[CONTRIBUTION FROM THE COLOR LABORATORY, BUREAU OF CHEMISTRY, No. 94]

THE INFLUENCE OF CONSTITUTIONAL VARIATION UPON THE ABSORPTION AND STABILITY TO HYDROGEN IONS OF CERTAIN HALOGENATED DERIVATIVES OF FLUORESCEIN[1]

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Of the materials examined in the present investigation, the tetrachloro-, tetrabromo- and tetra-iodo-fluoresceins (phthalic anhydride substitution) and the tetrabromo and tetra-iodo-eosins were prepared by Pratt and his collaborators.[2] They were supplied by the D. S. Pratt Fellowship, Mellon Institute of Industrial Research, of the University of Pittsburgh. The writer is also indebted to C. D. Garby of the Fixed Nitrogen Research Laboratory for a sample of deca-iodo-fluorescein. The other materials were prepared in the Color Laboratory. The fluorescein, eosin, erythrosin and tetrachloro-erythrosin were obtained by the recrystallization of technical products which had been shown by analysis to be of good purity. The dibromo-fluorescein (resorcinol substitution), tetrachloro-eosin and tetra-iodo-erythrosin were made from pure inter-

TABLE I

ABSORPTION MAXIMA ($\mu\mu$)

	Aqueous solution				Alcoholic solution			
		E. Vogel[3]	Formanek and Grandmougin[4]	Moir[5]		E. Vogel[3]	Formanek and Grandmougin[4]	Nichols and Spencer[6]
Fluorescein	490	491	489.3	493.5	499	500	498.5	501
Dibromo-fluorescein	505	504	504.8	509	510	510	...	...
Eosin	516	517.5	516.0	521	523	522	527.6	523
Erythrosin	524	523	522.2	525	530.5	530	533.9	...
Tetrachloro-fluorescein	509.5	510	...	513	520	522	...	523
Tetrabromo-fluorescein	510	510	...	514	521	522	...	...
Tetra-iodo-fluorescein	511	...	...	...	522	...	...	...
Tetrachloro-eosin	537.5	537	537.3	544	549	548	549.9	553
Tetrabromo-eosin	539	537	...	544	550	548	...	...
Tetra-iodo-eosin	540	...	...	...	551	...	...	...
Tetrachloro-erythrosin	548	548	547.7	548	557	560	558.1	...
Tetra-iodo-erythrosin	550	...	...	...	558	...	...	...
Deca-iodo-fluorescein	549.5	...	...	...	556.5	...	...	...

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[1] Presented before the Dye Section of the American Chemical Society at its meeting April 21–26, 1924.

[2] Pratt and others, THIS JOURNAL, **40**, 236, 245 (1918); **41**, 1293 (1919).

[3] Vogel, *Ann. Phys. Chem.* (*Wied.*), [2] **43**, 449 (1891).

[4] Formanek and Grandmougin, "Unt. Nach. org. Farb. auf Spekt. Wege," J. Springer, Berlin, **1908**, Part I.

[5] Moir, *Trans. Roy. Soc. S. Afr.*, **10**, [3] 159 (1922).

[6] Nichols and Spencer, THIS JOURNAL, **36**, 707 (1914).

mediates. The analysis of their color acids, dried to constant weight at 125°, gave 32.4% of bromine for the first (calc., 32.6%), 17.9% of chlorine and 40.2% of bromine for the second (calc., 18.1% and 40.7%) and 75.1% of iodine for the third (calc., 75.9 %).

The absorption of the dipotassium salts of these materials in dilute and slightly alkaline aqueous and alcoholic solutions was measured throughout the visible spectrum by means of a Hilger wave-length spectrometer provided with a Nutting photometer. In the region of principal absorption the measurements were carried out at spectral intervals of 1$\mu\mu$. The absorption maxima determined are recorded in Table I, together with corresponding data by previous investigators in instances in which such are available.

The measurements obtained are in excellent general agreement with those of Vogel and of Formanek and Grandmougin. (The high values obtained by the latter for alcoholic solutions of eosin and erythrosin doubtless may be attributed to a failure to insure the presence of the normal dye salts by employing a slight excess of alkali.)

