

glacial acetic acid. Acid hydrolysis of the preceding compound gave 5-bromo-6-aminotetralin, m. p. 52.5°.

Determination of Halogen Reactivity.—The method employed was somewhat similar to the one already used by Nicolet.⁸ All the compounds were treated under as nearly the same conditions as possible, the only variable factor being the time. It was essential that the acid concentrations should be the same for all runs, since it is known that the rate of halogen removal is directly proportional to the acid concentration but is independent of the stannous chloride concentration.

In this work 0.02 mole of compound was dissolved in a boiling mixture of 25 cc. of glacial acetic acid and 20 cc. of concentrated hydrochloric acid. To this was added enough stannous chloride to give one mole of stannous chloride per gram atom of halogen in the compound being examined. The solution was then boiled in an all-glass apparatus under reflux for a suitable period of time. On boiling, some hydrogen chloride was lost. However, since all the experiments were carried on as nearly as possible under the same conditions, no attempt was made to avoid this loss. The reaction mixture subsequently was transferred to a beaker, treated with concentrated hydrochloric acid and cooled. The precipitated hydro-

chloric acid salt or stannichloride of the amine was filtered off and treated with sodium hydroxide. The liberated amine was either identified as such or as the acetyl derivative. Its weight was determined and sometimes also its halogen content. The mother liquor from the above amine hydrochloride was treated with sodium hydroxide and any precipitate produced was examined from the standpoint of identity, weight and halogen content.

Summary

Some brominated derivatives of α - and β -naphthylamine, 5-aminohydrindene and 6-aminotetralin have been examined from the standpoint of "positive" bromine content. It is believed that this reaction is useful in detecting double bond fixation. The results indicate that the structures of the naphthalene derivatives are based on the Erlenmeyer formula, and that the preferred structures for the hydrindene derivatives are based on the Mills and Nixon formula. No definite conclusion was reached regarding tetralin.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF ALBERTA]

The Structure of Fluorescein, Sulfonefluorescein and Some of their Halogenated Derivatives

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Recently C. G. Le Fèvre and R. J. W. Le Fèvre² have determined the dipole moments of xanthone and some of its 2,7-disubstituted derivatives. They conclude that a nearly complete fixation of double and single bonds occurs in the xanthone skeleton (structure (I)). This is in accord with the theoretical prediction of Mills and Nixon:³ "that fusion of a benzene ring with a 6-membered ring tends toward the stabilization in the former of that Kekulé individual which has a double bond between the points of attachment of the side nucleus."⁴

With the work of Le Fèvre and Le Fèvre in mind, it occurred to the authors of this paper that the xanthene dyes such as fluorescein and sulfonefluorescein and which in the lactoid modifications have a xanthone skeleton, might from a chemical standpoint show ring stabilization.⁵

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(2) Le Fèvre and Le Fèvre, *J. Chem. Soc.*, 196 (1937).

(3) Mills and Nixon, *ibid.*, 2510 (1930).

(4) In the case of tetralin, however, it has been found that tetralin, like all ordinary benzene derivatives, can react in both Kekulé forms. Fieser and Lothrop, *THIS JOURNAL*, **58**, 2050 (1936); Sidgwick and Springall, *J. Chem. Soc.*, 1532 (1936).

