

*2-Methyl-3-i-butyl-5-nitro-4-ketodihydroquinazoline,*

$$O_2N.C_6H_5 \begin{cases} N = C-CH_3 \\ | \\ CO-N-C_4H_9(i) \end{cases}$$
, from nitroacetantranil and iso-  
 butylamine, melts at 202-203° (corr.). Nitrogen found, 16.08.  
 Calculated for  $C_{13}H_{15}O_3N_3$ , 16.09.

*2-Methyl-3-secondary butyl-5-nitro-4-ketodihydroquinazoline,*

$$O_2N.C_6H_5 \begin{cases} N = C-CH_3 \\ | \\ CO-N-C_4H_9(\text{secondary}) \end{cases}$$
, from nitroacetantranil and  
 secondary butylamine, melts at 209-210° (corr.) Nitrogen found,  
 15.84. Calculated for  $C_{13}H_{15}O_3N_3$ , 16.09.

*2-Methyl-3-i-amyl-5-nitro-4-ketodihydroquinazoline,*

$$O_2N.C_6H_5 \begin{cases} N = C-CH_3 \\ | \\ CO-N-C_5H_{11}(i) \end{cases}$$
, from nitroacetantranil and iso-  
 amylamine, melts at 213-214° (corr.). Found: C, 60.82, 60.74;  
 H, 6.12, 6.41; N, 15.17, 15.35. Calculated for  $C_{14}H_{17}O_3N_3$ : C,  
 61.09; H, 6.18; N, 15.27.

*2-Methyl-3-allyl-5-nitro-4-ketodihydroquinazoline,*

$$O_2N.C_6H_5 \begin{cases} N = C-CH_3 \\ | \\ CO-N-C_3H_5 \end{cases}$$
, from nitroacetantranil and allyl-  
 amine, melts at 160-161° (corr.). It is dimorphous, crystallizing in  
 rhombic plates or long needles. Nitrogen found, 16.95. Cal-  
 culated for  $C_{12}H_{11}O_3N_3$ , 17.14.

HAVEMEYER LABORATORIES, COLUMBIA UNIVERSITY,  
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[CONTRIBUTIONS FROM THE HAVEMEYER LABORATORIES OF COLUMBIA  
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## SOME EXPERIMENTS ON THE NITRO DERIVATIVES OF FLUORESCEIN.<sup>1</sup>

BY MARSTON TAYLOR BOGERT AND RALPH GARRIGUE WRIGHT.

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A STUDY of the nitro and amino derivatives of fluorescein has been undertaken, to determine the influence of these groups upon the fluorescence of this well-known substance, both as re-

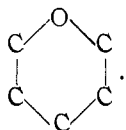
<sup>1</sup> Read at the General Meeting of the American Chemical Society, June 22, 1905. The fluorescein for this investigation was generously supplied by the firm of Kuttroff, Pickhardt & Co., to whom we wish to express our thanks.

gards the number of the groups and their position in the molecule. It is not unlikely, for example, that the influence of the groups present on the phthalic acid nucleus will be found to be quite different from that of similar groups on the resorcinol nuclei.

As the work must now be interrupted for several months, it seems advisable to present the results of our preliminary experiments to date, incomplete as they are. Considerable difficulty was encountered in the reduction of some of the nitro compounds, and much time was consumed in fruitless efforts to isolate these reduction products in sufficient purity to analyze.

Fluorescence is believed to be due to the absorption of light of a certain wave-length and its emission from the absorbing medium as light of a different wave-length. There have been many theories advanced from time to time to account for this phenomenon. Those of R. Meyer<sup>1</sup> and of Hewitt<sup>2</sup> are particularly interesting to organic chemists.