From the identical values obtained by Vogel in both aqueous and alcoholic solutions for tetrachloro- and tetrabromo-fluorescein and for tetrachloro- and tetrabromo-eosin, it would be concluded that in the instance of substitution within the phthalic anhydride residue the nature of the halogen is without influence upon the magnitude of the displacement of the absorption maximum. The values recorded by Moir for aqueous solutions of tetrachloro- and tetrabromo-eosin are also identical, although slightly different maxima are reported for the corresponding derivatives of fluorescein. The more comprehensive data of the present investigation indicate clearly that the nature of the halogen exerts a slight but measurable effect. The values obtained with the tetrahalogen derivatives of both fluorescein and eosin in both aqueous and alcoholic solutions record a distinct progression in displacement in the direction which would be anticipated with each substitution of one halogen for another.

The influence of the position in which bromine substitution occurs in the fluorescein molecule has been investigated by Moir[5] who has had remarkable general success in establishing a quantitative correlation of constitution and color (absorption) in a variety of classes of dyes. Each position in the molecule is assigned its specific "dicyclic color factor." The absorption maximum of any bromine derivative is to be calculated by means of the successive multiplication of the absorption maximum of fluorescein (expressed in $\mu\mu$) by each substitution factor involved. A conversion factor is also supplied whereby the maxima of chlorine derivatives may be calculated from those of the corresponding bromine compounds. It is to be inferred that a similar factor may be employed for iodine derivatives.

The agreement between the observed values obtained in the present investigation and values calculated by the application of the Moir factors to the writer's value for fluorescein is only approximate.

It is evident that factors of this type can be completely effective only if the effect of substitution in any one position, or group of positions, is constant and unaffected by substitution elsewhere in the molecule. From the data of the present investigation it may be concluded that such is not the case. The spectral interval between the aqueous maxima of eosin and fluorescein is 26 $\mu\mu$, whereas the spectral intervals between the maxima of tetrachloro-, tetrabromo- and tetra-iodo-eosins and the corresponding fluoresceins are, respectively, 28.5, 29 and 29 $\mu\mu$. The interval between the aqueous maxima of erythrosin and fluorescein is 34 $\mu\mu$, whereas the intervals between tetrachloro- and tetra-iodo-erythrosins and the corresponding fluoresceins are, respectively, 38.5 and 39 $\mu\mu$. Halogen substitution in the resorcinol residues effects an appreciably greater displacement in the maxima of fluoresceins which have undergone substitution in the phthalic anhydride residue than it does in the instance of fluorescein itself.

The same effect may be observed in certain of the data for substituted fluoresceins supplied by Moir; a precisely analogous one may be noted in the measurements on halogenated derivatives of phenolphthalein recorded by Howe and Gibson.[7] The influence of bromine or iodine substitution in the phenol residues was shown to be decidedly greater with phenol-tetrachlorophthalein than with phenolphthalein.

It is equally apparent that a general conversion factor cannot be supplied for the accurate calculation of the maxima of derivatives of one halogen from those of corresponding derivatives of another halogen. The effect of the replacement of chlorine by bromine, or of bromine by iodine, is appreciably greater in the resorcinol residues than it is in the phthalic anhydride residue.

The simple principles of color calculation proposed by Moir, accordingly, are inadequate for the precise estimation of the positions of the absorption maxima of the halogenated derivatives of fluorescein.

The deca-iodo-fluorescein, supplied by Garby, is believed to be the first completely substituted derivative of fluorescein which has ever been prepared. Its absorption is of particular interest in view of the direct evidence which it affords respecting the effect of substitution in the 1 and 8 (h and i) positions in the resorcinol residues. By inference from γ-orcinolphthalein and phloroglucinolphthalein, Moir estimated that the color factors of these positions would be about 0.990. On this supposition the conversion of octa-iodo-fluorescein into deca-iodo-fluorescein should exert a decided hypsochromic effect. If the absorption maximum of

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[7] Howe and Gibson, *Phys. Rev.*, 10, 770 (1917).

octa-iodo-fluorescein is accepted as 550 $\mu\mu$, that of the deca derivative should be approximately 539 $\mu\mu$. The observed value is 549.5 $\mu\mu$ and it may be concluded that substitution in the h and i positions is practically without effect on the position of the absorption band (as is the case with substitution in the 4^1 (b) position in the phthalic anhydride residue).

