Reduction of the Oxime $C_{11}H_9O_2N_3$, Formula XI, to the Corresponding Amine 2-Phenyl-4-Aminomethyl-6-oxypyrimidine $C_{11}H_{11}ON_3$. Formula XIII.—The reduction of the oxime is easily brought about by the action of tin chloride in hydrochloric acid solution. For 2 g. of the oxime we used 6 g. of the tin salt. The reaction is allowed to proceed at ordinary temperature for about 24 hours and finally at 45–50° for 2 hours. The tin is then removed in the usual manner by precipitation as sulfide, and the aqueous solution evaporated to dryness *in vacuo*. The pyrimidine base is obtained in the form of a stable, crystalline hydrochloride, which is easily purified by crystallization from 95% alcohol. This salt is colorless and melts with decomposition at 263–5°.

Subs., dried at 100°.

Calc. for $C_{11}H_{11}ON_3.HCl$: N, 17.72. Found: 17.6.

Summary.

1. Benzamidine condenses with ethyl γ-diethoxy-aceto-acetate in the presence of alkali to give the pyrimidine, 2-phenyl-4-diethoxy-methyl-6-oxypyrimidine (VI).

2. Hydrolysis of this pyrimidine (VI) leads to the formation of the corresponding pyrimidine aldehyde which exists in the form of a stable hydrate (XII).

3. The pyrimidine aldehyde-2-phenyl-6-oxy-4-aldehydo-pyrimidine (XII) interacts normally with hydroxylamine to give an oxime, which is converted by reduction into 2-phenyl-4-aminomethyl-6-oxypyrimidine (XIII).

4. Benzamidine and ethyl γ-diethoxy-aceto-acetate interact in neutral solution with formation of diethoxy-acetoacetyl-benzamidine (VIII).

NEW HAVEN, CONN.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE JAMES BUCHANAN BRADY UROLOGICAL INSTITUTE, JOHNS HOPKINS HOSPITAL.]

MERCURY DERIVATIVES OF PHTHALEINS.[1]

BY EDWIN C. WHITE.

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During the past 3 years the writer and collaborators have been engaged on studies of organic mercury compounds, particularly with reference to their use, both internally and locally, in the treatment of genito-urinary infections and of syphilis. The results obtained in the laboratory and the clinic with some of these compounds[2] have been of sufficient value to

[1] This work was carried out with the aid of funds granted by the United States Interdepartmental Social Hygiene Board for Research in the prevention and treatment of venereal diseases.

[2] E. G. Davis, E. C. White and R. Rosen, "Urinary Antisepsis," *J. Urology*, **2**, 277 (1918); J. E. Burns, E. C. White and J. G. Cheetham, "Experimental Nephropathy Produced by a Mercury Derivative of Phenolsulphonphthalein," *ibid.*, **3**, 1 (1919); H. H. Young, E. C. White and E. O. Swartz, "A New Germicide for Use in the Genito-Urinary Tract—Mercurochrome-220," *J. Am. Med. Assn.*, **73**, 1483 (1919).

warrant a description of the preparation and properties of these substances, particularly because little of a chemical nature has heretofore appeared in regard to mercurated phthaleins and because the methods used by the writer differ from the method described in the meager literature available.

This literature consists solely of 2 German patents. Pauly and Traumann[1] prepared derivatives containing as many as 4 "or more" atoms of mercury by the action of mercuric chloride on the sodium salt of fluorescein or halogenated fluorescein. Their statement that the latter is used instead of "phthalsäure fluorescein" indicates that the halogen was in the phthalic acid residue, not in the phenolic part of the molecule. The process was as follows. The nearly neutral solution of the phthalein (*i. e.*, one containing about 2 molecules of sodium hydroxide to one molecule of phthalein) was boiled with the desired amount of mercuric chloride, the precipitate first formed was dissolved by treatment with sodium carbonate and the solution was evaporated to dryness. No analysis or evidence of structure is given in the patent, nor is there any indication of the nature of the inorganic group held by the second bond of the mercury atom, a most important consideration when substances of this kind are to be used for pharmacological purposes.

Fahlberg, List and Co.[2] extend the process of the earlier patent to include a number of other phthaleins and succineins. The materials covered by these patents were studied pharmacologically by Klages and Schreiber,[3] Titze and Wedemann,[4] and Hahn and Kostenbader.[5] The mercury content of these compounds as given in the last article indicates that many of them were not single, definite compounds, but were probably mixtures. In only a few cases is the inorganic group held by the mercury indicated, so that the nature of the substances worked with is not fully shown.