Meyer attributes the fluorescence of organic compounds in solution to the presence in their molecules of a so-called "fluorophore" group. In the fluoresceins, for example, it is the pyrone ring,



The fluorophore group, however, is not in itself sufficient to cause fluorescence. It must be united with other, generally denser, atomic complexes. Its effect is influenced by substitution, and by the character of the solvent used. The influence of substituting groups in weakening the fluorescence is usually directly proportional to the molecular weight of the substituting group. The nitro group, however, seems to constitute a notable exception, its effect upon the fluorescence being much greater than that of the heavier bromine and iodine atoms. Thus, while the eosins and erythrosins are strongly fluorescent, the entrance of a single nitro group into fluorescein weakens the fluorescence very greatly, while in dinitrofluorescein<sup>3</sup> fluorescence is totally lacking. Rather strangely, however, the so-called "tetranitrofluorescein," although showing no fluorescence in

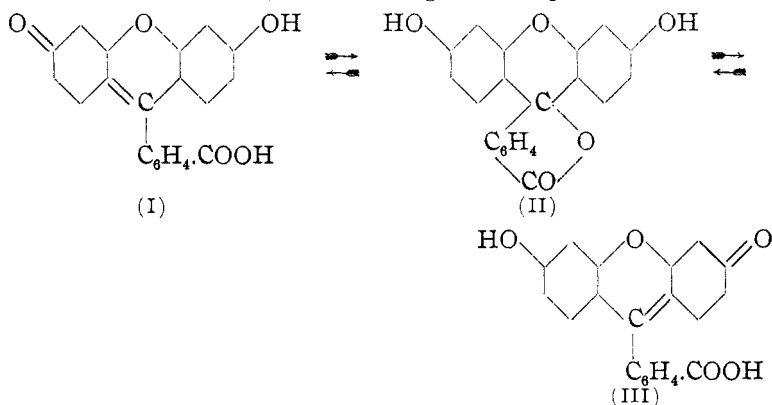
<sup>1</sup> *Z. physik. Chem.*, **24**, 468.

<sup>2</sup> *Ibid.*, **34**, 1.

<sup>3</sup> Baeyer: *Ann. Chem. (Liebig)*, **183**, 29.

alkaline solution, gives a strongly fluorescent acetone solution, and the crystalline compound obtained from the acetone solution is colorless, but possesses a distinct yellowish-green fluorescence in the solid state. By the reduction of the nitro fluoresceins, compounds are obtained which give fluorescent solutions again.

Hewitt<sup>1</sup> ascribes fluorescence to a molecular vibration, which he likens to the oscillation of a pendulum. Thus a fluorescent compound must possess a symmetrical structure of such a character that it may shift freely back and forth from one form to another identical one, through a common intermediate form. In the case of fluorescein, the following would represent such forms:



Substances having the required structure but exhibiting no fluorescence, and substances exhibiting fluorescence without possessing the doubly symmetrical tautomeric structure of fluorescein, are regarded as only apparent exceptions requiring further investigation.

In the present paper, certain nitrofluoresceins are described, together with their behavior on reduction.

Mononitrofluoresceins were prepared from 3- and from 4-nitrophthalic acids and resorcinol. They both dissolve in alkali with a yellowish red color, and weak but distinct green fluorescence. Dinitrofluorescein dissolves in alkalis with a deep blue color and no trace of fluorescence. Attempts to prepare the so-called "tetranitrofluorescein" gave a product which separated from acetone as a white crystalline solid with a distinct yellowish green fluorescence. Analysis of this substance, and of its tetracetyl

<sup>1</sup> *Loc. cit.*

derivative, point to its being a pentanitro derivative, but further proof is necessary to decide this question.

Reduction of the nitrofluoresceins proved a very troublesome and time-consuming task. It was hoped that by suitable modification of well-known reduction processes we could not only obtain direct reduction of the compounds in hand, but might also prepare some of the nitramino bodies. Our results are still very incomplete and unsatisfactory. Some of the products, although crystalline and evidently pure, have not been identified. In other cases, the amino bodies probably in the reduction products could not be isolated.

