

over two moles of anhydrous aluminum chloride are used for each mole of acid anhydride that is allowed to react.

2. The ketones are obtained in good yields and boil almost constant on the first distillation.

URBANA, ILLINOIS

[CONTRIBUTION FROM THE BAKER LABORATORY OF CHEMISTRY, CORNELL UNIVERSITY]

## SULFONEFLUORESCEIN AND DIHYDROXYBENZOYL-BENZENE- ORTHO-SULFONIC ACID, AND SOME OF THEIR DERIVATIVES<sup>1</sup>

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In 1884 Remsen<sup>2</sup> discovered sulfonefluorescein and called this class of compounds the sulfonephthaleins. Remsen and Hayes<sup>3</sup> in 1887 attempted to isolate sulfonefluorescein, but obtained the ammonium salt of dihydroxybenzoyl-benzene-*o*-sulfonic acid, as shown in 1889 by Remsen and Linn<sup>4</sup> who prepared sulfonefluorescein by heating dihydroxybenzoyl-benzene-*o*-sulfonic acid at 185°. Their analyses of the *amorphous* material showed it to contain a molecule of water. Fahlberg and Barge<sup>5</sup> in 1889 state that instead of the reaction between resorcinol and *o*-sulfobenzoic acid being analogous to that of the formation of fluorescein, it gave a product resulting from the condensation of four molecules of resorcinol with one of *o*-sulfobenzoic acid. Blackshear<sup>6</sup> in 1892 made sulfonefluorescein by the method of Remsen and Linn and his analyses of the *amorphous* product agreed with those of these authors. White<sup>7</sup> in 1895 made sulfonefluorescein by the same method and attempted to prepare a bromine derivative. He repeated the work of Fahlberg and Barge and obtained similar results. Remsen and McKee<sup>8</sup> in 1896 found that both chlorides of *o*-sulfobenzoic acid gave sulfonefluorescein when heated with resorcinol. They state that when dried in the air their product contained a molecule of water. Sisley<sup>9</sup> in 1897 made sulfonefluorescein by heating "saccharin" with resorcinol and sulfuric acid and states that he obtained a tetrabromo compound. Sohon<sup>10</sup> in 1898 made sulfonefluorescein from resorcinol and the

<sup>1</sup> From a dissertation presented by R. S. Vose in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

<sup>2</sup> Remsen, *Am. Chem. J.*, **6**, 180 (1884).

<sup>3</sup> Remsen and Hayes, *ibid.*, **9**, 372 (1887).

<sup>4</sup> Remsen and Linn, *ibid.*, **11**, 73 (1889).

<sup>5</sup> Fahlberg and Barge, *Ber.*, **22**, 754 (1889).

<sup>6</sup> Blackshear, *Am. Chem. J.*, **14**, 455 (1892).

<sup>7</sup> White, *ibid.*, **17**, 545 (1895).

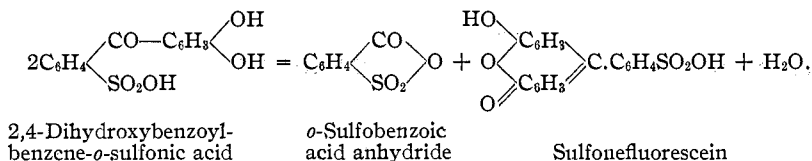
<sup>8</sup> Remsen and McKee, *ibid.*, **18**, 802 (1896).

<sup>9</sup> Sisley, *Bull. soc. chim. Paris*, [3] **17**, 822 (1897).

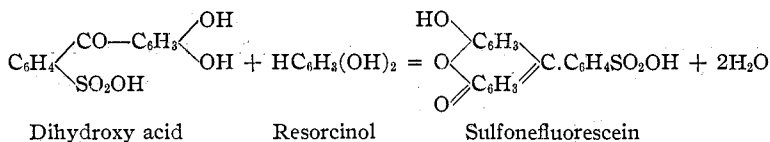
<sup>10</sup> Sohon, *Am. Chem. J.*, **20**, 257 (1898).

anhydride of *o*-sulfobenzoyl-benzene-*o*-sulfonic acid. It will be seen from the above review of the literature that sulfonefluorescein has never been obtained in a crystalline condition, and it is doubtful whether any of the products analyzed were pure. Nothing is known about the derivatives of sulfonefluorescein.

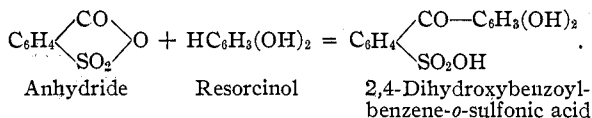
We have studied the conditions of the formation of sulfonefluorescein from dihydroxybenzoyl-benzene-*o*-sulfonic acid, and find that it is readily formed by heating the acid to 160–170° for two hours, *o*-sulfobenzoyl anhydride and water being eliminated. It may also be prepared by heating



the dihydroxy acid with resorcinol at 160–170°,



and, contrary to Fahlberg and Barge<sup>5</sup> and White,<sup>7</sup> by heating *o*-sulfobenzoyl anhydride with resorcinol at 135–140°. The dihydroxy acid then

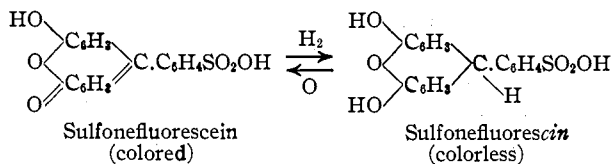


reacts with a second molecule of resorcinol to form sulfonefluorescein as shown above. A 52% yield of sulfonefluorescein is obtained by heating resorcinol with "saccharin" and sulfuric acid at 135–140° for seven hours; while a 41% yield is obtainable by heating resorcinol with the chlorides of *o*-sulfobenzoyl anhydride on the water-bath for 12 hours.

Sulfonefluorescein crystallizes in purple needles; but in a finely divided condition, as when first precipitated or pulverized, it is bright yellow. It reacts as a tautomeric substance, giving rise to both colored quinoid and colorless lactoid derivatives. The compound itself does not occur in the colorless lactoid form, but is probably a solid solution of the quinoid form as an oxonium or carbonium salt and the quinoid hydrate.<sup>11</sup> It forms a series of highly-colored salts which dissolve in water with a pronounced green fluorescence; they have a quinoid structure, the sulfonefluorescein acting as a monobasic acid.

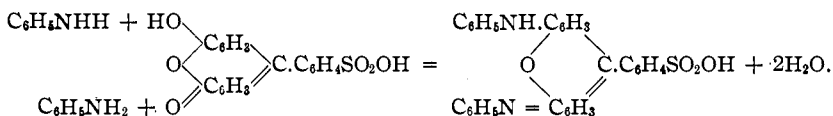
On reduction with zinc dust in water, the sulfonefluorescein is converted into the colorless zinc salt of sulfonefluorescein.

<sup>11</sup> THIS JOURNAL, 45, 490 (1923).



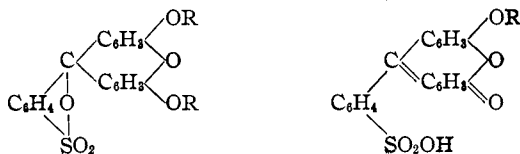
Sulfonefluorescein differs from sulfonefluorescein in being readily soluble in water.

On boiling with aniline, sulfonefluorescein is converted into a di-anilino compound, according to the equation,



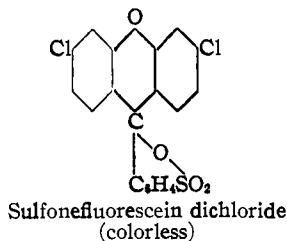
This is probably an inner ammonium salt since it is but slightly soluble in sodium hydroxide solution. It dissolves in cold water to form a yellow solution with a greenish fluorescence; in boiling water the color is cerise, the greenish fluorescence being less pronounced. Sulfonefluorescein does not react with boiling dimethylaniline, in which it is practically insoluble.