Since some of the halogen derivatives of fluorescein find application as indicators, and all are qualified in some degree for service in that respect, their relative stability to hydrogen ions is of interest.

An examination of the data recorded by Formanek and Grandmougin for the absorption of technical products[8] reveals the fact that the general stability of the dyes of the group to the hydrogen ion is greater in aqueous than in the alcoholic solvents and also greater in ethyl alcohol than in amyl alcohol. It is disclosed, furthermore, that in alcoholic solution the stability of those derivatives which are substituted within the resorcinol residues alone is less than that of those which are substituted within both the resorcinol residues and the phthalic anhydride residue. In aqueous solution, as will be shown, the relative stability of these two classes of derivatives is of an opposite order.

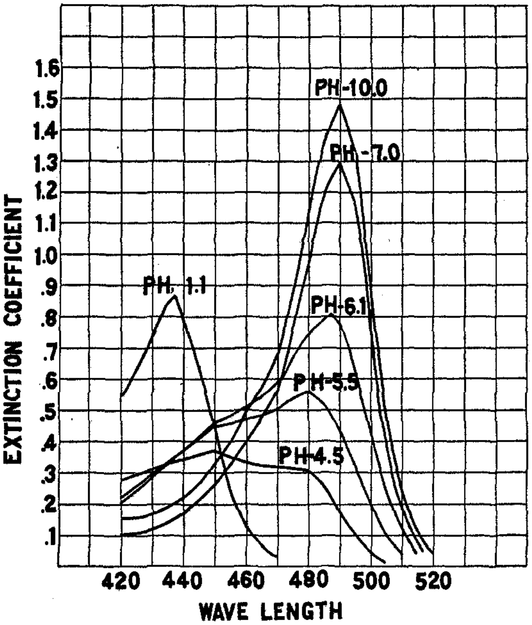

Fig. 1.—Influence of variation in hydrogen-ion concentration on the absorption of fluorescein.

Stock solutions were prepared of the normal dyes (dipotassium salts) containing from 10 to 20 mg. of dye per 100 cc. The solutions for spectro-photometric evaluation were prepared by diluting 5cc. aliquot portions of these solutions to 50 cc. with a series of Clark and Lubs' standard buffer solutions.

The data of measurements made on the freshly prepared solutions are given in Table II. The figures give the relative values of the extinction coefficients of the solutions at the normal aqueous maxima of the respective dyes, with the maximum extinction coefficients expressed as unity.

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[8] "Untersuch. und Nachweis organ. Farbstoffe auf spekt. Wege," J. Springer, Berlin, 1913, Part II, Issue II.

The progressive decrease in values obtained with increasing hydrogen-ion concentration provides an approximate index of the percentage removal of the dyes, as such, from solution.

TABLE II

THE IMMEDIATE INFLUENCE OF VARIATION IN SÖRENSEN (P_H) VALUE UPON ABSORPTION INTENSITIES

P_H	Fluorescein	Di-bromo-fluorescein	Eosin	Erythrosin	Tetra-chloro-fluorescein	Tetra-bromo-fluorescein	Tetra-iodo-fluorescein	Tetra-chloro-eosin	Tetra-bromo-eosin	Tetra-iodo-eosin
11.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
9.3	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
8.65	1.00	1.00	1.00	1.00	1.00	1.00	0.97	1.00	1.00	1.00
7.1	0.92	0.99	1.00	1.00	0.90	0.89	.83	1.00	1.00	0.98
6.25	.64	.94	1.00	1.00	.67	.63	.51	1.00	1.00	.93
6.0	.47	.87	1.00	0.99	.51	.50	.29	1.00	0.99	.92
5.0	.20	.59	0.98	.95	.19	.17	.09	0.98	.96	.85
4.0	..	..	.92	.67	.00	.00	.00	.88	.62	.11
3.0	..	..	.41	.12	..	..	..	.06	.00	.00

The influence of substitution within the resorcinol residues is very well defined, both with fluorescein itself and with fluoresceins substituted within the phthalic anhydride residue. The resulting increase in stability to the hydrogen ion is decided in all instances.