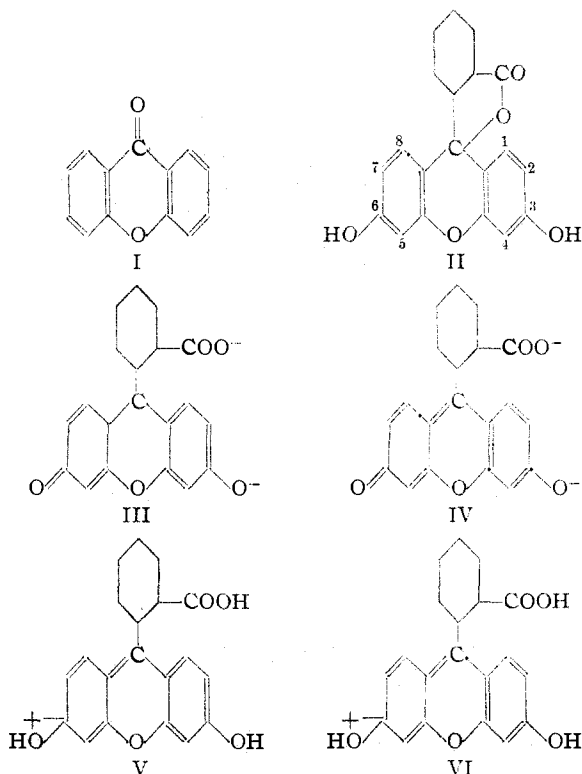
(5) For earlier work on the structure of fluorescein and sulfone-

The regulated dibromination of fluorescein has been examined and has been found to occur in the 4,5-positions.⁶ This is contrary to what one would ordinarily expect from a consideration of a steric effect.⁷ It is believed by the authors that this type of dibromination is an indication of ring stabilization. If the lactoid structure of fluorescein is assumed, then it is believed that structure (II) is the preferred structure. If, on the other hand, it is the quinoid form which is brominated, then it is assumed that either structure (IV) (two resonating negative ions) or structure (VI) (two fluorescein, see (a) Orndorff, Gibbs and Shapiro, *THIS JOURNAL*, **50**, 819 (1928); (b) Gibbs and Shapiro, *ibid.*, **50**, 1755 (1928); (c) Ramart-Lucas, *Compt. rend.*, **205**, 1409 (1937).

(6) Several text-books on organic chemistry give the structure of mercurochrome to be a mercurated dibromofluorescein in which the bromine atoms occupy the 2,7-positions. Orndorff and Hemmer, *THIS JOURNAL*, **49**, 1272 (1927), give the structure of dibromofluorescein as a 4,5-dibromo compound. However, Orndorff and Vose, *ibid.*, **46**, 1896 (1924), give a 2,7-structure for the dibromosulfonefluorescein and diodosulfonefluorescein diacetates.

(7) That the structure of dibromofluorescein is a 4,5-dibromo one has also been shown by Harris, Marriot and Smith, *J. Chem. Soc.*, 1838 (1936). However, they gave no definite proof that the compound they obtained by the alkali fusion of dibromofluorescein was 2-bromoresorcinol which has a melting point of 102–103°. The melting point of 4-bromoresorcinol is almost identical with that of the 2-bromo compound, *viz.*, 103°.

resonating positive carbonium ions) represents the preferred structure. In the case of sulfone-fluorescein, only dibromination occurs and again the structure has been shown to be a 4,5-dibromo structure. Since no tetrabromination of sulfone-fluorescein takes place no matter how much bromine is used, it is conceivable that ring stabilization is much more pronounced in this case than in the case of fluorescein.



Hewitt and Woodforde⁸ have shown that the dinitration of fluorescein occurs at the 4,5-positions, although previous workers had believed that nitration occurred at the 2,7-positions. More recently Ingrassia⁹ has shown that fluorescein yields a benzenemonazofluorescein and a benzenebisazofluorescein when allowed to couple with a phenyldiazonium salt. He concludes that fluorescein has a lactoid structure and that the azo derivatives are 2,7-substituted derivatives. However, the authors of this paper believe that the azo derivatives must be 4,5-compounds because on reduction Ingrassia has found that the diamino-fluorescein dihydrochloride produced is identical with that prepared from dinitrofluorescein. The authors of this paper also suggest that the structure (IV) (two resonating negative ions),

(8) Hewitt and Woodforde, *J. Chem. Soc.*, **81**, 803 (1902).