Sulfonefluorescein gives a green mono-acetate and a diacetate which is light yellow. A colored monobenzoate is formed on boiling with benzoyl chloride. The diacetate has a lactoid structure, while the mono-acetate and monobenzoate are quinoid derivatives.

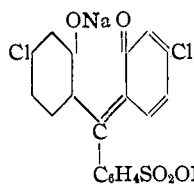


where R = COCH<sub>3</sub> or COC<sub>6</sub>H<sub>5</sub>.

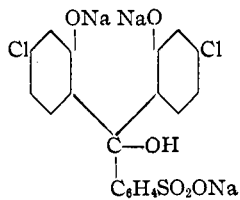
On treatment with benzoyl chloride or with phosphorus pentachloride, colorless sulfonefluorescein dichloride is produced. This has the lactoid structure.



The chlorine atoms in this compound are not removed by boiling with 20% sodium hydroxide solution, although it dissolves, forming both colored and colorless salts of sulfonefluorescein dichloride.

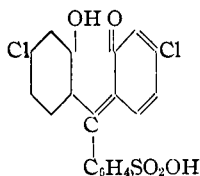
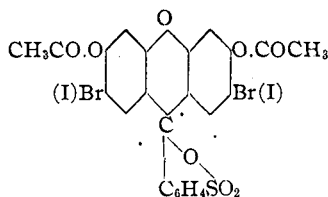


Colored salt of sulfonefluorescein dichloride



Colorless salt of sulfonefluorescein dichloride

When solutions of these salts are acidified, the quinoid hydrate of sulfonefluorescein dichloride is precipitated as a red solid.

Hydrate of sulfonefluorescein dichloride  
(colored)Dibromo-sulfonefluorescein and di-iodo-sulfonefluorescein diacetates  
(colorless)

On bromination, sulfonefluorescein yields a dibromo compound; under no conditions could the formation of the tetrabromo compound claimed by Sisley<sup>9</sup> be observed, even with the use of a large excess of bromine. With iodine, di-iodo-sulfonefluorescein is formed. The dibromo and the di-iodo derivatives form diacetates (see above) which are almost colorless and thus possess the lactoid structure.

**Comparison between the Fluorescence of Fluorescein and that of Sulfonefluorescein.**<sup>12</sup>—The fluorescence of a solution of sulfonefluorescein containing 1 mg. to 100 cc. of a 0.00011 *N* solution of potassium hydroxide was studied by the aid of a Lummer-Brodhun Spectrophotometer. A 40-watt tungsten lamp was used for excitation and a tungsten lamp was also used as a comparison source. Measurements were also made under identical conditions with a solution of pure, *yellow* fluorescein (1 mg. to 100 cc. of a 0.00012 *N* solution of potassium hydroxide). With the unaided eye it was difficult to distinguish between the two solutions.

In each case the fluorescence spectrum consisted of a single band, the maximum lying at  $\lambda = 5249$  for sulfonefluorescein and at  $\lambda = 5209$  for fluorescein. The maximum for sulfonefluorescein was almost exactly 10% more intense than the maximum for fluorescein. The two bands had nearly the same shape, although that for sulfonefluorescein was somewhat steeper on the side of short wave length. The fluorescein band showed some slight indication of being complex, that is, as if a weak band at about

<sup>12</sup> We are indebted to Professor E. Merritt of the Department of Physics for this report.

$\lambda = 5512$  were superposed on the principal band. For sulfonefluorescein the band extended approximately from  $\lambda = 5000$  to  $\lambda = 5900$ . With fluorescein the width was nearly the same.

The absorption band for sulfonefluorescein was shifted toward the red, as compared with fluorescein, somewhat more than the fluorescence band, and was noticeably steeper on the red side than the absorption band for fluorescein. Since in each case the absorption region extended toward the red a little beyond the position of maximum fluorescence, this difference in the steepness of the absorption band may account for the difference in the shape of the two fluorescence bands.

At the crest of the absorption band the transmission for the sulfonefluorescein solution was about 2.5% (with a layer 2 cm. thick) while the transmission for the fluorescein solution was about 3%. The conditions did not permit great accuracy, however.

### Experimental Part<sup>13</sup>

**Ammonium Salt of Dihydroxybenzoyl-benzene-*o*-sulfonic Acid.**—This salt was prepared by the method used by Remsen and Linn<sup>4</sup> and by Blackshear.<sup>6</sup> According to Remsen and Linn it crystallizes with 1.5 molecules of water, while Fahlberg and Barge<sup>5</sup> found 2 molecules of water. It contains 2 molecules of water, as shown by the following analyses.

*Anal.* Subs., 1.8027, 0.9112: loss at 150°, H<sub>2</sub>O, 0.1860, 0.0945. Subs., 0.3978, 0.4282: cc. of 0.1 *N* HCl, 11.54, 12.37. Subs., 0.5254 (dry), 0.4948 (dry): BaSO<sub>4</sub>, 0.3955, 0.3704. Calc. for C<sub>13</sub>H<sub>13</sub>O<sub>6</sub>SN + 2H<sub>2</sub>O: H<sub>2</sub>O, 10.38, N, 4.03; S, 10.30. Found: H<sub>2</sub>O, 10.32, 10.37; N, 4.06, 4.05; S, 10.34, 10.28.

**Preparation of 2,4-Dihydroxybenzoyl-benzene-*o*-sulfonic Acid from the Ammonium Salt.**—The ammonium salt was dissolved in water, using about 10 cc. per g. Hydrogen chloride was passed into the filtered solution until the concentration of the hydrochloric acid was sufficient to cause the free dihydroxy acid to crystallize, but not the ammonium chloride. The acid was recrystallized by dissolving it in water and passing in hydrogen chloride. This method takes only a few minutes for both preparing and purifying the acid. It gives a well-crystallized product, the crystals having a cream color with a silky luster. When powdered it is colorless. If the solution is cooled slowly the crystals are large, but more yellow than the lustrous plates obtained by cooling the solution quickly.

The acid was dried in the air for a week and then analyzed. When heated to 98° it slowly fused, giving off water and becoming colored. The water could not be determined by drying, however. The sulfur determinations, as well as titration of the acid with 0.1 *N* sodium hydroxide solution, show that its composition is represented by the formula,<sup>6</sup> C<sub>13</sub>H<sub>10</sub>O<sub>6</sub>S + 3H<sub>2</sub>O.

*Anal.* Subs., 0.4936, 0.4693: BaSO<sub>4</sub>, 0.3253, 0.3137. Calc. for C<sub>13</sub>H<sub>10</sub>O<sub>6</sub>S + 3H<sub>2</sub>O: S, 9.21. Found: 9.06, 9.18.

With methyl red the acid titrates as a *monobasic* acid.

<sup>13</sup> We are indebted to the Monsanto Chemical Co. for the Saccharin used in this investigation. It was a very pure product and contained but a trace of *p*-sulfo-amino-benzoic acid.

	Acid	0.1 N NaOH Cc.	0.1 N NaOH per g. Cc.	Mol. wt. found
I	0.5378	15.40	28.64	349.2
II	.6891	19.70	28.59	349.8
III	.4350	12.45	28.62	349.4
IV	.4654	13.30	28.58	349.9

It titrates as a *dibasic* acid, however, with phenolphthalein.

