the –MgX group to nitrogen in pyrrylmagnesium halides. The ultimate disposition of substituents on the α-carbon is accordingly no proof for a –CMgX linkage.

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A NEW SERIES OF HALOGENATED SULFONEPHTHALEINS

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Introduction

Sulfonephthaleins with halogen substituted in the phenolic residues have been prepared by several investigators since the initial discovery of the parent body by Remsen.[1] Since certain of the phthaleins and sulfonephthaleins have interesting application as dyes, as indicators, and in certain clinical procedures, a study was begun in 1927 of the preparation of a series of sulfonephthaleins having halogen substituted in the *o*-sulfobenzoic anhydride residue. The selective elimination by the kidney of phenolsulfonephthalein offered a potential lead to direct pyelography if this property were retained in compounds rendered opaque to x-rays by substituted halogen.

In 1929 there appeared a paper by Harden and Drake[2] describing some work on such a series, including four of the compounds described here. They gave but meager details of the preparation, however, and it was felt that the compounds and methods described below might still prove of interest.

Preparation and Properties of the Parent Substances (Tetra-iodo-*o*-sulfobenzoic Anhydride and Tetrabromo-*o*-sulfobenzoic Anhydride.—As has evidently been the experience of other workers, it was found that halogen could be not introduced directly into the *o*-sulfobenzoic anhydride residue of a sulfonephthalein. Attempts to do so resulted in the destruction of the molecule. Consequently the halogen was substituted in the *o*-sulfobenzoic anhydride molecule before condensation, using a slight modification of the Juvalta method, as described by Pratt and Shupp.[3] Iodine and bromine derivatives were thus prepared with comparative ease. No attempt was made to prepare the fluorine or chlorine derivatives, though the latter could doubtless have been made, as Harden and Drake[2] report the preparation of some of them.

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[1] Remsen, *Am. Chem. J.*, **6**, 180 (1884); Lubs and Acree, THIS JOURNAL, **38**, 2773 (1916); White and Acree, *ibid.*, **41**, 1190 (1919); Orndorff and Sherwood, *ibid.*, **45**, 486 (1923); Orndorff and Shapiro, *ibid.*, **46**, 2856 (1924).

[2] Harden and Drake, *ibid.*, **51**, 562 (1929).

[3] Pratt and Shupp, *ibid.*, **40**, 254 (1918).

Tetra-iodo-*o*-sulfobenzoic Anhydride.—108 g. of *o*-sulfobenzoic anhydride (Eastman, practical grade) and 300 g. of powdered iodine (4 moles of iodine per 1 mole of anhydride) were mixed in a long-necked flask (an 800-cc. Kjeldahl flask serves very well), and 500 cc. of fuming sulfuric acid containing 50% free SO_3 was added, with stirring. Acid of this strength is ordinarily partly or completely solid, and may require melting. The flask, into the neck of which, to prevent the escape of too much iodine or sulfuric acid, a test-tube was loosely fitted by means of a winding of asbestos cord, was slowly heated in an oil-bath, and the temperature kept between 110 and 120° until the evolution of sulfur dioxide had practically ceased (five to ten hours). The temperature was then raised to 175–180° and held for about thirty minutes. The whole apparatus was then allowed to cool, and when cool the practically solid contents poured into about 1.5 liters of water, and washed with successive portions of water containing sulfurous acid to remove the excess iodine. It was allowed to stand overnight with the final portion of wash water, the solid filtered off, washed with a little water, and dried in air; yield, 333 g. or 88%, calculated on the basis of the amount of *o*-sulfobenzoic anhydride used. The product so obtained is pure enough for making sulfonephthaleins.

Tetra-iodo-*o*-sulfobenzoic anhydride is somewhat soluble in water, acetic acid, tetrachloro-ethane, chloroform and benzene. It is quite soluble in acetone and in alcohol, but cannot be recrystallized from either. When dissolved in acetone it forms a dark brown mass, owing, apparently, to the formation of an addition product, since tetra-iodo-*o*-sulfobenzoic anhydride cannot be recovered from the mass except by the action of some drastic reagent, such as warm nitric acid. In alcohol it supersaturates, forming, when concentrated, a yellow varnish-like mass, which is quite unmanageable. When recrystallized from chloroform it has a melting point of 132° (uncorr.), as against the melting point of the crude product of about 260°, indicating the probable formation of some sort of addition product.

It can, however, be recrystallized from a mixture of alcohol and acetic acid (60 g. will require about 350 cc. of a mixture consisting of 100 cc. of 95% ethyl alcohol and 250 cc. of glacial acetic acid).

