Some Properties of Fluorescein

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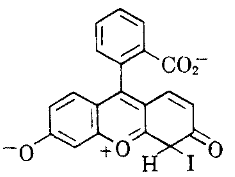
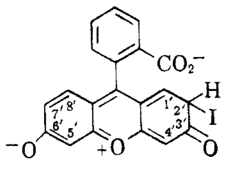
Previous investigators[1] have shown that electrophilic disubstitution, such as dinitration and dibromination, of fluorescein and sulfonefluorescein occurs in the hindered 4′- and 5′-positions. It has also been found that sulfonefluorescein, unlike fluorescein, does not undergo tetrabromination.[2] In order to account for the preference of 4′,5′- over 2′,7′-disubstitution it has been suggested[1b] that the xanthene dyes have a lactoid or quinoid structure in which there is fixation of double and single bonds. It was considered that a fixed structure for fluorescein and sulfonefluorescein was an extension of, and in accord with the theoretical prediction of Mills and Nixon.[3] However there has been much speculation regarding the reality of the Mills and Nixon effect. The structure of fluorescein nevertheless takes on an added interest, in view of the recent work of Davies and Jones[4] on the infrared absorptions of fluorescein and some alkali derivatives. Their results indicate that the classical lactoid structure is to be preferred. They also conclude that the dipolar ion structure is not the correct representation of fluorescein.

In the present work the iodination of fluorescein with excess iodine in ammonium hydroxide has been examined, and there is evidence that fluorescein is most susceptible to electrophilic attack by iodine at the hindered 4′- and 5′- positions. In ammonium hydroxide, fluorescein has shown a marked resistance to tetra substitution. The uptake of iodine at the end of 24 hr. was 95% of the amount calculated for diiodofluorescein, although the amount of iodine used was sufficient to form the tetraiodo compound.[5] At the end of 7 days the uptake of iodine was 103% of the amount calculated for diiodofluorescein. In a similar manner the compounds 4′,5′-dibromo- (I), 2′,7′-dibromo (II), and 2′,7′-dichloro-fluorescein (III) have been treated with iodine. At the end of 7 days, I showed an uptake of iodine which was approximately 4%. The calculated amount for 4′,5′-dibromo-2′,7′-diiodofluorescein is 34.2%. On the other hand, II and III showed an iodine uptake which was almost the required amount for diiodination. From these results it is clear that there is definite resistance to 2′,7′-disubstitution. However, it should be mentioned that the halogens already present at the 4′- and 5′-positions are deactivating.

A tentative explanation for the preferred 4′,5′-substitution is the formation of an intermediate (IV) with naphthalene-like resonance stabilization, and which is therefore energetically favorable. Addition at a 2′- or 7′-position would give an intermediate (V) with a quinoid structure which would be less favorable.



IV



V

The present authors also suggest that the difference in properties between fluorescein and sulfonefluorescein, such as the nontetrabromination of sulfonefluorescein, may be due to the difference in nucleophilicity of the carboxylate and sulfonate ion.

EXPERIMENTAL

Materials. Fluorescein was prepared and purified by the procedure of Orndorff and Hemmer.[6] The dibromination of fluorescein was carried out according to Phillips[7] and afforded 4′,5′-dibromofluorescein (I) which was purified through the diacetate. The tetrabromination of fluorescein was carried out according to Orndorff and Hemmer.[6] The procedure of Kolthoff, Lauer, and Sunde[8] for the preparation of 2′,7′-dichlorofluorescein (II) gave excellent results. The dichloro compound was purified through the diacetate. However, it was found best to hydrolyze the diacetate by heating it on a water bath for several hours with 85% sulfuric acid. 2′,7′-Dibromofluorescein[1b] (III) was made by heating tetrabromofluorescein with stannous chloride and hydrochloric acid in a glacial acetic acid–dioxane medium.