	Acid	0.1 N NaOH Cc.	0.1 N NaOH per g. Cc.	Mol. wt. found
I	0.4819	27.70	57.48	348.0
II	.4867	27.90	57.32	348.8
III	.5415	31.10	57.43	348.2
Calc. for $C_{13}H_{10}O_6S + 3H_2O$ : mol. wt., 348.3.				

**3,5-Dibromo-2,4-dihydroxybenzoyl-benzene-*o*-sulfonic Acid.**—To 10 g. of the dihydroxy acid in 100 cc. of glacial acetic acid, 6 cc. of bromine in 50 cc. of glacial acetic acid was added. The dihydroxy acid dissolved upon the addition of the bromine. The mixture was then heated for 15 hours on a boiling water-bath, the solution filtered, concentrated to a small volume, and diluted with water to about 200 cc. Hydrogen chloride was then passed in, while the solution was cooled, until the concentration was sufficient to crystallize the brominated dihydroxy acid. The acid was again crystallized in the same way and was then obtained as small, colorless crystals, which are soluble in glacial acetic acid, water, alcohol and dil. hydrochloric acid, but insoluble in concd. hydrochloric acid. They melt at 110–112°. After drying in the air for a week they were analyzed.

*Anal.* Subs., 0.2219, 0.2449; AgBr, 0.1683, 0.1850. Calc. for  $C_{13}H_8Br_2O_6S + 2.5H_2O$ : Br, 32.16. Found: 32.28, 32.15.

The dibromo product titrates as a *dibasic* acid with either methyl red or phenolphthalein.

With methyl red the following results were obtained.

	Acid	0.1 N NaOH Cc.	0.1 N NaOH per g. Cc.	Mol. wt. found
I	0.5025	20.10	40.00	500.0
II	0.5000	20.00	40.00	500.0

With phenolphthalein the results again show that the acid crystallizes with 2.5 molecules of water.

	Acid	0.1 N NaOH Cc.	0.1 N NaOH per g. Cc.	Mol. wt. found
I	0.4923	19.80	40.22	497.2
II	.5972	24.00	40.19	497.6
Calc. for $C_{13}H_8Br_2O_6S + 2.5H_2O$ : mol. wt., 497.1.				

**Preparation of Sulfonefluorescein by Heating Dihydroxy-benzoyl-benzene-*o*-sulfonic Acid.**—Sulfonefluorescein was prepared from dihydroxybenzoyl-benzene-*o*-sulfonic acid by heating it to 160–170° for two hours. The anhydride of *o*-sulfobenzoic acid sublimed as colorless needles, which melted at 128°.

The sulfonefluorescein was crystallized by dissolving it in sodium hydroxide solution, filtering the solution, and diluting with water to give one liter of solution for each 1.5 g. of the dihydroxy acid used. This solution was heated to boiling and acidified with hydrochloric acid. After several days the sulfonefluorescein separated in red crystals

which had a blue surface color. They were washed with water until free from chlorides, air-dried for a week and analyzed.

*Anal.* Subs., 0.3124: loss at 150°, 0.0078. Calc. for  $C_{19}H_{12}SO_6 + 0.5H_2O$ :  $H_2O$ , 2.39. Found: 2.50.

Subs., 0.3046 (dry):  $BaSO_4$ , 0.1926. Calc. for  $C_{19}H_{12}SO_6$ : S, 8.71. Found: 8.69.

The crystallized sulfonefluorescein dissolves to a slight extent in boiling water, in boiling glacial and in boiling dil. acetic acid, but only enough to give the solutions a yellow color. It is practically insoluble in all the ordinary organic solvents. It dissolves in concd. sulfuric acid with a red color and greenish fluorescence. When this solution is poured into a large volume of water a yellow solution with greenish fluorescence results, from which on standing amorphous *yellow* sulfonefluorescein precipitates. This soon changes to the red amorphous product. It dissolves to some extent in boiling pyridine to give a red solution with greenish fluorescence, and the red crystals become yellow, due to formation of the pyridine salt.

**Preparation of Sulfonefluorescein from Dihydroxybenzoyl-benzene-*o*-sulfonic Acid and Resorcinol.**—Sulfonefluorescein was also prepared by heating 3.5 g. of the dihydroxy acid with 5 g. of resorcinol at 160–170° for four hours, and stirring the mixture during the heating. Water was added and the mixture steam-distilled to remove resorcinol. The sulfonefluorescein was filtered from this solution, washed with water, dissolved in dil. sodium hydroxide solution, and the solution boiled with boneblack. The filtered solution was diluted with water to 12 liters, heated to boiling and acidified with hydrochloric acid. After 24 hours, the crystallized sulfonefluorescein was filtered off, washed with water until free from chlorides and air-dried for a week.

*Anal.* Subs., 0.4324: loss at 150°, 0.0111. Calc. for  $C_{19}H_{12}SO_6 + 0.5H_2O$ :  $H_2O$ , 2.39. Found: 2.57.

Subs., 0.4213 (dry):  $BaSO_4$ , 0.2655. Calc. for  $C_{19}H_{12}SO_6$ : S, 8.71. Found: 8.66.

This sulfonefluorescein is identical with that obtained by heating the dihydroxy acid alone, or by heating resorcinol with the anhydride of *o*-sulfobenzoic acid (see below).

When the ammonium salt of the dihydroxy acid is first prepared it always contains some sulfonefluorescein. The pure ammonium salt is colorless and dissolves in water without color. When first made its solution is yellow and shows a marked green fluorescence.

These results show that the formation of sulfonefluorescein from resorcinol and *o*-sulfobenzoic acid anhydride takes place in two steps, and that 2,4-dihydroxybenzoyl-benzene-*o*-sulfonic acid is the intermediate product.

**Preparation of Sulfonefluorescein from the Anhydride of *o*-Sulfobenzoic Acid and Resorcinol.**—Ninety g. of *o*-sulfobenzoic acid anhydride and 130 g. of resorcinol were heated for three hours at 135–140°, and the sulfonefluorescein was purified as usual. It was further purified by crystallization after conversion into a pure, crystallized zinc salt of sulfonefluorescein (see below).

The zinc salt of sulfonefluorescein was dissolved in water and the zinc precipitated with an excess of diammonium phosphate. The solution was filtered, made alkaline and boiled until the ammonia was expelled. It was then diluted to a large volume, heated to boiling, and air bubbled through until the sulfonefluorescein had been oxidized to sulfonefluorescein. The solution was then made acid with hydrochloric acid, and after a day or so, purple crystals of sulfonefluorescein were obtained. Eight g. was re-crystallized by dissolving in sodium hydroxide solution, filtering and diluting with water to 12 liters. This solution was heated to boiling and acidified with hydrochloric acid. The sulfonefluorescein precipitated on standing in well-formed, purple crystals which were air-dried.

*Anal.* Subs., 0.2321, 0.1790: loss at 150°, 0.0057, 0.0046. Calc. for  $C_{19}H_{12}O_6S + 0.5H_2O$ :  $H_2O$ , 2.39. Found: 2.46, 2.57.

Subs. (dry), 0.2648, 0.4881:  $BaSO_4$ , 0.1624, 0.3016. Calc. for  $C_{19}H_{12}O_6S$ : S, 8.71. Found: 8.42, 8.48.

**Preparation of Sulfonefluorescin from Saccharin and Resorcinol.**—Forty grams of Saccharin was heated with 50 g. of concd. sulfuric acid at 120° until dissolved and 54 g. of resorcinol added, which caused the temperature to rise to 140°. The mixture was heated at 135–140° for seven hours and stirred during this time, water was added, and the whole steam distilled to remove resorcinol and sulfuric acid. The insoluble material was dissolved in sodium hydroxide solution, the solution filtered, diluted to 10–11 liters with water and heated to boiling. It was then made acid with hydrochloric acid, which precipitated an amorphous sulfonefluorescin. This was filtered off, and the red solution allowed to stand overnight when purple crystals of sulfonefluorescin separated. The amorphous product was treated in the same way to obtain more of the crystallized sulfonefluorescin. After six such crystallizations, 43 g. had been obtained, a yield of 52%. Although the yield is satisfactory, this method necessitates the use of large volumes of water, and takes considerable time. For this reason the sulfonefluorescin is preferably prepared from the chlorides of *o*-sulfobenzoic acid.