(9) Ingrassia, *Gazz. chim. ital.*, **62**, 781 (1932).

as well as a fixed lactoid structure, will account for the formation of a bisazofluorescein.¹⁰

Some tetrahalogenated derivatives of fluorescein have also been examined from the standpoint of containing "positive" halogen.¹¹ Stannous chloride dissolved in hydrochloric acid, it is believed, can be used to detect bond fixation. For example, this reagent has no effect on 3-bromo- β -naphthylamine, whereas with the same reagent the bromine in 1-bromo- β -naphthylamine is very readily replaced by hydrogen. In the present work, tetrabromofluorescein dissolved in dioxane has been refluxed with excess stannous chloride hydrochloric acid reagent for as long as twenty-five hours and the 4,5-dibromo atoms are the only ones which are removed. A similar thing happens with 4,5-dibromo-2,7-dichlorofluorescein, from which only the two bromine atoms are removed. The results indicate that again there is the possibility of bond fixation in such a way that there is a double bond between the phenolic ring and the points of attachment of the side nucleus.

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Experimental Part

Preparation of Compounds.—The fluorescein used in this work was prepared and purified according to Orndorff and Hemmer.⁶ It was brominated to the dibromofluorescein stage according to Phillips,¹² purified through the diacetate and formed red plates, m. p. 285°. By using acetic anhydride and ethanol as a solvent for the purification of the dibromofluorescein diacetate, the present authors have obtained a melting point of 228–229°. Phillips¹² reports a melting point of 211°. The tetrabromination of fluorescein was carried out according to Orndorff and Hemmer.⁶

The preparation of 2,7-dichlorofluorescein was carried out according to Kolthoff, Lauer and Sunde.¹³ It was purified through the diacetate (m. p. 237° from benzene). It was found best to hydrolyze the diacetate by heating it on a water-bath for several hours with 85% sulfuric acid. When alcoholic potassium hydroxide was used as the hydrolyzing agent, the dichlorofluorescein produced invariably had a low chlorine content, probably due to a hydrolytic breakdown of the dichlorofluorescein molecule itself. When dichlorofluorescein was brominated with

(10) The authors have found that only a diiodofluorescein (presumably 4,5-diiodofluorescein) is produced when an ammoniacal solution of fluorescein is treated with excess iodine dissolved in aqueous potassium iodide. Evidence has also been found that under similar circumstances 2,7-dichlorofluorescein forms a diiodo derivative, whereas 4,5-dibromofluorescein does not. This work is being done now and will be reported on later.

(11) Nicolet and co-workers, *THIS JOURNAL*, **43**, 2081 (1921); **49**, 1796, 1801, 1806, 1810 (1927).

(12) Phillips, *J. Chem. Soc.*, 724 (1932).

(13) Kolthoff, Lauer and Sunde, *THIS JOURNAL*, **51**, 3273 (1929).

two moles of bromine, 4,5-dibromo-2,7-dichlorofluorescein was produced.

Anal. Calcd. for $C_{20}H_8Cl_2Br_2O_5$: AgCl + AgI, 41.3. Found: (Carius) 41.4.

Dibromosulfonefluorescein was prepared by the procedure of Orndorff and Vose.⁶ No tetrabromination, using excess bromine, was found to occur.

2-Bromoresorcinol was prepared according to Rice¹⁴ and melted at 100°.

4-Bromoresorcinol was made according to the procedure of Sandin and McKee¹⁵ and melted at 100–102°.

3-Bromoresorcinol and 2-bromoresorcinol behave quite differently on sublimation. The latter sublimes much more readily than the former. Also the latter on sublimation tends to produce long needles, whereas the former sublimes to form colonies of small crystals.

Hydrolysis of Dibromofluorescein and Dibromosulfonefluorescein.—The directions of Harris⁷ were followed and in both cases 2-bromoresorcinol was isolated and purified by sublimation. There was no depression of the melting point on mixing with synthetic 2-bromoresorcinol. However, when mixed with synthetic 4-bromoresorcinol, the melting point was as low as 75°.