The aminofluorescein obtained by reducing 4-nitrofluorescein dissolves in alkalis with a green fluorescence. We have not yet been able to isolate the reduction product of 3-nitrofluorescein, or of the supposed pentanitrofluorescein. Diaminofluorescein appears to be a good indicator, giving a bright purple with alkalis and a straw-yellow with acids. Its solutions are fluorescent.

#### EXPERIMENTAL.

##### *Nitrofluoresceins.*

*Mononitrofluorescein from 3-Nitrophthalic Acid.*—Equal parts by weight of 3-nitrophthalic acid and resorcinol were heated in an oil-bath at 195° until the mass solidified, when it was purified by dissolving in alkaline carbonate and reprecipitating. Crystallized from alcohol, it appeared as a dark brownish red, crystalline solid, melting at 215°. This melting-point agrees with that given for this compound by Reverdin.<sup>1</sup>

It dissolves in alkalis with a yellowish red color, and weak but distinct green fluorescence.

	Calculated for C <sub>20</sub> H <sub>11</sub> O <sub>7</sub> N.	Found.
Nitrogen.....	3.71	3.55

*Mononitrofluorescein from 4-Nitrophthalic Acid.*—This was prepared in a manner similar to the above, from 4-nitrophthalic acid and resorcinol, and, when pure, forms a crystalline, yellowish brown powder, which sinters and darkens above 300°. It has been patented recently by Kalle & Co.<sup>2</sup>

It gives a yellowish red solution in alkalis, with a weak but distinct green fluorescence.

<sup>1</sup> *Ber.*, 20, 334 (1887).

<sup>2</sup> D. R. P., 139428 (1903).

	Calculated for $C_{20}H_{11}O_7N_5$ .	Found.
Nitrogen.....	3.71	3.63

So far as we could tell by a superficial examination of these two mononitrofluoresceins, their fluorescence is approximately the same.

*Dinitrofluorescein* was prepared by the method of Hewitt and Perkin.<sup>1</sup> Crystallized from a mixture of alcohol and carbon disulphide, it forms beautiful red crystals, m. p. 152–153°. On crushing these crystals, an orange-colored powder results. The compound dissolves in alkalis with a deep blue color and no trace of fluorescence, and corresponds with the hydrated body described by Hewitt.

The ammonium salt was also prepared by the action of ammonia gas upon an alcoholic solution of the dinitrofluorescein. It forms red crystals, with a green metallic luster, melting at 243–244°, and agrees in other respects with the properties already recorded for this compound.

(*Tetra-, or Pentanitrofluorescein*).—An attempt was made to prepare tetranitrofluorescein<sup>2</sup> by allowing a sulphuric acid solution of fluorescein to remain for some time in contact with an excess of fuming nitric acid. The solution was then poured into water, the precipitate filtered out, washed, and then boiled with alcohol (to remove any dinitrofluorescein). The residue, crystallized from acetone, formed a white crystalline powder, with a distinct yellowish green fluorescence. It has no definite melting-point, but explodes violently when heated.

The difficulties of the analysis were such that no satisfactory figures could be secured for carbon and hydrogen. The following are the results for nitrogen:

Nitrogen.....	Calculated for $C_{20}H_9O_{16}N_5$ .	Found.		
		I.	II.	III.
.....	12.1	12.3	11.95	11.98

These figures check closely with the theoretical composition of a pentanitrofluorescein.

The compound turns a bright red on standing in the presence of moist air, or on the addition of water. Heating for several hours at 110° does not change this color.

*Tetracetyl (Tetra-, or Pentanitrofluorescein*).—On heating our pentanitrofluorescein with acetic anhydride, it dissolved at first,

<sup>1</sup> *J. Chem. Soc. (London)*, **77**, 1324.

<sup>2</sup> *Baeyer: Loc. cit.*

but almost immediately separated again as a fine white crystalline powder, which was recrystallized from acetone. The dried product is fully as, if not more, explosive than the pentanitrofluorescein itself. Its analysis by most of the ordinary methods was abandoned by Hewitt and Perkin<sup>1</sup> as a bad job. We tried a determination by the Kjeldahl method, but with similar lack of success.