*Anal.* Subs., 0.7698, 1.1648: loss at 125–140°, 0.0193, 0.0301. Calc. for  $C_{19}H_{12}O_6S + 0.5H_2O$ :  $H_2O$ , 2.39. Found: 2.51, 2.58.

Subs. (dry), 0.3642, 0.4654:  $BaSO_4$ , 0.2332, 0.2918. Calc. for  $C_{19}H_{12}O_6S$ : S, 8.71. Found: 8.80, 8.61.

Combustion analyses by de Roode's method gave the following results.

Subs. (dry), 0.1893, 0.3309:  $CO_2$ , 0.4288, 0.7494;  $H_2O$ , 0.0533, 0.0921. Calc. for  $C_{19}H_{12}O_6S$ : C, 61.94; H, 3.29. Found: C, 61.80, 61.78; H, 3.15, 3.12.

Sisley<sup>9</sup> crystallized his sulfonefluorescin from glacial acetic acid or alcohol. As sulfonefluorescin is practically insoluble in these solvents, this statement must be an error or else his product was not sulfonefluorescin.

**Preparation of Sulfonefluorescin from the Chlorides of *o*-Sulfobenzoic Acid.**—Sulfonefluorescin was prepared by heating 392.5 g. of the chlorides and 400 g. of resorcinol in a water-bath for about 12 hours. At 65–68° there was a vigorous reaction with the evolution of hydrochloric acid. The mixture was kept at 65° for about 3.5 hours until most of the hydrogen chloride was expelled, and the liquid had become a thick, red-purple mass. The temperature was kept at 85–90° for the remainder of the time. The mixture was thoroughly extracted with hot water, the insoluble material filtered off and purified by acidifying a hot, dilute aqueous solution of its sodium salt. This gave 255 g. of the pure sulfonefluorescin, a 41% yield.

Eight g. of this product was suspended in 10.5 liters of water and enough sodium hydroxide solution added to dissolve it. The solution was filtered, heated to boiling, acidified with hydrochloric acid, and allowed to stand overnight. This gave a well crystallized, purple sulfonefluorescin. It was dried at 150°.

*Anal.* Subs., 0.2557:  $CO_2$ , 0.5784;  $H_2O$ , 0.0747. Subs., 0.4350;  $BaSO_4$ , 0.2783. Calc. for  $C_{19}H_{12}O_6S$ : C, 61.94; H, 3.29; S, 8.71. Found: C, 61.71; H, 3.27; S, 8.79.

**The Crystalline Form of Sulfonefluorescin.**<sup>14</sup>—The crystals are mostly dendritic or pinnate aggregates, probably rhombic in crystallization. A blue surface color in the thicker crystals is modified in the thinner crystals by transmitted light to a reddish-purple. Pleochroism is strong, from yellow to fox-red. The red color is seen when the light vibrates across the sharp pointed pinnules of the aggregates, and when it is parallel

<sup>14</sup> This description of the crystalline form of sulfonefluorescin is by Professor A. C. Gill of the Department of Crystallography and Mineralogy.



to the longer diameter of the spatulate forms. Greater optical elasticity shows less absorption. The substance is thought to be rhombic, even though a few elongated crystals seem to show an extinction angle of a few degrees, since the crystal boundaries are not sharp.

**Yellow Sulfonefluorescein.**—Remsen and Linn<sup>4</sup> and Blackshear<sup>6</sup> found, upon dissolving sulfonefluorescein in potassium hydroxide solution and precipitating it with dil. sulfuric acid, that sometimes it came out in yellow, flocculent masses which quickly turned red-brown.

This work was repeated, with the result that the bright yellow precipitate did not seem to be flocculent, although it did coagulate rapidly into a somewhat flocculent, red mass. The acid precipitated the sulfonefluorescein in a very finely divided state. When this was stirred it quickly changed into larger aggregates which were red, whereas the sulfonefluorescein in the finely divided state was yellow. This agrees with the fact that when the purple crystals of sulfonefluorescein are finely ground, the resulting powder is yellow.

The precipitation of sulfonefluorescein from alkaline solution with dil. sulfuric acid was then observed under a microscope. The acid precipitated the sulfonefluorescein in a yellow, colloidal state; at least, the individual particles of the precipitate were not visible. This quickly broke up with the formation of tiny, globular masses of radiating, acicular crystals. These spherulites appeared opaque by transmitted light; single crystals appeared red to orange, depending upon the thickness. With reflected light the crystalline precipitate was orange. As soon as the sulfonefluorescein had crystallized, it no longer was yellow, except in the case of a few radiating crystals. These were very thin plates, since with the proper reflected light they glistened and displayed the iridescent colors of thin films. This shows that the color of sulfonefluorescein depends upon the state of division. When in a finely divided or colloidal state it is yellow. When the aggregates are larger it is red. Crystals which are very thin plates, approaching a film, are also yellow. When well crystallized it is red with a blue surface color, which causes it to appear purple.

**Ammonium Salt of Sulfonefluorescein.**—Four g. of sulfonefluorescein was dissolved in 100 cc. of ammonium hydroxide, the solution evaporated to a small volume, diluted to about 300 cc. with absolute ethyl alcohol, and enough ether added to precipitate the ammonium salt as a dark red powder.

The analyses show it to be the mono-ammonium salt.

*Anal.* Subs., 1.1794: loss at 130°; H<sub>2</sub>O, 0.1036. Subs., 0.4209, 0.4222: cc. of 0.1 N HCl, 9.92; 10.11. Calc. for C<sub>19</sub>H<sub>11</sub>SO<sub>6</sub>NH<sub>4</sub> + 2H<sub>2</sub>O: H<sub>2</sub>O, 8.55; N, 3.33. Found: H<sub>2</sub>O, 8.78; N, 3.30, 3.36.

**Barium Salt of Sulfonefluorescein.**—A mixture of 5.5 g. of sulfonefluorescein and an excess of barium carbonate was boiled with water until all of the former had dissolved. The barium carbonate was filtered off, the solution of the barium salt evaporated to dryness on a water-bath, the residue redissolved in water, and the solution filtered through asbestos and again evaporated to dryness on a water-bath. This was repeated until the barium salt dissolved completely in water. The dark, beetle-green residue was boiled with 500 cc. of methyl alcohol, the solution filtered, and distilled down to 100 cc. During the distillation an orange-colored barium salt crystallized. This was filtered off and dried in the air for several days.

Analyses show this to be a pure normal barium salt.

*Anal.* Subs., 0.3892, 0.3047: loss at 150–160°, 0.0395, 0.0317. Found: H<sub>2</sub>O, 10.15, 10.40.

Subs. (dry), 0.3550, 0.2730: BaSO<sub>4</sub>, 0.0950, 0.0727. Calc. for C<sub>19</sub>H<sub>11</sub>O<sub>6</sub>S-Ba-C<sub>19</sub>H<sub>11</sub>O<sub>6</sub>S: Ba, 15.76. Found: 15.75, 15.67.

Solutions of this barium salt give with hydrochloric acid a crystallized sulfonefluorescein, even though the precipitation is very rapid. The barium salt is very soluble in water, somewhat soluble in methyl alcohol, but insoluble in absolute ethyl alcohol, even at the boiling point.