Reduction of Tetrabromofluorescein and Dichlorodibromofluorescein.—To a solution of 50 g. of tetrabromofluorescein in 500 cc. of dioxane, 100 cc. of concentrated hydrochloric acid and 50 cc. of glacial acetic acid was added 150 g. of stannous chloride. The solution was refluxed for fifteen to twenty-five hours. At the end of about six hours, crystals of 2,7-dibromofluorescein began to settle out and the amount increased as the refluxing was continued. It was found that dibromofluorescein is much less soluble in dioxane than the tetrabromo compound. The reaction mixture was cooled, the crystals were filtered off and dissolved in dilute sodium hydroxide. The solution was filtered and the dibromo compound precipitated by the addition of hydrochloric acid. The freshly precipitated material was dark red in color. However, on heating on a water-bath for several hours the material became definitely crystalline and the color changed to a bright yellow. The appearance of the dibromofluorescein at this stage was very much like that of 2,7-dichlorofluorescein which has been digested with hydrochloric acid.

Anal. Calcd. for $C_{20}H_{10}O_5Br_2$: Br, 32.7. Found: Br (Carius), 31.8.

The diacetate was made by refluxing the dibromofluorescein with acetic anhydride. Crystallization of the diacetate from acetic anhydride gave the pure diacetate, m. p. 259° (with preliminary darkening of color).

(14) Rice, *THIS JOURNAL*, **48**, 3125 (1926).

(15) Sandin and McKee, *Org. Syntheses*, **17**, 23 (1937).

Anal. Calcd. for $C_{24}H_{14}O_7Br_2$: Br, 27.9. Found: Br (Carius), 27.7.

The diacetate was hydrolyzed with 85% sulfuric acid by heating on the water-bath for several hours. The reaction mixture was diluted with water, the dibromofluorescein filtered off and washed thoroughly with hot water. It formed beautiful yellow crystals, m. p. 300–301°.

Anal. Calcd. for $C_{20}H_{10}O_5Br_2$: Br, 32.7. Found: Br (Carius), 32.6.

The above purified dibromofluorescein was hydrolyzed according to the procedure of Harris.⁷ 4-Bromoresorcinol was isolated from the hydrolysis product and its identity established by carrying out mixed melting points with synthetic 2- and 4-bromoresorcinols. Moreover, on sublimation it behaved like the 4-bromoresorcinol. There was also isolated what is believed to be 2-(5'-bromo-2',4'-dihydroxybenzoyl)-benzoic acid, m. p. 240–241° (with preliminary darkening of color and softening). Harris⁷ and co-workers give the melting point of 2-(3'-bromo-2',4'-dihydroxybenzoyl)-benzoic acid as 200° with softening at 187°.

Anal. Calcd. for $C_{14}H_8O_5Br$: Br, 23.7. Found: Br (Carius), 23.4.

The reduction of dichlorodibromofluorescein was carried out in the same manner. From the reaction mixture there was isolated nearly pure dichlorofluorescein.

Anal. Calcd. for $C_{20}H_{10}O_5Cl_2$: Cl, 17.8. Found: Cl (Carius), 17.5.

Fluorescein dissolved in glacial acetic acid was refluxed with stannous chloride and concentrated hydrochloric acid. At the end of fifteen hours the fluorescein was isolated almost quantitatively as the pure fluorescein diacetate. Evidently stannous chloride has a negligible effect on the conjugated system of double bonds as found in the quinoid modification of fluorescein.

Summary

1. Fluorescein and sulfonefluorescein have been dibrominated and the bromine atoms have been shown to occupy the 4,5-positions. Tetrabromofluorescein and dichlorodibromofluorescein have been reduced with stannous chloride and only the 4,5-dibromo atoms have been removed.

2. To explain these results it is suggested that these compounds have fixed structures according to the Mills and Nixon theory.

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