The influence of substitution within the phthalic anhydride residue is opposite in effect. With the derivatives of fluorescein itself this effect is clearly defined only in the instance of tetra-iodo-fluorescein, but all of the derivatives of eosin are appreciably less stable than is eosin itself.

The influence of the nature of the halogen is also clearly defined. The bromine derivatives of both fluorescein and eosin are somewhat less stable to acidity than the corresponding chlorine derivatives, while the iodine derivatives are decidedly less stable than the corresponding bromine derivatives.

The immediate modification in the absorption of the acid solutions of the dyes, recorded in Table II, was followed by a further gradual decrease in absorption, accompanied, in some instances, by visible precipitation. The immediate measurements have been accepted as affording an index of the true hydrogen-ion equilibria and the subsequent modifications attributed to a gradual process of increase in molecular aggregation. Values obtained in a second examination of the solutions after a period of one week confirmed all the conclusions that were based upon their immediate examination, respecting the influence of the type of substitution and the nature of the substituent upon the relative stability of the dyes.

Summary

1. Absorption maxima are supplied for a comprehensive selection of halogenated derivatives of fluorescein in aqueous and alcoholic solutions.

2. In the instance of substitution within the phthalic anhydride residue the displacement of the maxima is influenced by the nature of the halogen concerned in a definite, but appreciably less decided, degree than is the case in the instance of substitution within the resorcinol residues.

3. The effect of substitution in the resorcinol residues is not constant in degree under all conditions but is modified by substitution within the phthalic anhydride residue.

4. Substitution in the 1 and 8 (h and i) positions in the resorcinol residues is without appreciable effect upon the absorption.

5. Substitution within the resorcinol residues increases the stability of the dyes in aqueous solution to the hydrogen ion, whereas substitution within the phthalic anhydride residue is of opposite effect.

6. The relative stability of the dyes to acids is increased by the replacement of iodine by bromine and by that of bromine by chlorine.

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ACTION OF AROMATIC ALCOHOLS ON AROMATIC COMPOUNDS IN THE PRESENCE OF ALUMINUM CHLORIDE. III. CONDENSATION OF BENZYL ALCOHOL AND PHENOL

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Earlier publications in this series[1] described the condensation of primary and secondary aromatic alcohols with benzene to form diphenylmethane and its derivatives. In this article is described the application of the method to the preparation of benzylphenol and its ethers.

The condensation of benzyl alcohol and phenols has been accomplished by the use of zinc[2] and sulfuric and acetic acids[3] and zinc chloride.[4] Zinc and zinc chloride have also been used in the condensation of simple aliphatic alcohols with phenols.[5]

Merz and Weith[6] found that aluminum chloride reacted with phenol to give a 10 to 12% yield of diphenyl ether. This reaction takes place when the mixture is heated under a reflux condenser. The present in-

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[1] THIS JOURNAL, **38**, 2525 (1916); **40**, 785 (1918).

[2] Paterno, (a) *Gazz. chim. ital.*, **2**, 2 (1872); (b) *Ber.*, **5**, 288 (1872). (c) Paterno and Mazzara, *Gazz. chim. ital.*, **8**, 303 (1878).

[3] Paterno and Fileti, *Gazz. chim. ital.*, **5**, 381 (1875).

[4] Liebmann, *Ber.*, **14**, 1844 (1881). Venturi, *Gazz. chim. ital.*, **31**, 1, 469 (1901).

[5] Dennstedt, *Ber.*, **23**, 2569 (1890). Auer, *Ber.*, **17**, 669 (1884). Senkowski, *Ber.*, **24**, 2974 (1891). Bauer, *Ber.*, **27**, 1614 (1894). Anschütz and Beckerhoff, *Ber.*, **28**, 407 (1895).

[6] Merz and Weith, *Ber.*, **14**, 189 (1881).