**Zinc Salt of Sulfonefluorescein.**—A mixture of 10 g. of sulfonefluorescein and 15 g. of zinc carbonate was boiled with 1 liter of water for four hours and the zinc carbonate filtered off. The solution of the zinc salt was then evaporated to crystallization. Crystals do not form until the solution is very concentrated, and any insoluble matter that separated before this was filtered off. Upon recrystallizing the product thrice from water all of the insoluble material was removed, giving a well crystallized, orange zinc salt of sulfonefluorescein that was entirely soluble in water.

The crystals were dried in the air for about ten days before analysis.

*Anal.* Subs., 0.4396, 0.4358: loss at 125–130°, 0.0576, 0.0575. Calc. for  $C_{19}H_{11}O_6S \cdot Zn \cdot C_{19}H_{11}O_6S \cdot 7H_2O$ :  $H_2O$ , 13.62. Found: 13.10, 13.19.

Subs. (dry), 0.3820, 0.3783:  $ZnNH_4PO_4$ , 0.0857, 0.0838;  $Zn_2P_2O_7$ , 0.0730, 0.0714. Calc. for  $C_{19}H_{11}O_6S \cdot Zn \cdot C_{19}H_{11}O_6S$ : Zn, 8.17. Found: (as  $ZnNH_4PO_4$ ), 8.22, 8.11; (as  $Zn_2P_2O_7$ ), 8.20, 8.10.

**The Reduction Product of Sulfonefluorescein—Sulfonefluorescein.**—When sulfonefluorescein is suspended in water and boiled with zinc dust it first forms the orange zinc salt of sulfonefluorescein described above. On further boiling with the zinc dust and water this salt is reduced to the colorless zinc salt of sulfonefluorescein. Upon oxidation the quinoid structure is again established.

**Zinc Salt of Sulfonefluorescein.**—When 17.5 g. of sulfonefluorescein in 500 cc. of water was boiled with an excess of zinc dust, the color soon changed from a deep red with a green fluorescence to a light yellow lacking fluorescence. On standing, the solution became red and showed a green fluorescence. After about 24 hours, crystallization started and continued for the next four or five days. This gave a well-crystallized, cream-colored zinc salt that became yellow in the air. After the crystals had been dried in the air in the dark for a week they were analyzed.

A sample dried in a current of air at 120–125° changed to orange-red, the color of the zinc salt of sulfonefluorescein made from zinc carbonate. The samples analyzed were dried in a current of carbon dioxide, but became somewhat yellow.

*Anal.* Subs., 1.0680, 0.7438: loss at 120–125°, 0.1730, 0.1210. Found: 16.20, 16.27.

Subs. (dry), 0.6565, 0.4071:  $ZnNH_4PO_4$ , 0.1453, 0.0891;  $Zn_2P_2O_7$ , 0.1231, 0.0761. Calc. for  $C_{19}H_{13}O_6S \cdot Zn \cdot C_{19}H_{13}O_6S$ : Zn, 8.13. Found: (as  $ZnNH_4PO_4$ ), 8.11, 8.02; (as  $Zn_2P_2O_7$ ), 8.04, 8.02.

**Preparation of Sulfonefluorescein.**—A mixture of 17.5 g. of sulfonefluorescein and 500 cc. of water was boiled with zinc dust for an hour and the solution filtered. The zinc was precipitated with hydrogen sulfide, the zinc sulfide filtered off, and the solution evaporated to crystallization in a current of carbon dioxide. It is to be noted that while sulfonefluorescein is very difficultly soluble in water, sulfonefluorescein is extremely soluble. A volume of 50 cc. gave straw-colored, well-formed crystals of sulfonefluorescein. These when filtered off and washed with water were colorless. They melted at 164° and very soon turned yellow upon exposure to the air. When dried in the air the crystals became light brown. They were therefore placed over concd. sulfuric acid in a desiccator filled with carbon dioxide and dried to constant weight under a vacuum. They were then faintly yellow.

*Anal.* Subs., 0.5891, 0.6415.  $BaSO_4$ , 0.3645, 0.3988. Calc. for  $C_{19}H_{14}O_6S$ : S, 8.66. Found: 8.50, 8.54.

Sulfonefluorescin is insoluble in ether and benzene. It is very soluble in water, absolute methyl or ethyl alcohol, and in acetone. It is somewhat soluble in cold glacial acetic acid, and dissolves readily when warmed. Dil. sodium hydroxide solution dissolves it very readily, giving a colorless solution, which shows a faint trace of fluorescence. On standing, this solution becomes yellow and the green fluorescence becomes very marked. When the *dry* sulfonefluorescin is heated above 130° it turns red, and at 240° it becomes very dark, although it does not fuse when heated to 300°.

**The Diacetate of Sulfonefluorescin.**—Sulfonefluorescin dissolves very readily in acetic anhydride with the evolution of heat. This solution was boiled for an hour and then concentrated until crystallization commenced. The material was recrystallized from acetic anhydride and obtained as light yellow plates soluble in water, giving a yellow solution. It also dissolves easily in glacial acetic acid to give a yellow solution. It is not very soluble in methyl or ethyl alcohol. When heated, it shows a color change at 205°, and fuses with decomposition at 230°.

The analyses of the product dried to constant weight in a desiccator over ground sodium hydroxide show it to be a diacetate.

*Anal.* Subs., 0.2989, 0.3648: BaSO<sub>4</sub>, 0.1498, 0.1850. Calc. for C<sub>19</sub>H<sub>12</sub>O<sub>6</sub>S(COCH<sub>3</sub>)<sub>2</sub>: S, 7.06. Found: 6.89, 6.97.

**Action of Ammonia on Sulfonefluorescin.**—Dry sulfonefluorescin was exposed for 48 hours to ammonia that had been dried with solid sodium hydroxide and sodium wire. It absorbed about four molecules of ammonia.

*Anal.* Subs., 0.4630, 0.3762: NH<sub>3</sub> absorbed, 0.0684, 0.0565. Calc. for C<sub>19</sub>H<sub>12</sub>O<sub>6</sub>S.4NH<sub>3</sub>: NH<sub>3</sub>, 15.61. Found: 14.77, 15.02.

When placed in a desiccator over concd. sulfuric acid and allowed to come to constant weight, it then contained one molecule of ammonia.

*Anal.* Subs., 0.4630, 0.3762: NH<sub>3</sub>, 0.0218, 0.0173. Calc. for C<sub>19</sub>H<sub>12</sub>O<sub>6</sub>S.NH<sub>3</sub>: NH<sub>3</sub>, 4.42. Found: 4.71, 4.60.

**Action of Aniline on Sulfonefluorescin.**—Ten g. of pure sulfonefluorescin was boiled with 100 cc. of freshly distilled aniline for eight hours. The aniline derivative separated in well-formed, green crystals during the heating. It was filtered off, washed with ether until free from aniline, and dried. The substance lost no weight when heated to 120°.

*Anal.* Subs., 0.5450, 0.6081. BaSO<sub>4</sub>, 0.2420, 0.2725. Calc. for C<sub>31</sub>H<sub>22</sub>O<sub>4</sub>SN<sub>2</sub>: S, 6.18. Found: 6.10, 6.16.

It dissolves somewhat in dilute alkalis, in glacial acetic acid, and in hydrochloric acid to give a cerise-colored solution showing a dark green fluorescence. The aniline derivative of sulfonefluorescin is insoluble in ether. It dissolves in absolute methyl or ethyl alcohol, giving a deep red solution with a dark green fluorescence. The crystals of the aniline derivative are blue-violet by transmitted light. The surface color, however, is green. They do not melt when heated to 320°.

