

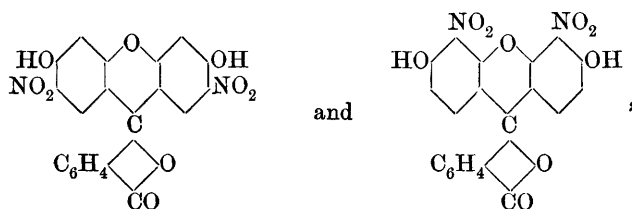
XCI.—*Bromonitro-derivatives of Fluorescein.*

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SOME eighteen months ago, one of the authors of the present communication endeavoured to explain why dinitro- and tetranitro-fluoresceins, in contradistinction to the parent substance, gave non-fluorescent solutions when dissolved in dilute alkalis (Hewitt and Perkins, *Trans.*, 1900, **77**, 1324). In the paper in question, it was shown that, not only does the hydrate of dinitrofluorescein give non-fluorescent solutions, but that if dinitrofluorescein itself, as obtained by the acid hydrolysis of its diacetyl derivative and containing an intact pyrone ring, be dissolved in alkali, the brown solution shows no fluorescence.

At the time, the following explanation was given: If an *o*-nitrophenol be dissolved in an alkali the metal becomes attached to the nitroxyl and not to the hydroxyl group; in consequence of the tautomeric quinonoid structure so set up, the usual double symmetrical tautomerism of fluorescein and its derivatives is obscured and the fluorescence disappears.

One thing is certain, the nitro-groups will enter the fluorescein nucleus in the ortho-position relatively to hydroxyl on account of the orientating influence of these groups. Eosin is the only case in which the orientation of substituent groups in fluorescein has been fully worked out. The bromine atoms occupy the four ortho-positions relatively to the hydroxyl groups (Heller, *Ber.*, 1895, **28**, 315; R. and H. Meyer, *Ber.*, 1896, **29**, 2624). Of the two possible formulæ,



for dinitrofluorescein, the first was regarded as the more probable it is now shown, however, that the latter must be regarded as correct.

In effecting the fission by fusion with alkali, Hewitt and Perkins isolated a minute quantity of a substance melting at  $114^{\circ}$ ; since 4-nitroresorcinol melts at  $115^{\circ}$ , it was supposed that the substance obtained was identical with this. Almost immediately after the publication of the paper referred to, one of the authors received a letter from M. Reverdin pointing out that L. Matras had some years previously obtained 2-nitroresorcinol by the fission of dinitrofluorescein (*Chem.*

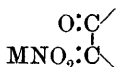
*Zeit.*, 1895, **19**, 408 ; *Arch. Sci. phys. nat.*, 1895, [iii], **33**, 285), and on repeating the experiment his results proved to be correct. Dinitro-fluorescein or its hydrate is dissolved in hot 10 per cent. sodium hydroxide solution and warmed until the deep blue colour has disappeared and is replaced by a dirty brown shade ; the solution is then acidified with dilute sulphuric acid and distilled in a current of steam. Nitro-resorcinol is found in the distillate ; some separates in a crystalline form, the remainder may be extracted with ether, and after a further recrystallisation from this solvent the orange-red plates can be identified by their melting point, 85°. A specimen obtained in this way was analysed, with the following result, the gas being measured over 50 per cent. potassium hydroxide solution :

0.1342 gave 10.6 c.c. nitrogen at 19° and 742 mm.  $N = 8.79$ .

$C_6H_5O_4N$  requires  $N = 9.05$  per cent.

The other product of fission is difficult to isolate ; doubtless, however, it was the substance in a more or less pure condition which showed a melting point of 114° and gave rise to the mistaken idea that dinitro-fluorescein was a derivative of 4-nitroresorcinol.

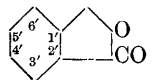
The argument with respect to the formulæ of the alkali salts, representing these substances as possessing the grouping



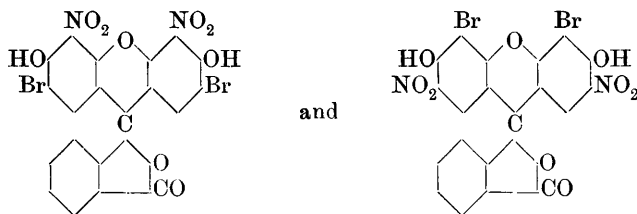
(where  $M = \text{Na}, \text{K}, \&c.$ ), is in no way affected, since whether the nitro-groups are in the positions 2 and 7 or 4 and 5, they are in either case in ortho-positions relatively to the hydroxyl groups of fluorescein.\* Another point is, however, raised ; if the nitro-groups in positions 4 and 5 are favourable to the opening of the pyrone ring, will they have a similar action when in positions 2 and 7 ?

