 115° and the 1:3:2derivative at 85° (Weselsky, Annalen, 1872, 164, 1; Monatsh., 1880, 1, 887; Flitz, Ber., 1875, 8, 631).

Five grams of dinitrofluorescein hydrate were dissolved in 20 grams of 50 per cent. caustic potash solution and heated in a nickel basin until the blue colour had given place to a yellowish-brown shade. During the fusion, a certain amount of ammonia was evolved. The product was dissolved in water and acidified with hydrochloric acid, upon which a strong smell of hydrocyanic acid was noticeable. The liquid was filtered, the solid residue boiled with water, allowed to cool, and filtered, the operation being repeated several times. Nitroresorcinol, if produced during the fusion, should be found in the aqueous extract, which was in consequence repeatedly shaken with On distillation of the benzene extract, a small residue was benzene. obtained which, after two recrystallisations from water, with the addition of animal charcoal, gave a sufficient quantity of minute yellow crystals for the determination of a melting point. This was found to be 114° (uncorr.).

One point further has to be considered, namely, the position in the molecule where water is added on in the production of the so-called hydrates. In 1876, von Baeyer assumed that, in the production of fluorescein, oxide or ether formation took place between hydroxyl groups of the two resorcinol molecules, which condense with one molecule of phthalic anhydride. On the conversion of a fluorescein compound into a hydrate, this oxygen atom was supposed to take up a molecule of water, the change being represented by the equation

$$\mathbf{C}_{6}\mathbf{H}_{4} \underbrace{<}_{\mathbf{CO} \cdot \mathbf{C}_{6}}^{\mathbf{CO} \cdot \mathbf{C}_{6}} \underbrace{\mathbf{H}_{3}(\mathbf{OH})}_{\mathbf{OH}} \underbrace{>} \mathbf{O} + \mathbf{H}_{2}\mathbf{O} = \mathbf{C}_{6}\mathbf{H}_{4} \underbrace{<}_{\mathbf{CO} \cdot \mathbf{C}_{6}}^{\mathbf{CO} \cdot \mathbf{C}_{6}} \underbrace{\mathbf{H}_{3}(\mathbf{OH})_{2}}_{\mathbf{OH}},$$

On the substitution of the lactone for the diketonic formula for fluorescein, the idea seems to have arisen that water was added to the lactone group. For example, in Beilstein's *Handbuch* (2te Aufl. Bd. II., 1296) dinitrofluorescein is formulated as

 $O[C_6H_2(NO_2)OH]_2C(OH) \cdot C_6H_4 \cdot CO_2H.$

Such an expression seems to us to be untenable; in its place we would substitute a formula in which the addition of water resulted in the splitting of the pyrone ring in the following manner:



The grounds on which this view is based are as follows.

1. Fluorescein itself is very stable towards alkali, the tetrabromoderivative takes up water more easily than fluorescein, whilst with dinitrofluorescein the hydrate is the stable form and with tetranitrofluorescein the hydrate is the only form we have so far been able to isolate. Negative groups introduced into a benzene nucleus are known to influence other groups in the same nucleus and especially when the substituents are in ortho- or para-positions relatively to one another. That the negative groups should influence the extranucleal lactone ring, however, is, so far as we are aware, without analogy.

2. The pyrone ring of fluorescein is evidently weakened by the introduction of nitro-groups as shown by the fact that an acridine derivative results when dinitrofluorescein is treated with cold ammonia solution (Reverdin, *Ber.*, 1897, **30**, 332).

3. The blue colour of the solution of dinitrofluorescein hydrate in alkali is not dissimilar to the colour exhibited by the phthaleins; at any rate, it resembles this far more than the red or brown solutions produced when fluorescein or fluorone derivatives are dissolved in an alkali. This argument does not lose in weight by the fact that dinitrofluorescein itself dissolves in cold alkali with an orange shade.

4. The formation of a tetra-sodium salt from dinitrofluorescein hydrate affords fairly conclusive evidence in favour of this view; had the lactone ring been opened a trisodium salt would be expected, as is shown on consideration of the two possible formulæ:



As a result of this work the conclusion may be drawn that the introduction of nitro-groups into the fluorescein molecule in the orthoposition relatively to the hydroxyl groups prevents fluorescence in alkaline solutions, owing to the usual phenol-lactone and quinonoidcarboxylic acid tautomerism of fluorescein being inhibited by a secondary tautomerism between the nitro- and phenolic hydroxyl groups. At the same time, the pyrone ring is so much weakened that water is readily added on with production of a true phthalein.

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