**Sulfonefluorescin Mono-acetate.**—Ten g. of sulfonefluorescin was boiled for six hours with 500 cc. of acetic anhydride, giving a dark green solution. This was filtered and concentrated until crystallization commenced. The dark green crystals were filtered off, recrystallized twice from acetic anhydride, dried to constant weight in a desiccator over ground sodium hydroxide and analyzed.

*Anal.* Subs., 0.5243, 0.6382: BaSO<sub>4</sub>, 0.2946, 0.3600. Calc. for C<sub>19</sub>H<sub>11</sub>O<sub>6</sub>S(COCH<sub>3</sub>): S, 7.81. Found: 7.72, 7.75.

Sulfonefluorescin mono-acetate dissolves in boiling glacial acetic acid giving a dark green solution. If this solution is boiled for about an hour the green color disappears

with the separation of sulfonefluorescein from the solution. It is not very soluble in cold water, but dissolves easily in boiling water. Upon standing overnight this solution gives well-crystallized sulfonefluorescein. Dil. sodium hydroxide solution saponifies it at once. Unlike the diacetate it is insoluble in toluene. The acetate decomposes without melting when heated above 300°.

**Sulfonefluorescein Diacetate.**—Five g. of sulfonefluorescein was boiled for 15 hours with 500 cc. of acetic anhydride. It dissolved at first giving a dark green solution, but at the end of the heating the solution was very much lighter in color. It was filtered, concentrated until crystallization commenced and the crystals were recrystallized twice from acetic anhydride. This gave a light yellow product. Further recrystallization from acetic anhydride did not remove the yellow color. It was recrystallized four times from dry toluene using boneblack to decolorize the solution, but the yellow color was not removed. It is soluble in dry toluene only when first moistened with acetic anhydride.

These crystals do not melt, but decompose sharply at 209°. Professor Gill reports on them as follows. The orange-yellow crystals vary in habitus from nearly square prisms with oblique ends to elongated plates. The large tabular crystals have a plane angle of about 72°, as measured on a microscope stage, the greater elasticity which is also the direction of less absorption, making an angle of 14° or 15° with the long diameter in the acute (72°) angle of the plate. On edge, the plane angle is 78°, and the extinction is in the obtuse (102°) angle. Pleochroism is fairly strong, from clear greenish yellow near the elongation to orange-yellow for the transverse vibration. The double refraction of the plates is near 0.1000, probably a little more than that. A few twinned crystals were observed with a longitudinal composition plane. The occurrence of more than one end face at only one termination of the crystals is frequent enough to indicate, but not prove, that they have triclinic pedial structure, rather than the pinacoidal symmetry. The substance is not very soluble in water, although when boiled with water some of it goes into solution, and after standing overnight well crystallized sulfonefluorescein separates. It is also saponified when boiled with dil. acetic acid, and very readily by dil. sodium hydroxide solution. It was dried to constant weight over ground sodium hydroxide in a vacuum desiccator and analyzed. Analyses show that it is the diacetate.

*Anal.* Subs., 0.4911, 0.5069: BaSO<sub>4</sub>, 0.2473, 0.2579. Calc. for C<sub>19</sub>H<sub>10</sub>SO<sub>6</sub>(CO-CH<sub>3</sub>)<sub>2</sub>: S, 7.09. Found: 6.92, 6.99.

**Sulfonefluorescein Monobenzoate.**—Five g. of sulfonefluorescein and 30 cc. of benzoyl chloride were heated to boiling for 4½ hours. The excess of benzoyl chloride was evaporated in a water-oven through which a current of dry air passed, the brown mass was extracted with hot benzene, and then the yellow solid remaining was crystallized from dry benzene.

*Anal.* Subs., 0.5395, 0.5039: BaSO<sub>4</sub>, 0.2689, 0.2446. Calc. for C<sub>19</sub>H<sub>11</sub>O<sub>8</sub>S(CO-C<sub>6</sub>H<sub>5</sub>)<sub>2</sub>: S, 6.80. Found: 6.85, 6.67.

The yellow crystals of the monobenzoate on standing in the air for some time turned light brown, and the color continued to darken on further exposure to the air. When the substance was allowed to stand in a desiccator over concd. sulfuric acid the yellow color did not change. The crystals do not melt when heated to 300°. They are insoluble in water, but dissolve in alcohol giving a deep wine-red solution, and in benzene giving an orange-colored solution. They are somewhat soluble in hydrochloric acid forming light yellow solutions. They dissolve in 10% sodium hydroxide solution giving a deep bluish-purple color, and upon standing for a short time saponification takes place.

**Sulfonefluorescein Dichloride.**<sup>15</sup>—Sulfonefluorescein reacts both with benzoyl chloride and with phosphorus pentachloride to form sulfonefluorescein dichloride.

<sup>15</sup> This substance is in reality 3,6-dichloro-sulfonefluoran.

**Dichloride of Sulfonefluorescein Formed by the Action of Benzoyl Chloride upon Sulfonefluorescein.**—Ten g. of crystallized sulfonefluorescein and 60 cc. of benzoyl chloride were heated for ten hours at 200°. For the first few minutes a small amount of hydrogen chloride bubbled off due to the water in the sulfonefluorescein. This then stopped and there was no further indication of a reaction other than a darkening in the color of the solution. The excess of benzoyl chloride was evaporated in a water-oven through which a current of dry air passed. This caused a large quantity of benzoic acid to sublime. The material was then crystallized several times from benzene, giving colorless needles. There was no loss in weight when the substance was heated to 120°. When heated to 250–260° the compound became yellowish-brown, on further heating reddish-brown, and at 320° it was much darker due to decomposition.

The analyses show that it is the dichloride of sulfonefluorescein.

*Anal.* Subs., 0.5110, 0.4849: BaSO<sub>4</sub>, 0.2922, 0.2824. Subs., 0.2245, 0.2127: AgCl, 0.1600, 0.1520. Calc. for C<sub>19</sub>H<sub>10</sub>O<sub>4</sub>Cl<sub>2</sub>S: S, 7.91; Cl, 17.50. Found: S, 7.86, 8.00; Cl, 17.63, 17.68.

**Action of Phosphorus Pentachloride on Sulfonefluorescein.**—From his analyses, White<sup>7</sup> seemed to think that when sulfonefluorescein is treated with phosphorus pentachloride, "four chlorine atoms enter in, replacing the anhydride oxygen as well as the phenol hydroxyls." This work was repeated.

A mixture of 25 g. of sulfonefluorescein and 100 g. of phosphorus pentachloride was heated at 125–130° for three hours and when cold treated with water. The insoluble matter was filtered off, washed thoroughly with water, dried and recrystallized from benzene until it was colorless.

*Anal.* Subs., 0.3040, 0.2914. AgCl, 0.2155, 0.2060. Calc. for C<sub>19</sub>H<sub>10</sub>O<sub>4</sub>Cl<sub>2</sub>S: Cl, 17.50. Found: 17.54, 17.49.

The analyses show that the product is identical with the dichloride formed from sulfonefluorescein by the action of benzoyl chloride.

Sulfonefluorescein dichloride is insoluble in petroleum ether, and only slightly soluble in dil. or concd. hydrochloric acid or water, giving yellow solutions. It is soluble in absolute methyl or ethyl alcohol giving a greenish-yellow solution. Its solution in chloroform is yellow. The solutions in ether, benzene, toluene, or acetone are colorless. It dissolves in glacial acetic acid and in acetic anhydride. The crystals are insoluble in a boiling dilute solution of sodium hydroxide. Crystals from toluene are colorless to faint yellow, well-formed, lath-shaped prisms with square ends and beveled sides. They are either monoclinic or triclinic and have moderately strong double refraction. The maximum extinction angle is about 35° and the index of refraction is about 1.73.