However, as the compound is easily saponified, even by hot water, we saponified it with phosphoric acid, and distilled with steam into standardized caustic soda solution. This method gave reasonably satisfactory results. No trace of nitrates or nitrites could be discovered in the distillate, and, as the process was carried out with great care, we believe that we obtained only the acetic acid in our distillate.

	Calculated for		Found.	
	$C_{28}H_{17}O_{20}N_5$ .	$C_{28}H_{18}O_{18}N_4$ .		
CH <sub>3</sub> CO <sub>2</sub> H.....	32.3	34.48	32.45	31.18

Considering the difficulties of the analysis, these results agree fairly well, and indicate a tetracetyl-pentanitrofluorescein. Fuller analytical data are necessary before we can decide whether we are dealing with tetra- or pentanitro derivatives in this and its antecedent compound.

#### *Reduction of the Nitrofluoresceins.*

*Reduction of 4-Nitrofluorescein.*—Four grams of the nitrofluorescein from 4-nitrophthalic acid were dissolved in 40 cc. glacial acetic acid, and treated with 10 grams stannous chloride and 30 cc. concentrated hydrochloric acid. After long standing, a mass of yellowish brown crystals separated, which, on treatment with water, yielded a yellow powder. This powder was purified by repeated solution in alcohol and reprecipitation with water, until it was free from ash. It melts sharply at 281°, and dissolves in alkalis with a green fluorescence. It is probably the aminofluorescein, but the amount of pure substance available was not sufficient for an analysis.

*Reduction of 3-Nitrofluorescein.*—The reduction was conducted in the same manner as the above. The crude product gave a fluorescent alkaline solution, but the pure amino compound could not be isolated.

<sup>1</sup> *Loc. cit.*

*Reduction of Dinitrofluorescein.*—Five grams of dinitrofluorescein were dissolved in 35 cc. glacial acetic acid, the solution cooled, and a solution of 12 grams stannous chloride in 40 cc. hydrochloric acid gradually added. Evolution of heat accompanied the reduction, and, after long standing, the solution was filled with a mass of red crystals with a brilliant blue metallic luster. These crystals are apparently the double tin salt of the diaminofluorescein. On treating them with water, a clear solution was obtained only with difficulty and after long stirring. Hardly had the last of the substance dissolved when the solution again became turbid, and in a few minutes was filled with a mass of fine grayish blue needles with brilliant bronze-green luster. Recrystallized from alcohol, these needles changed to plates, of slightly darker color but with the same brilliant luster, melting at 198–199°. In ether or glacial acetic acid, the compound is slightly soluble, and the solutions exhibit a strong greenish fluorescence. In dilute alkalis, the solution appears reddish with violet fluorescence. In strong alkalis, it dissolves with a bright purple color, which changes to a straw-yellow on acidifying. It is apparently a good indicator. In spite of numerous recrystallizations, it was impossible to free the crystals from a trace of some very stubborn impurity, so that the analytical results were uniformly low.

An alcoholic solution of dinitrofluorescein, reduced with stannous chloride and hydrochloric acid gas, gave a brown powder, from which ether extracted a substance forming bright yellow crystals, melting at 249°. Not enough of the pure substance was obtained for analysis.

*Reduction of (Tetra-, or) Pentanitrofluorescein.*—The pentanitrofluorescein was subjected to the action of various reducing agents—sodium amalgam, magnesium amalgam, aluminum amalgam, tin and hydrochloric acid, stannous chloride, etc. In every instance deep red solutions resulted with no apparent fluorescence in the concentrated state. On diluting, and examining a thin film of the solution, such as is obtained by pouring the liquid slowly down the side of a beaker, the red solution took on a deep and pure blue color, probably due to fluorescence, but no pure reduction product could be separated.