To decide the matter, it was necessary to obtain compounds of the desired type, and the most readily obtainable substances for purposes of comparison appeared to be the two dibromodinitrofluoresceins of the respective formulæ

\* With respect to the nomenclature of complicated fluorescein derivatives, it seems as if it would be best to look upon them as derivatives of fluoran, the ring of which must then be numbered in a systematic manner. If Richter's numbering for the xanthen ring be adopted and positions in the phthalic acid residue be represented by dashed numbers, the positions in fluoran will then be as follows :



Fluorescein will become 3:6-dihydroxyfluoran, and eosin 2:4:5:7-tetrabromo-3:6-dihydroxyfluoran, or shortly 2:4:5:7-tetrabromofluorescein. This nomenclature has been adopted throughout the paper.



*2:7-Dibromo-4:5-dinitrofluorescein.*—The dinitrofluorescein used for the bromination experiments was prepared essentially according to the modification given by Hewitt and Perkins (*Trans.*, 1900, **77**, 1326) of Baeyer's original method (*Annalen*, 1876, **183**, 32). The process may, however, be somewhat shortened by dissolving 5 grams of fluorescein in 100 c.c. of concentrated sulphuric acid, adding 10 grams of nitric acid (sp. gr. 1·36) without any especial precaution with regard to cooling, and then allowing the whole to stand for a short time until a drop of the acid mixture boiled with an excess of caustic soda shows a clear blue colour but no trace of fluorescence. The whole is then poured into water, collected, washed, dissolved in 10 per cent. sodium hydroxide solution, heated on the water-bath until the solution has turned blue, cooled, precipitated with acetic acid, and the precipitate collected and recrystallised from dilute spirit.

The bromination was carried out by mixing solutions in glacial acetic acid of 4 parts of dinitrofluorescein hydrate and 3 parts of bromine. The mixture was heated to boiling and allowed to cool, yellowish-brown needles separated which, although turning dark red about 270°, do not melt at 310°. The preparation obtained in this way is the anhydrous dibromodinitrofluorescein, the ring apparently being closed by the boiling with acetic and hydrobromic acids. For analysis, the substance was again recrystallised from ethyl acetate :

0·1620 gave 0·2444 CO<sub>2</sub> and 0·0293 H<sub>2</sub>O. C = 41·14; H = 2·01.

0·2871 „ 12·7 c.c. nitrogen at 16° and 748 mm. N = 5·12.

0·1386 „ 0·0902 AgBr. Br = 27·65.

C<sub>20</sub>H<sub>8</sub>O<sub>9</sub>N<sub>2</sub>Br<sub>2</sub> requires C = 41·38; H = 1·38; N = 4·83; Br = 27·58

The substance dissolves in alcohol, ether, acetone, ethyl acetate, or pyridine (? formation of a salt). Caustic soda, if dilute, dissolves it in the cold with a brown colour and no fluorescence, the solution readily turns blue on warming, the shade developed is rather more inclined to green than in the case of 4:5-dinitrofluorescein.

*Dibromodinitrofluorescein hydrate*, C<sub>20</sub>H<sub>10</sub>O<sub>6</sub>Br<sub>2</sub>(NO<sub>2</sub>)<sub>2</sub>, is obtained by acidifying of the blue alkaline solution. The precipitate furnished the following numbers after collection, washing, and drying in a desiccator (five days over sulphuric acid) :

0.1868 gave 0.2700  $\text{CO}_2$  and 0.0275  $\text{H}_2\text{O}$ .  $\text{C} = 39.42$  ;  $\text{H} = 1.63$ .

$\text{C}_{20}\text{H}_{10}\text{O}_{10}\text{N}_2\text{Br}_2$  requires  $\text{C} = 40.13$  ;  $\text{H} = 1.67$  per cent.

This substance dissolves in cold dilute caustic soda solution with an immediate blue colour.