That the two phenol hydroxyl groups have been replaced by chlorine is shown by the fact that this compound cannot be acetylated by boiling it with acetic anhydride.

Sulfonefluorescein dichloride was boiled for an hour with acetic anhydride. The solution was concentrated to crystallization, giving colorless needles which dissolve in concd. sodium hydroxide solution with the same color that sulfonefluorescein dichloride gives. After drying to constant weight over concd. sulfuric acid and ground sodium hydroxide in a vacuum desiccator it was analyzed. The analysis shows that it was the unchanged dichloride.

*Anal.* Subs., 0.2077: AgCl, 0.1450. Calc. for C<sub>19</sub>H<sub>10</sub>O<sub>4</sub>Cl<sub>2</sub>S: Cl, 17.50. Found: 17.27.

The colorless crystals were boiled with a 20% sodium hydroxide solution free from chlorides, and the green-blue solution was acidified with nitric acid. This gave a red solution, which when tested with silver nitrate showed no trace of chlorides. This was repeated, the alkaline solution being boiled until it was only faintly yellow. When it was

acidified with nitric acid, a red solution was obtained which gave no test for chlorides. From this, on standing, the red hydrate of sulfonefluorescein dichloride precipitated.

**Hydrate of Sulfonefluorescein Dichloride.**—The colorless crystals of sulfonefluorescein dichloride were boiled with a 20% solution of sodium hydroxide until the solution was only a light yellow. It was then acidified with hydrochloric acid, the red precipitate was filtered off and, after being washed with water until free from chlorides, dried in a water-oven. It was dried to constant weight at 120–125° and analyzed. The analyses show that the dried compound still contained a molecule of water.

*Anal.* Subs., 0.6356, 0.4968: loss at 120–125°, 0.0680, 0.0520. Found: H<sub>2</sub>O, 10.70, 10.47.

Subs. (dry), 0.2275, 0.1960: AgCl, 0.1553, 0.1321. Calc. for C<sub>19</sub>H<sub>10</sub>O<sub>4</sub>Cl<sub>2</sub>S.H<sub>2</sub>O: Cl, 16.76. Found: 16.89, 16.67.

The filtrate from the red hydrate was concentrated and allowed to stand until crystallization took place. The crystals were orange to greenish-orange by transmitted light. They formed clusters of well-formed, obtuse or square-ended prisms (first and second order). They are probably orthorhombic, exhibiting parallel extinction, strong double refraction and faint pleochroism. The index of refraction is high and is greater for vibrations parallel to the elongation.

The crystals of the hydrate dissolve in a dilute solution of sodium hydroxide forming a yellow solution with greenish fluorescence.

**Dibromo-sulfonefluorescein.**—To 10 g. of sulfonefluorescein suspended in 100 cc. of glacial acetic acid was added 17.5 g. of bromine in 50 cc. of glacial acetic acid. This mixture was boiled for six hours until the evolution of hydrogen bromide had ceased. After standing overnight, the red crystallized product was filtered off. Five g. of this material was dissolved in 5% sodium hydroxide solution, the filtered solution diluted to about 10.5 liters with water, heated to boiling and acidified with hydrochloric acid. On standing overnight red needles having a silky luster separated. The substance was then recrystallized twice in the same manner except that dil. sulfuric acid was used for acidifying. The air-dried crystals were analyzed.

*Anal.* Subs., 0.4547, 0.3455: loss at 125–130°, 0.0195, 0.0146. Calc. for C<sub>19</sub>H<sub>10</sub>Br<sub>2</sub>SO<sub>6</sub>.1.25H<sub>2</sub>O: H<sub>2</sub>O, 4.11. Found: 4.29, 4.23.

Subs. (dry), 0.4067, 0.2830: AgBr, 0.2910, 0.2021. Subs., 0.3046: BaSO<sub>4</sub>, 0.1372. Calc. for C<sub>19</sub>H<sub>10</sub>Br<sub>2</sub>SO<sub>6</sub>: Br, 30.38; S, 6.09. Found: Br, 30.45, 30.39; S, 6.19.

The analyses show that the product is the dibromo-sulfonefluorescein, although the amount of bromine used was sufficient to form the tetrabromo compound.

Since Sisley<sup>9</sup> claims to have made a tetrabromo compound by brominating in alcohol, this work was repeated. A mixture of 12 g. of sulfonefluorescein, 1 liter of absolute alcohol and 30 g. of bromine was boiled for eight hours. The product was recrystallized as before, dried to constant weight at 125–130° and analyzed.

*Anal.* Subs. (dry), 0.2219, 0.2168: AgBr, 0.1589, 0.1542. Calc. for C<sub>19</sub>H<sub>10</sub>Br<sub>2</sub>SO<sub>6</sub>: Br, 30.38. Found: 30.47, 30.27.

The above results show that the product is dibromo-sulfonefluorescein, although the amount of bromine used was sufficient to form the tetrabromo compound.

An attempt was made to prepare the tetrabromo compound by brominating the dibromo-sulfonefluorescein. The product obtained gave 30.43% and 30.29% of bromine, showing that it was the unchanged dibromo product. Sisley's tetrabromo compound could not be made.

Dibromo-sulfonefluorescein does not melt when heated to 300°. It is practically insoluble in water, alcohol and glacial acetic acid. It dissolves very readily in a dilute alkaline solution. By reflected light this solution is intensely green; by transmitted

light it is orange to red, depending upon the concentration. When the alkaline solution is heated, however, the color changes to a blue-violet. When some of this solution is diluted with water and allowed to stand the color changes to orange (or yellow) with green fluorescence. Considering this reaction to be similar to that which Baeyer found for fluorescein, it can be represented as an opening up of the pyrone ring and then closing again in the dilute alkali. Dibromo-sulfonefluorescein dissolves in boiling aniline giving a red solution, but does not form a dianilino compound similar to that obtained from sulfonefluorescein.

**Dibromo-sulfonefluorescein Diacetate.**—Five g. of dibromo-sulfonefluorescein was boiled for nine hours with 250 cc. of acetic anhydride. The solution was filtered and distilled down to about 75 cc. for crystallization. The material was recrystallized twice from acetic anhydride, giving well-formed crystals having a light cream color. Upon standing in the air they quickly turned yellow and then orange. Some freshly crystallized material was dried to constant weight in a vacuum desiccator over concd. sulfuric acid and ground sodium hydroxide and then analyzed.

*Anal.* Subs., 0.3779, 0.3077: AgBr, 0.2323, 0.1883. Subs., 0.5551: BaSO<sub>4</sub>, 0.2089. Calc. for C<sub>19</sub>H<sub>9</sub>Br<sub>2</sub>SO<sub>6</sub>(COCH<sub>3</sub>)<sub>2</sub>: Br, 26.20. S, 5.26. Found: Br, 26.16, 26.04; S, 5.16.

Hence the product is the diacetate. It does not melt when heated to 300°. It dissolves in dil. sodium hydroxide solution, undergoing saponification. It is not very soluble in methyl or ethyl alcohol, although it gives yellow solutions in these solvents. It is insoluble in ether. It dissolves to some extent in glacial acetic acid, only enough to color the solution, however. It is somewhat soluble in hot, dil. acetic acid forming an orange-red solution. Dibromo-sulfonefluorescein crystallizes from this solution on standing. It is also hydrolyzed by water. Although it is not very soluble in cold water, upon boiling it dissolves, giving a red solution from which dibromo-sulfonefluorescein crystallizes on standing.

**Action of Ammonia on Dibromo-sulfonefluorescein.**—Dry dibromo-sulfonefluorescein was exposed for 48 hours to dry ammonia. It absorbed 4 molecules of ammonia.