Iodination of fluorescein. To a solution of 4.0 g. (.012 mole) fluorescein in concentrated ammonium hydroxide (250 ml.) was added 12.2 g. (0.048 mole) of iodine dissolved in a solution of potassium iodide (25 g.) in water (50 ml.). The reaction mixture was allowed to stand at room temperature for 7 days. During this period the mixture was stirred occasionally and *care was taken to prevent the accumulation of explosive nitrogen triiodide on the inside walls of the container.* At the end of 24 hr., any solid material had usually dissolved. After 7 days the clear solution was poured with stirring into a mixture of concentrated hydrochloric acid (400 ml.) and ice (1 kg.). After warming gently, the precipitate was filtered off and washed thoroughly with hot water. The solid was dissolved in dilute sodium hydroxide and the precipitation, filtration, and washing procedure repeated. The material was

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(1) (a) R. M. Harris, G. J. Marriott, and J. C. Smith, *J. Chem. Soc.*, 1838 (1936). (b) R. B. Sandin, A. Gillies, and S. C. Lynn, *J. Am. Chem. Soc.*, **61**, 2919 (1939). (c) J. T. Hewitt and A. W. G. Woodforde, *J. Chem. Soc.*, **81**, 893 (1902).

(2) W. R. Orndorff and R. S. Vose, *J. Am. Chem. Soc.*, **46**, 1896 (1924).

(3) W. H. Mills and I. G. Nixon, *J. Chem. Soc.*, 2510 (1930).

(4) M. Davies and R. L. Jones, *J. Chem. Soc.*, 120 (1954).

(5) In the present work there is no rigid proof for the structure of diiodofluorescein. However, on the basis of a similarity between iodination and other electrophilic substitution reactions such as bromination and nitration, the structure is assumed to be 4′,5′-diiodofluorescein. Also the fact that substituents in the 2′,7′-positions do not hinder iodination is in favor of a 4′,5′-diiodo structure.

(6) W. R. Orndorff and A. J. Hemmer, *J. Am. Chem. Soc.*, 49, 1272 (1927).

(7) M. A. Phillips, *J. Chem. Soc.*, 724 (1932).

(8) I. M. Kolthoff, W. M. Lauer, and C. J. Sunde, *J. Am. Chem. Soc.*, **51**, 3273 (1929).

dried and no attempt was made to purify it, since the purpose of this work was to determine only the extent of iodination.

Anal. Calcd. for $C_{20}H_{10}O_5I_2$: I, 43.5. Found: I, 44.8, 44.9.

In a similar manner, after 24 hr., the reaction mixture afforded a crude diiodofluorescein which was analyzed.

Anal. Calcd. for $C_{20}H_{10}O_5I_2$: I, 43.5. Found: I, 41.4, 41.3.

Iodination of I, II, *and* III. Compounds I, II, and III were allowed to react for 7 days with excess iodine in ammonium hydroxide, in the manner described for fluorescein, and the crude products were analyzed. In the case of I, some iodination took place.

Anal. Found for 0.2086 g. of $C_{20}H_8O_5Br_2I_2$: Ag halide, 0.1688.

The combined AgI and AgBr was converted into AgCl.[9] Found: AgCl, 0.1264 g. On this basis the extent of iodination is 4.1%.

Calcd. for $C_{20}H_8O_5Br_2I_2$: I, 34.2.

Compounds II and III showed an uptake of iodine which was close to the required amount.

Anal. Found for 0.3548 g. of $C_{20}H_8O_5Cl_2I_2$: Ag halide, 0.3914 g. Found: AgCl, 0.3014 g. The extent of iodination was 35.2%. Calcd. for $C_{20}H_8O_5Cl_2I_2$: I, 38.8.

Anal. Found for 0.4034 g. of $C_{20}H_8O_5Br_2I_2$: Ag halide, 0.4570 g. Found: AgCl, 0.3126 g. The extent of iodination was 32.3%. Calcd. for $C_{20}H_8O_5Br_2I_2$: I, 34.2.

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[9] E. P. Treadwell and W. T. Hall, *Analytical Chemistry*, Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1935, p. 310.