Of the compounds described in the present paper, only one, a derivative of phenolphthalein, is crystalline. The others are amorphous and their chemical identification depends on a knowledge of the parent phthalein, determination of mercury content, and in some cases, of the second group attached to the mercury atom. Melting points, which in the case of the crystalline mercury derivatives of certain phenols and amines afford, in connection with analytical data, a means of rigidly establishing chemical and physico-chemical identity, are not shown by any of the mercurated phthaleins. In other words, the chemical data on these compounds can establish only identity of composition as between 2 substances of this

[1] Pauly and Trauman, *D. R. P.*, 201,903.

[2] Fahlberg, List & Co., *D. R. P.*, 308, 335; *C. A.*, 13, 1621 (1919).

[3] Klages and Schreiber, *17th Internat. Cong. Med.*, 1913. Report of Section of Therapeutics, p. 95.

[4] Titze and Wedemann, *Centr. Bakteriol.*, 57, (I), Referate 179 (1913).

[5] Hahn and Kostenbader, *Z. Chemotherapie*, Originalabhandlungen, 2, (I), 71(1912).

class, and cannot show possible differences such as isomerism or variation in the state of molecular aggregation. Differences of this kind have a fundamental bearing on the biological behavior of dyes, as is shown, for example, in the work of Schuleman[1] on vital staining. It should be remembered that salts of acids of high molecular weight, such as we have in most dyes, show in solution more or less pronounced colloidal properties. The physico-chemical characteristics of a dye are, therefore, almost as important as the purely chemical ones when the dye is to be used for pharmacological study or for clinical administration. Identity of composition may or may not mean identity of biological behavior, and for this reason in our work we have adopted the safe course of considering as different individuals any 2 substances which, though of the same composition, are not made by precisely the same procedure. In at least one case we have found that 2 presumably identical samples made by different methods showed a marked difference in toxicity.

In order to distinguish the substances described in this paper as well as other mercurated dyes to be reported later, the generic name "Mercurochrome" has been coined, the individual member of the group being indicated by suffixing the laboratory number. The purpose of this nomenclature is practical rather than scientific, since one member of the group—dibromo-hydroxymercury-fluorescein, number 220—has come into considerable clinical use as a local urinary disinfectant[2] and the chemical name is too unwieldy for the clinician.

The position of the mercury in these compounds has not been rigidly demonstrated, but there is strong presumptive evidence that the metal enters the phenolic residues, in position *ortho* to the hydroxyl or the quinone oxygen. Mercuration in general is a process quite analogous to halogenation, nitration and sulfonation; and since none of these processes leads to substitution in the phthalic acid residue of phthaleins, but goes only as far as tetra substitution in the phenolic residues it is to be presumed that mercury also enters these residues. Moreover, if the 4 *ortho* positions mentioned above are occupied, as in the case of eosin, mercury cannot be substituted in the molecule by any method tried in this work. The method used in the German patents, the second of which makes a claim for a mercury derivative of tetrabromo-fluorescein was also tried several times with eosin, and although mercuric chloride was used in one case and mercuric acetate in another, in both experiments a mercury salt of the dye, as indicated by its insolubility in alkali, was the only product obtained. (Possibly the tetrabromo-fluorescein of the patent was the derivative of tetrabromo-phthalic acid.) If, on the other hand, 2 of the *ortho* positions are occupied, as in the dibromo-fluorescein or *o*-

[1] Schuleman, *Biochem. Z.*, 80, 1 (1917).

[2] Young, White and Swartz, *loc. cit.*

cresol-phthalein, exhaustive mercuration leads to a product containing no more than 2 atoms of mercury, corresponding to the 2 unoccupied positions. Finally, if the phthalein contains no substituent groups in the *ortho* positions, as in fluorescein or phenolsulfon-phthalein, 4 atoms of mercury, and no more, can be made to enter the molecule by the methods used in this work. The behavior of phenolphthalein is rather peculiar, as the product of exhaustive mercuration in this case seems to contain only 3 atoms of the metal.