*Anal.* Subs., 0.2196, 0.2328: NH<sub>3</sub>, absorbed, 0.0257, 0.0271. Calc. for C<sub>19</sub>H<sub>10</sub>Br<sub>2</sub>SO<sub>6</sub>.4NH<sub>3</sub>: NH<sub>3</sub>, 11.47. Found: 11.70, 11.64.

When placed in a desiccator over concd. sulfuric acid and allowed to come to constant weight, it then contained one molecule of ammonia.

*Anal.* Subs., 0.2196, 0.2328: NH<sub>3</sub>, 0.0074, 0.0076. Calc. for C<sub>19</sub>H<sub>10</sub>Br<sub>2</sub>SO<sub>6</sub>.NH<sub>3</sub>: NH<sub>3</sub>, 3.14. Found: 3.37, 3.27.

**Di-iodo-sulfonefluorescein.**<sup>16</sup>—Twelve cc. of 30% sodium hydroxide solution was added to 12 g. of sulfonefluorescein suspended in 120 cc. of water. Fifty-five g. of iodine suspended in 120 cc. of water was decolorized by treatment with 70 cc. of 30% sodium hydroxide solution. The two solutions were combined, heated nearly to boiling, and 55 g. of glacial acetic acid was added drop by drop while the mixture was stirred. The solution was then partially neutralized with 25 cc. of 30% sodium hydroxide solution and after an hour, heated nearly to boiling for 3/4 hour. It was then added to 2.5 liters of nearly boiling water and made strongly acid by the addition of 200 cc. of 1:1 hydrochloric acid. After the mixture had stood overnight, 28 g. of the iodine derivative was filtered off. This material could not be crystallized by acidifying a hot, dilute solution of its sodium salt. It was obtained in red needles, however, by preparing the crystallized acetate (see di-iodo-sulfonefluorescein diacetate) and hydrolyzing this with boiling water.

<sup>16</sup> This is the method used by Gomberg and Tabern to prepare *tetraiodofluorescein*, *J. Ind. Eng. Chem.*, **14**, 1115 (1922).

When the red solution was concentrated and allowed to stand, the di-iodo compound crystallized. This was dried to constant weight at 125–130° and analyzed; the analyses showed that it was di-iodo-sulfonefluorescein.

*Anal.* Subs., 0.1864, 0.1937: AgI, 0.1425, 0.1476. Calc. for  $C_{19}H_{16}I_2SO_6$ : I, 40.94. Found: 41.32, 41.19.

Di-iodo-sulfonefluorescein does not melt when heated to 300°. It is not very soluble in water, alcohol or glacial acetic acid. It dissolves in dil. sodium hydroxide solution forming a pink-to-red solution having a green fluorescence.

**Di-iodo-sulfonefluorescein Diacetate.**—Fifteen g. of di-iodo-sulfonefluorescein was boiled for ten hours with 500 cc. of acetic anhydride. The solution was filtered and distilled until crystallization commenced. The brown crystals obtained were recrystallized from acetic anhydride until they were only faintly cream-colored. This material was dried to constant weight in a vacuum desiccator over concd. sulfuric acid and ground sodium hydroxide, and then analyzed.

*Anal.* Subs., 0.2319, 0.2413: AgI, 0.1554, 0.1627. Calc. for  $C_{19}H_{16}I_2SO_6(CO-CH_3)_2$ : I, 36.05. Found: 36.22, 36.45.

The analyses showed that the product was the diacetate. Crystals of di-iodo-sulfonefluorescein diacetate are short, yellow, thick, strongly doubly refractive prisms, apparently monoclinic or triclinic. They do not melt, but when heated to 240° they are light orange in color. At 315° the color becomes dark red. This acetate seems to be stable in the air for some time.

It is insoluble in cold water, but is hydrolyzed by boiling water, giving an orange-red solution from which on concentration, the di-iodo-sulfonefluorescein crystallizes. The diacetate is also insoluble in cold, dil. sodium hydroxide solution. Upon standing, however, it hydrolyzes slowly, dissolving with the bluish-red color of alkaline solutions of di-iodo-sulfonefluorescein. This solution when diluted is pink and shows a marked light green fluorescence.

### Summary

The results of this investigation may be briefly stated as follows.

1. 2,4-Dihydroxybenzoyl-benzene-*o*-sulfonic acid has been made by decomposing its ammonium salt in aqueous solution with hydrogen chloride. This compound is colorless and forms a colorless monosodium and disodium salt.

2. 3,5-Dibromo-2,4-dihydroxybenzoyl-benzene-*o*-sulfonic acid has been prepared. It is colorless and forms a colorless disodium salt.

3. It has been shown that 2,4-dihydroxybenzoyl-benzene-*o*-sulfonic acid is an intermediate product in the formation of sulfonefluorescein. When heated alone or with resorcinol it gives sulfonefluorescein.

4. Pure, crystallized sulfonefluorescein has been made from the intermediate acid, from Saccharin, from the chlorides of *o*-sulfobenzoic acid, and from the anhydride of *o*-sulfobenzoic acid. It has been shown that sulfonefluorescein when crystalline is purple, but when in a finely divided state it is bright yellow.

5. Sulfonefluorescein, like the other phthaleins, acts as a tautomeric substance and yields *colorless* derivatives of the *lactoid* modification and *colored* derivatives having a *quinoid* structure. Sulfonefluorescein, its



salts, the mono-acetate and monobenzoate and the dianilino derivative are colored and have the quinoid structure. The diacetate and the dichloride are derivatives of the lactoid modification.

6. Sulfonefluorescin, its zinc salt and its diacetate have been made. They are colorless. Sulfonefluorescin is very readily oxidized by the air to sulfonefluorescein.

7. With ammonia, sulfonefluorescein forms a stable mono-ammonium salt.

8. Dibromo-sulfonefluorescein and di-iodo-sulfonefluorescein have been made and studied. Like sulfonefluorescein they are tautomeric substances, having themselves the colored quinoid structure and yielding diacetates having the lactoid structure. The tetrabromo-sulfonefluorescein described by Sisley could not be made.

9. With ammonia, dibromo-sulfonefluorescein forms a stable mono-ammonium salt.

10. It has been shown that the fluorescence of sulfonefluorescein is about 10% more intense than that of fluorescein.

ITHACA, NEW YORK

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF COLUMBIA UNIVERSITY, No. 445]

## RESEARCHES ON SELENIUM ORGANIC COMPOUNDS. IV THE SYNTHESIS OF BENZO-BIS-SELENAZOLES AND SELENAZOLO-BENZOTHAZOLES, NEW HETEROCYCLIC SYSTEMS

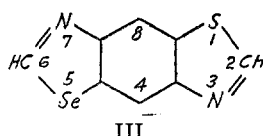
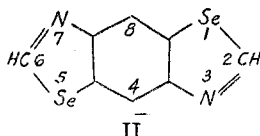
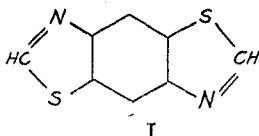
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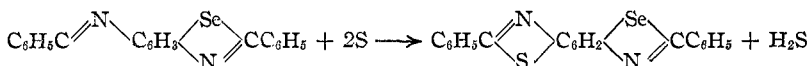
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### Introductory

Benzo-bis-thiazoles (I) have been described previously,<sup>1,2</sup> but benzo-bis-selenazoles (II) and selenazolo-benzothiazoles (III) have been unknown hitherto.



The synthesis of derivatives of heterocycles II and III has been accomplished by fusion of the 6-benzalamino-2-phenyl-benzoselenazole with selenium or sulfur.



<sup>1</sup> Green and Perkin, *J. Chem. Soc.*, **83**, 1207 (1903).

<sup>2</sup> Bogert and Abrahamson, *THIS JOURNAL*, **44**, 826 (1922).