The purified product so obtained does not melt, but decomposes at from 305 to 310°. It is somewhat lighter in color than tetra-iodophthalic anhydride, resembling iodoform, except for a slight greenish tinge. The crystals, when examined under the microscope, are seen to be small, imperfect platelets, more opaque than the coffin-shaped crystals of tetra-iodophthalic anhydride.

The compound was analyzed for iodine by the method of Baubigny and Chavanne,[4] other methods, including that of Carius, proving unsatisfactory.

Anal. Calcd. for $C_7O_4I_4S$: I, 74.0; S, 4.6. Found: I, 73.8, 74.1; S, 4.8.

The toxic dose for a rabbit appeared to lie in the vicinity of 0.2 g. per kilogram of body weight when injected intravenously. The substance is not bacteriocidal for *B. coli* except in very high concentrations. One of us (B) took 0.5 g. by mouth without ill effect; subsequently, it could not be demonstrated in the urine.

Tetra-bromo-*o*-sulfobenzoic Anhydride.—Twenty-five grams of *o*-sulfobenzoic anhydride, 1 g. of iodine, and 125 cc. of fuming sulfuric acid containing 50% free SO_3 were mixed together in a long-necked flask of about 300 cc. capacity. About 39 cc. (124 g.) of bromine was measured out into a flask and added as described below.

About two-thirds of the bromine was added, and the mixture heated to 80° in an oil-bath until the bromine was all absorbed. The apparatus was then allowed to cool to room temperature, when two-thirds of the remaining bromine was added, and the mixture heated to 100° until the bromine was absorbed, as before. The apparatus was again

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[4] Lassar-Cohn, "Organic Laboratory Methods," Oesper–Adams–Clarke, Williams and Wilkins, Baltimore, **1928**, p. 443.

allowed to cool, two-thirds of the remaining bromine added, the mixture heated to 130°. until absorption of the bromine took place, and again allowed to cool. The next time all of the remaining bromine was added, and the mixture heated to 170° for thirty minutes, and finally allowed to cool. The treatment from this point on is the same as for the iodine derivative; yield, 56 g., or 81%.

The crystals, after washing and drying the crude preparation, have a faint brownish color which deepens on exposure to light, but the pure compound (recrystallized three times from glacial acetic acid) is pure white. The crystals are easily seen under the low power of the microscope to be small, imperfect, hexagonal prisms. After the recrystallized product had been dried in a desiccator over sodium hydroxide to remove traces of acetic acid, the melting point was found to be 219° (uncorr.).

The neutral equivalent was found to be 258, which corresponds to the calculated molecular weight of 516. The compound was analyzed for bromine by the method of Carius.

Anal. Calcd. for $C_7O_4I_4S$: Br, 61.9. Found: Br, 61.8, 61.8.

The substance is soluble in alcohol, acetone, glacial acetic acid and to some extent in water.

Preparation and Properties of the Halogenated Sulfonephthaleins.—For the condensation of the parent substances with phenols, the best condensing agent, both with respect to quantity and to quality of product, was found to be fuming stannic chloride. These anhydrides are much more difficult to condense than the corresponding phthalic anhydride derivatives, and the actual yields were all small.

The decision as to the best condensing agent and also as to optimum temperature was made by treating equal volumes of a standard mixture of melted phenol and anhydride with different reagents at different temperatures, dissolving the product in a slight excess of alkali, diluting to a definite volume, and comparing the resultant colors in the colorimeter.

The preparation of phenol-tetra-iodo-sulfonephthalein may be taken as typical.

Properties of the New Series.—Practically all of these sulfonephthaleins decompose gradually at very high temperatures, but have no real melting points, so as a rule melting points are not given.

*P*H ranges were determined either by adding a given amount of an alcoholic solution of the substance in question to buffered solutions prepared according to the directions of Folin,[5] or by adding a given amount of 0.04% aqueous solution of the sodium salt of the phthalein. Results obtained by these two methods were identical for the purpose in question.

The buffer mixtures were prepared according to the directions of Clark[6] except that in the *P*H range 4.6–5.6 the acetic acid mixtures described by Folin[5] were used.

Into these tetra-iodo and tetra-bromosulfonephthaleins more halogen

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[5] Folin, "Laboratory Manual of Biological Chemistry," 4th ed., Appleton and Co., New York, **1925**, pp. 49–53.

[6] Clark, "The Determination of Hydrogen Ions," 3d ed., Williams and Wilkins Co., Baltimore, **1928**, pp. 192–220.

can be introduced. Bromine can be introduced by boiling the phthalein in acetic acid solution with a slight excess of bromine, pouring into water, extracting with alkali and purifying, as before. Iodine can be introduced, in alkaline solution, by adding a solution of iodine in potassium iodide solution.