The effect produced on color by the introduction of mercury into the phthalein appears to be qualitatively the same as that produced by halogens, but is much less intense. It it is considerable only in compounds containing at least 2 mercury atoms. Thus, the tetramercury derivative of fluorescein with alkali gives a notably redder tint than fluorescein itself produces, but far less red than is that of eosin. The monomercury derivative of fluorescein, on the other hand, appears to the eye to have practically the same color as fluorescein, though it is possible the spectroscope might show some difference. The same is true in the case of phenolphthalein, whose trimercury derivative gives an intense bluish-purple salt, whereas the monomercury derivative gives a salt of the same pink color as is given by the parent substance. The dimercury derivative of *o*-cresol-phthalein, which represents the maximum degree of mercuration, likewise gives an intense bluish-purple color with alkali. The monomercury derivative of phenolsulfon-phthalein shows the same color and about the same range of color change on the hydrogen-ion scale as does phenolsulfon-phthalein, but the salt of the tetramercury derivative shows a much bluer nuance, the difference being most marked as the solution is diluted. The last-named compound is interesting, also, because it shows the effect of organically-bound mercury in decreasing solubility. Although phenolsulfon-phthalein is fairly soluble in water, to form a yellow solution, the tetramercury derivative is entirely insoluble. It can be boiled for hours without imparting the slightest trace of color to the water, even though the filtrate is made alkaline.

The readiness with which phthaleins undergo substitution by mercury is shown by the variety of conditions under which the reaction will proceed. The dye may be in solution, either in alcohol as the free acid or in water as the salt, or it may be used in a state of suspension in water. The mercury may be used in an insoluble form, as the oxide, or in solution, preferably the acetate. The nature of the product and maximum degree of substitution attainable will vary with the conditions chosen. Two general reactions were used, the action of yellow mercuric oxide on a solution of a phthalein salt and the action of mercuric acetate on the free phthalein. The first of these reactions appears to proceed as far as the substitution of 3 atoms of mercury in phenolsulfon-phthalein, but

does not go beyond the second stage of substitution in fluorescein. In phenolphthalein the substitution of even the first atom of mercury takes place with some difficulty, and in dibromo-fluorescein and eosin no substitution at all occurs by this method.

The second reaction will lead to the maximum degree of substitution permitted by the free *ortho*-positions, with the exception, already noted, of phenolphthalein. The phthalein may be used in alcoholic solution, or if it is insoluble in alcohol, it may be suspended in water. In the former case, an *acetoxy-mercury* derivative, $RHgOOCCH_3$ is formed, but in the latter case the acetic acid is partially removed by hydrolysis and there results a mixture of acetoxy-mercury and hydroxymercury derivatives. In the case of the monomercury derivative prepared in this way from dibromo-fluorescein—"Mercurochrome-220"—the hydrolysis is practically complete.

Method of Analysis.—When the mercury is bound to acetic acid the latter is determined by distillation with steam in the presence of phosphoric acid, a method used by Brieger and Schuleman,[1] who, however, give no details. The following procedure was found satisfactory About 0.5 g. of the substance is weighed into a 300-cc. Claisen flask, 10 to 15 cc. of syrupy phosphoric acid is added, then about 100 cc. of water. The steam-delivery tube passes through the long neck of the flask, the short neck being closed by a rubber stopper. By bending slightly the delivery tube leading to the condenser the flask is tilted towards the steam generator, the short neck thus serving as an excellent trap to catch any spray containing phosphoric acid. The steam distillation is continued until no more acetic acid comes over, as indicated by titration of the distillate with 0.1 *N* alkali. If the steam current is vigorous, usually the process is complete when about 300 cc. of distillate has been collected.

In the determination of mercury, the organic matter is destroyed by permanganate and sulfuric acid. About 0.2 g. of the substance is weighed into an 800-cc. Pyrex Kjeldahl flask and 10 cc. of conc. sulfuric acid is added to the flask which is tilted to allow the acid to run along the neck slowly, so as to wash down any adherent particles. To mix the materials the flask is shaken gently and heated until the acid begins to fume moderately. This treatment should yield a clear solution or an even suspension. The mixing must be continued until no lumps are left. *Finely powdered* potassium permanganate is now added little by little; each time as much as can be held on the end of a pen-knife blade is used, and the materials are thoroughly mixed after each addition. The slight flash which sometimes occurs is of no danger. The addition of permanganate is continued until a considerable excess of the brown oxide is present, which may be recognized by the development of a green color (manganate) on further application of heat. From one to 1.5 grams of permanganate is required. The

[1] Brieger and Schuleman, *J. prakt. Chem.* (2) **89**, 97 (1914).

heating must be regulated so that the bottom of the neck of the flask does not become hot to the touch; there is then no danger of loss of mercury by volatilization. When the mixture has cooled to room temperature it is diluted with about 100 cc. of water and powdered oxalic acid is added until a clear solution is obtained. This is transferred to a beaker, and filtered, if necessary, to remove any undissolved particles of oxide. The volume is made up to about 200 cc. with the rinsings, and when the solution is cool, the mercury is precipitated by hydrogen sulfide and weighed. The Gooch crucible is preferably prepared with a disc of filter paper under the asbestos; the mercury sulfide can then be filtered with the greatest ease. Controls run by this method, with various phthaleins and known amounts of mercuric chloride, showed that 98–99% of the mercury is recovered.