The *acetyl* derivative,  $\text{C}_{20}\text{H}_6\text{O}_5\text{Br}_2(\text{NO}_2)_2(\text{O}\cdot\text{CO}\cdot\text{CH}_3)_2$ , was obtained by boiling the substance in a reflux apparatus for about 1 hour with four times the weight of acetic anhydride. The substance, which is sparingly soluble in all the usual solvents, separates from the acetic anhydride during the progress of the acetylation ; for purification, it was dissolved in a large quantity of acetone, a small quantity of alcohol added, and the acetone evaporated off at a gentle heat. White crystals were obtained melting at  $276^\circ$  with decomposition :

0.1136 gave 0.0651  $\text{AgBr}$ .  $\text{Br} = 23.76$ .

$\text{C}_{24}\text{H}_{12}\text{O}_{11}\text{N}_2\text{Br}_2$  requires  $\text{Br} = 24.10$  per cent.

The *benzoyl* derivative,  $\text{C}_{20}\text{H}_6\text{O}_5\text{Br}_2(\text{NO}_2)_2(\text{O}\cdot\text{CO}\cdot\text{C}_6\text{H}_5)_2$ , was prepared by boiling dibromodinitrofluorescein in a reflux apparatus with four times its weight of benzoyl chloride until the evolution of hydrogen chloride had ceased (about 1 hour). The product was poured into spirit ; a pasty mass separated, turning to a yellow, crystalline powder when stirred. This was filtered off, washed with spirit, and recrystallised from benzene. Pale yellow crystals are thus obtained ; a subsequent recrystallisation from ethyl acetate furnishes colourless needles which melt at  $315^\circ$  with decomposition :

0.1284 gave 0.2419  $\text{CO}_2$  and 0.0318  $\text{H}_2\text{O}$ .  $\text{C} = 51.37$  ;  $\text{H} = 2.75$ .

$\text{C}_{34}\text{H}_{16}\text{O}_{11}\text{N}_2\text{Br}_2$  requires  $\text{C} = 51.77$  ;  $\text{H} = 2.03$  per cent.

*Salts of 2:7-Dibromo-4:5-dinitrofluorescein.*—A solution of the sodium salt was obtained by dissolving the acid to saturation in boiling sodium carbonate solution ; on sufficient concentration, crystals (apparently regular tetrahedra) were deposited. After drying in the air, these furnished the following numbers on analysis :

0.1807 lost 0.0310  $\text{H}_2\text{O}$  at  $110^\circ$ .  $\text{H}_2\text{O} = 5.82$ .

$\text{C}_{20}\text{H}_6\text{O}_9\text{N}_2\text{Br}_2\text{Na}_2\cdot 2\text{H}_2\text{O}$  requires  $\text{H}_2\text{O} = 5.46$  per cent.

0.1497 dried salt gave 0.0331  $\text{Na}_2\text{SO}_4$ .  $\text{Na} = 7.17$ .

$\text{C}_{20}\text{H}_6\text{O}_9\text{N}_2\text{Br}_2\text{Na}_2$  requires  $\text{Na} = 7.38$  per cent.

This salt dissolves in water with an orange-brown shade, obviously as a derivative of a substance containing the pyrone ring intact. On boiling the aqueous solution, the colour does not alter ; addition of sodium hydroxide to the boiling aqueous solution immediately changes the colour to dark blue. The orange solution furnishes the following precipitates.

*Silver Nitrate*.—Orange precipitate, soluble in boiling water and separating as a crimson, crystalline powder on cooling.

*Mercurous Nitrate*.—Flocculent red precipitate. Insoluble in boiling water, but becoming more crimson in shade when heated.

*Lead Acetate*.—Orange precipitate, soluble in much boiling water. Separates on cooling as small crystals possessing a bronzy glance.

*Mercuric Chloride*.—No precipitate with moderate concentrations either hot or cold.

*Cadmium Sulphate*.—Orange precipitate, soluble in boiling water, separates as an orange powder on cooling.

*Copper Sulphate*.—Yellowish-orange precipitate, becomes crystalline on boiling.

*Aluminium Sulphate*.—Orange precipitate, dissolves to a certain extent on boiling.

*Chromium Chloride* (green solution).—Dirty brown precipitate.

*Ferric Chloride*.—Flocculent orange-brown precipitate.

*Ferrous Sulphate*.—Flocculent orange precipitate.

*Zinc Sulphate*.—No precipitate with moderate concentration either hot or cold.

*Barium and Calcium Chlorides*.—Orange precipitates, somewhat soluble.

*Magnesium Sulphate*.—No precipitate with moderate concentrations.