Phenol-tetra-iodo-sulfonephthalein.—Eighty grams (0.12 mole) of tetra-iodo-*o*-sulfobenzoic anhydride was dissolved in 320 g. (3.4 moles) of freshly distilled, melted phenol, 80 g. of fuming stannic chloride added, and the mixture heated in an oil-bath for about eight hours. The flask was kept closed by a ground-glass stopper carrying a sealed-in glass tube. Moisture was excluded by a calcium chloride tube.

At the end of about eight hours the mixture was poured into about 6 liters of hot water, and washed repeatedly by decantation and boiling with fresh water. This washed raw product was then extracted with 2% sodium hydroxide solution until only an insoluble residue remained, the extract filtered, and the phthalein precipitated by the addition of hydrochloric acid. This precipitate was then washed with hot water, as before, and this alternation of solution in alkali, precipitation and washing repeated until the product remained solid in hot water, and was completely soluble in alkali. It was then recrystallized from glacial acetic acid. Harden and Drake report recrystallization from benzene, but this in the experience of the present writers was unsuccessful.

Phenol-tetra-iodo-sulfonephthalein, when pure, consists of small imperfect crystals of a reddish-black color. It decomposes at about 210°. The crystals contain 8 to 10% moisture, which can be driven off by prolonged heating at 180°.

It is readily soluble in methyl alcohol, ethyl alcohol, ethyl acetate, acetone and acetic acid. It is somewhat soluble in benzene, carbon tetrachloride, tetrachloroethane, chloroform and ether. It is only very slightly soluble in water. It dissolves in alkali with an intense purplish color. By dissolving the compound in the calculated amount of alkali and evaporating the solution to dryness, the disodium salt can be obtained, as a dark reddish-black mass, with a bluish tinge. The phthalein dissolves, apparently without permanent change, in concentrated sulfuric acid, with the formation of an intense purplish color. The compound has a useful *P*H range of 7.0 to 8.2.[7] Its color changes in this interval from a brownish-yellow to a wine-purple.

Phenol-tetra-iodo-sulfonephthalein will dye silk a color which varies from brownish-yellow to reddish-walnut, depending upon the method of application. Dyeing from a 5% aqueous acetic acid solution is perhaps the simplest laboratory method.

Injected intravenously, as the disodium salt, it is, in part, eliminated through the kidney. It is not toxic to rabbits in doses of 0.2 g. per kilogram of body weight.

Anal. Calcd. for $C_{19}H_{10}O_5I_4S$: I, 59.3. Found: I, 59.0, 58.8, 59.1.

Tetra-iodo-phenol-tetra-iodo-sulfonephthalein.—This compound consists of small, irregular crystals of a reddish-brown color. It is soluble in alcohol, methyl alcohol, ethyl acetate, acetone and acetic acid, but in all to a less extent than is phenol-tetra-iodo-sulfonephthalein. It dissolves in alkali with a green color, and will dye silk a greenish-brown color. It is but slightly soluble in water (the disodium salt) and this renders it unsuited for the ultimate purpose of the study.

The *P*H range is from 6.2 to 7.0 but as the color simply ranges from one shade of green to another, it is hardly possible that the compound could be of value as an indicator.

Anal. Calcd. for $C_{19}H_6O_5I_8S$: I, 74.6. Found: I, 74.4, 74.2.

Tetra-nitro-phenol-tetra-iodo-sulfonephthalein.—This compound crystallizes in

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[7] Harden and Drake (Ref. 2) report for the crude product *P*H 6.4–8.0.

small brown crystals, melting with decomposition at 190°. It dyes silk a peculiar greenish-black. It dissolves in alkali with a purple color. Its *P*H range is probably between 6 and 7, but it is quite unsuited for use as an indicator. It can be analyzed for nitrogen by the Kjeldahl method by first adding zinc dust to the digestion mixture, so as to bring the nitrogen into the reduced form.

Anal. Calcd. for $C_{19}H_6O_{13}I_4N_4S$: N, 5.39. Found: N, 5.20, 5.23.

Diacetyl-phenol-tetra-iodo-sulfonephthalein.—This substance is light brown in color and melts at 136° (uncorr.). It is soluble in the usual organic solvents but practically insoluble in water.

Anal. Calcd. for $C_{23}H_{14}O_7I_4S$: I, 54.0. Found: I, 53.8.

Tetra-bromo-phenol-tetra-iodo-sulfonephthalein, ($C_{19}H_6O_5Br_4I_4S$).—This substance is brown in color, gives a green color in alkali and dyes silk a greenish-brown color, similar to that given by tetra-iodo-phenol-tetra-iodo-sulfonephthalein, but greener. The disodium salt is practically insoluble in water.

***o*-Cresol-tetra-iodo-sulfonephthalein.**—The properties of this compound are not essentially different from those of phenol-tetra-iodo-sulfonephthalein. It gives a similar purple color in alkali, and dyes silk a shade darker. Its *P*H range was found to be 8.8–9.6.[8] The water solubility is low.

Anal. Calcd. for $C_{21}H_{14}O_5I_4S$: I, 57.4. Found: I, 57.0, 57.2.

Resorcin-tetra-iodo-sulfonephthalein (Tetra-iodo-sulfonefluorescein).—This compound is very difficult to obtain in crystalline form. After washing, on spreading the crystals out to dry, they liquefy to a viscous mass and eventually harden again to a black mass. The solid material is black. It is soluble in the usual solvents. Its solution in alkali is red with a splendid greenish fluorescence. Its *P*H range is about 8 to 10, but it would be quite valueless as an indicator. It dyes silk a golden-reddish-brown.

Anal. Calcd. for $C_{19}H_8O_6I_4S$: I, 58.4. Found: I, 58.4.

Salicylic-acid-tetra-iodo-sulfonephthalein.—This compound consists of small, imperfect crystals of a rose-beige color. It is soluble in ethyl alcohol, methyl alcohol, acetone and ethyl acetate. It is not as soluble in acetic acid, chloroform and ether as the compounds previously described. It dyes silk a golden-brown.

Its useful *P*H range is 7.2–8.0, the color changing from yellow-brown to pink. It might prove moderately satisfactory as an indicator.

Anal. Calcd. for $C_{21}H_{10}O_9I_4S$: I, 53.7. Found: I, 53.6.

Phenol-tetra-bromo-sulfonephthalein.—This substance gives very small reddish-black crystals of high but indefinite melting point; its properties are very similar to those of phenol-tetra-iodo-sulfonephthalein. It gives a purple color in alkali, and a golden-yellow on silk. Its *P*H range was found to be shorter than that of phenol-tetra-iodo-sulfonephthalein, its color changing between *P*H 7.2 and 8.0[9] from a light brown to a purple, so abruptly that its turning point might almost be fixed at *P*H 7.4. It might possibly be of some value as an indicator.

Anal. Calcd. for $C_{19}H_{10}O_5Br_4S$: Br, 47.7. Found: Br, 47.5.

Tetra-bromo-phenol-tetra-bromo-sulfonephthalein.—This substance dissolves in alkali with a bluish-green color, and dyes silk a similar shade, but is not otherwise very different from phenol-tetra-bromo-sulfonephthalein. It is less soluble in the usual solvents.

Its *P*H range was found to be 5.6–7.0,[10] but the colors are a series of shades of bluish-

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[8] Harden and Drake report *P*H 7.0–8.6.

[9] Harden and Drake give *P*H 6.6–8.2.

[10] Harden and Drake report *P*H 3.0–4.6.

green at all the hydrogen-ion concentrations within this range, so its usefulness as an indicator would probably be small.

Anal. Calcd. for $C_{19}H_6O_5Br_8S$: Br, 64.8. Found: Br, 64.6.

Resorcin-tetra-bromo-sulfonephthalein.—This substance is a black solid, with a greenish sheen. It is soluble in the usual solvents, dissolving in alkali to give a red solution with a magnificent greenish fluorescence. It dyes silk a light reddish-brown. The *P*H range was difficult to determine, but was about 8–10. The compound would hardly be useful as an indicator.

Anal. Calcd. for $C_{19}H_8O_6Br_4S$: Br, 46.7 Found: Br, 46.5.

o-**Cresol-tetra-bromo-sulfonephthalein** was also prepared but offered nothing distinctive in its properties.

Tetra-iodo-saccharin.—This compound is not really a member of the present series, but was made while working on the parent substances. It can be prepared by the Juvalta method as described for them.

The substance consists of silky greenish-yellow crystals, somewhat soluble in acetone, and practically insoluble in everything else, including sodium hydroxide solution. It has a faint bitter taste.

The crystals did not melt or otherwise alter even at 345°. They contained carbon, sulfur, iodine and nitrogen, as demonstrated by the usual tests. Analysis for nitrogen was carried out by the Kjeldahl method, a rather long time being required for digestion.

Anal. Calcd. for $C_7HO_3I_4NS$: N, 2.03. Found: N, 1.99.

Attempts to condense this substance with phenolic substances were made, but unlike saccharin itself the substance would not condense.

Summary

Methods of preparing the parent substances of a new series of sulfonephthaleins having halogen substituted in the *o*-sulfobenzoic anhydride residue are described, together with methods of preparing the phthaleins themselves.

Certain properties of members of the new series, and of another substance, tetra-iodo-saccharin, are given.

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