*Dyeing Experiment*.—Silk was dyed with a  $2\frac{1}{2}$  per cent. solution of 2 : 7-dibromo-4 : 5-dinitrofluorescein in the form of its hydrate. The colour produced is orange, the shade is altogether much yellower than that given by eosin, and much resembles that given by tetranitrofluorescein. Dinitrofluorescein hydrate gives a pale yellow on silk, the colours produced generally by nitrofluoresceins with nitro-groups in positions 4 and 5 are those generally associated with nitrophenols.

4 : 5-Dibromo-2 : 7-dinitrofluorescein is obtained either by the nitration of 4 : 5-dibromofluorescein or by the action of bromine on tetranitrofluorescein. The necessary dibromofluorescein was prepared by Baeyer's method and recrystallised from a mixture of alcohol and acetic acid. In addition to Baeyer's description of this compound, we may add that we obtained the *benzoyl* derivative by boiling the substance in a reflux apparatus with twice its weight of benzoyl chloride until the evolution of hydrogen chloride ceased. The product was poured into alcohol, and when the mass had set hard it was roughly purified by solution in benzene and precipitation with petroleum spirit, and then twice recrystallised from benzene. Small, colourless needles melting at  $240-244^{\circ}$  were obtained :

0.1123 gave 0.2420  $\text{CO}_2$  and 0.0273  $\text{H}_2\text{O}$ .  $\text{C} = 58.77$ ;  $\text{H} = 2.50$ .

$\text{C}_{34}\text{H}_{28}\text{O}_7\text{Br}_2$  requires  $\text{C} = 58.45$ ;  $\text{H} = 2.58$  per cent.

*Nitration of Dibromofluorescein.*—Ten grams of dibromofluorescein were dissolved in 300 grams of concentrated sulphuric acid and cooled to  $0^\circ$ . A mixture of 5 grams of nitric acid (sp. gr. 1.36) and 20 grams of concentrated sulphuric acid was added drop by drop, the mixture being well stirred meanwhile. The acid was then poured on to crushed ice, the precipitate collected, well washed, and recrystallised from hot acetone or a mixture of acetone and 50 per cent. acetic acid. The yield of dried crude product, adopting the precaution of cooling above mentioned, is 115 per cent. on the weight of the dibromofluorescein or 90 per cent. of the theoretical:

0.1847 gave 0.2788  $\text{CO}_2$  and 0.0246  $\text{H}_2\text{O}$ .  $\text{C} = 41.68$ ;  $\text{H} = 1.47$ .

$\text{C}_{20}\text{H}_8\text{O}_9\text{N}_2\text{Br}_2$  requires  $\text{C} = 41.38$ ;  $\text{H} = 1.38$  per cent.

The substance, although becoming dark red in colour when heated, does not melt even at  $310^\circ$ ; it is very sparingly soluble in the usual organic solvents, but taken up fairly easily by hot acetone, especially when freshly precipitated. The solution in alkalis is a magnificent crimson in shade and exhibits no fluorescence. Silk is dyed a crimson purple from neutral solution in presence of sodium sulphate, but the colour is readily discharged by mineral acids. The alkaline solution, even if boiled for some time, shows no indication of opening of the pyrone ring, from which we conclude that the nitro-groups, if in para-positions relatively to the pyrone oxygen atom, do not conduce to the same easy hydrolysis with alkali which they do if they are in the ortho-position.

*Action of Bromine on Tetranitrofluorescein.*—If tetranitrofluorescein be covered with eight times its weight of glacial acetic acid and its own weight of bromine be added, oxides of nitrogen are evolved on gentle warming and the reaction is soon completed. The substance may be collected and purified in the usual manner. The fact that two nitro-groups may be eliminated and replaced by bromine has already been noticed by Baeyer; it is not, however, perfectly clear from his description whether the same product is obtained as that produced when nitric acid acts on dibromofluorescein, or whether it is the substance formed on brominating dinitrofluorescein. That confusion has arisen is obvious from the following account of the preparation of dinitrodibromofluorescein given in Morley and Muir's *Dictionary* (1888, vol. I, p. 591). "From dibromofluorescein and  $\text{HNO}_3$  or from dinitrofluorescein and  $\text{Br}$ ." It would be remarkable if the same substance were produced by the two different methods; experiment shows that the two compounds are entirely distinct in properties. The identity of the substance obtained with that produced by nitrating dibromofluorescein was

established by a dye trial, the skeins dyed with the two preparations showing precisely the same shade, quite different from that produced by the isomeric compound.

*Acetyl Derivative.*—Two grams of 4 : 5-dibromo-2 : 7-dinitrofluorescein were boiled in a reflux apparatus for 3 hours with 8 grams of acetic anhydride. On pouring into 50 c.c. of spirit, the acetyl derivative separated in brownish needles. For purification, the substance was dissolved in boiling ethyl acetate, the solution filtered, and an equal bulk of spirit added. The crystals deposited were collected ; a further crop was obtained from the mother liquor :

0.1415 gave 0.2210  $\text{CO}_2$  and 0.0320  $\text{H}_2\text{O}$ .  $\text{C} = 42.59$  ;  $\text{H} = 2.51$ .

$\text{C}_{24}\text{H}_{12}\text{O}_{11}\text{N}_2\text{Br}_2$  requires  $\text{C} = 43.37$  ;  $\text{H} = 1.83$  per cent.

The substance melts at  $215^\circ$  and is but sparingly soluble in most solvents ; esters and benzenoid hydrocarbons take it up with greater ease than do the alcohols.

The *benzoyl* derivative was prepared by boiling the dibromodinitro-compound with five times its weight of benzoyl chloride for half-an-hour, pouring the product into spirit, and stirring until hard. Purification was effected by solution in chloroform, adding spirit, and allowing the substance to crystallise slowly. Small, colourless needles were obtained ; when heated, these redden and finally melt at  $301^\circ$  with decomposition :

0.1118 gave 0.2120  $\text{CO}_2$  and 0.0256  $\text{H}_2\text{O}$ .  $\text{C} = 51.75$  ;  $\text{H} = 2.54$ .

$\text{C}_{34}\text{H}_{16}\text{O}_{11}\text{N}_2\text{Br}_2$  requires  $\text{C} = 51.77$  ;  $\text{H} = 2.03$  per cent.

The *sodium* salt was prepared by dissolving 4 : 5-dibromo-2 : 7-dinitrofluorescein to saturation in hot sodium carbonate solution. The salt produced appears to crystallise in an anhydrous state ; after drying in the air, it lost only 1.02 per cent. at  $110^\circ$ . The dried salt was analysed, with the following result :

0.1020 gave 0.0210  $\text{Na}_2\text{SO}_4$ .  $\text{Na} = 6.68$ .

$\text{C}_{20}\text{H}_6\text{O}_9\text{N}_2\text{Br}_2\text{Na}_2$  requires  $\text{Na} = 7.17$  per cent.

An aqueous solution exhibited the following reactions with solutions of salts :

*Silver Nitrate.*—A crimson precipitate somewhat soluble in water.

*Mercurous Nitrate.*—A scarlet, insoluble precipitate.

*Mercuric Chloride.*—No precipitate with cold dilute solutions, turbidity produced on boiling.

*Lead Acetate.*—Crimson precipitate, somewhat soluble in water.

*Copper Sulphate.*—Greyish-brown, insoluble precipitate.

*Cadmium Sulphate.*—Pink, insoluble precipitate.

*Aluminium Sulphate*.—Pink precipitate.

*Ferrous Sulphate*.—Reddish-brown precipitate.

*Ferric Chloride*.—Reddish-brown, flocculent precipitate.

*Zinc Sulphate*.—Red, crystalline precipitate, distinctly soluble in water.

*Barium* and *calcium chlorides* give no precipitates at moderate dilutions in the cold, but crimson, crystalline powders are deposited on heating.

*Magnesium Sulphate*.—A flocculent, light pink precipitate.

One further point deserves attention. Reverdin has shown that 4 : 5-dinitrofluorescein, when treated with ammonia solution, turns orange owing to the formation of a yellow dyestuff, in which the pyrone oxygen atom is replaced by an imino-group (*Ber.*, 1897, 30, 332).

2 : 7-Dibromo-4 : 5-dinitrofluorescein apparently behaves in a perfectly similar fashion ; when covered with strong ammonia and then heated, a brown solution is obtained, the colour of which is not changed to blue even on boiling with caustic soda. The behaviour of 4 : 5-dibromo-2 : 7-dinitrofluorescein is, however, quite different ; even boiling with strong ammonia solution results only in the development of the same shade as is obtained with caustic soda ; the oxygen of the pyrone ring is firmly attached in this compound, so that the nitro-groups must necessarily be in ortho-positions relatively to the pyrone oxygen atom before exerting any appreciable influence upon it.

In conclusion, we wish to state that the expenses incurred in this work have been defrayed out of a grant received from the Government Grant Committee of the Royal Society.

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