A similar method was used by Rupp and Kropat,[1] who, however, carried out the oxidation in alkaline solution. In either method, if halogens are absent, the mercury may be determined volumetrically with potassium thiocyanate.

Experimental.

Action of Mercuric Oxide on Phenolphthalein.—Three g. of phenolphthalein was dissolved in 25 cc. of *N* sodium hydroxide solution, the solution was made up to about 150 cc. with water and boiled with 3 grams of yellow oxide of mercury for 3 hours. Some reduction of the oxide or decomposition of the mercuration product took place; this was indicated by the formation of a small amount of very fine gray precipitate which appeared to be mercury or perhaps mercurous oxide. This material, which occurs to some extent in every case of the action of mercuric oxide on a phthalein salt, could not be removed by filtration. It was, therefore, allowed to settle in a tall cylinder for several days, the clear supernatant liquid was poured off, and centrifuged to remove remaining traces of the gray substance. The mercurated phthalein, precipitated by carbon dioxide, settles out as a slightly purplish, milky precipitate. It was separated and washed by centrifuging, dried on the water-bath, then washed with alcohol, and dried at 110°.

Subs., 0.2000: HgS, 0.0820.
Calc. for $C_{20}H_{13}O_4$ (Hg OH): Hg, 37.3. Found: 35.3.

The substance was insoluble in all the usual solvents except glacial acetic acid, in which it gave a turbid solution. It could not be crystallized. Its color with alkali is the same as that of phenolphthalein. The analysis indicates a monomercurated derivative containing a small amount of unchanged phenolphthalein.

Action of Mercuric Oxide on Fluorescein.—Two hundred cc. of 0.1 *N* sodium hydroxide solution was saturated with about 3.5 g. of fluorescein

[1] Rupp and Kropat, *Apotheker Ztg.*, 1912, p. 377.

and without filtering off the excess of fluorescein, 5 g. of mercuric oxide was added. The mixture was boiled for 4 hours, while water was added to replace that lost by evaporation. After it was clarified by centrifuging and evaporated to dryness on the steam-bath, it gave an iridescent green scaly product.

Subs., 1.5278: Loss, 0.1215 at 115°.
Subs., 0.2000: HgS, 0.1068.
Calc. for $C_{20}H_8O_5Na(HgOH)_2$: Hg, 49.6. Found: Hg (calc. to dry basis) 49.9; H_2O, 7.9.

This experiment was repeated with 10 g. of mercuric oxide. When the mixture was boiled for 9 hours, it gave a solution which contained some of the oxide in colloidal suspension. It could not be completely clarified by centrifuging or by adsorbents, so it was evaporated to dryness after removing as much as possible by means of the centrifuge. Even with the extra mercury thus contained, the amount was less than that required for a trisubstitution product, which showed that the substitution of mercury by this reaction does not proceed beyond the second stage. Of course, if only one equivalent of mercuric oxide is used, mono-hydroxymercury-fluorescein can be prepared. By treatment of the solution of the salt with dil. hydrochloric acid, if it is stirred thoroughly and an excess of acid is avoided, chloromercury-fluorescein is precipitated as a caseous mass. After washing the material until it is free of chlorides by centrifuging, a sample was dried and analyzed for chlorine by the lime method. In this way all the mercury is removed by volatilization, so that no mercury is left to interfere with the precipitation of silver halide.

Subs., 0.1110: AgCl, 0.0282.
Calc. for $C_{20}H_{11}O_5(HgCl)$: Cl, 6.26. Found: 6.28.

If it is desired to isolate the free hydroxymercury-fluorescein, very dil. sulfuric acid is used instead of hydrochloric acid; the precipitate is of the same pasty nature in both cases. The salt of dihydroxymercury-fluorescein shows a rather pronounced eosin-like color, though the red is not nearly so intense as that of eosin.

Action of Mercuric Oxide on Phenolsulfon-phthalein.—Five cc. of *N* sodium hydroxide solution was diluted to about 150 cc. and the solution was saturated with phenolsulfon-phthalein—about 2 g.—so as to insure the absence of any dibasic salt. Without filtering the excess, 6 g. of mercuric oxide was added and the mixture was boiled for 4 hours. The purplish solution was centrifuged and evaporated to dryness on the water-bath; the residue was powdered and dried at 110°. The material formed a purple powder with a bronze luster.

Subs., 0.2000: HgS, 0.1333.
Calc. for $C_{19}H_{10}O_5SNa(HgOH)_3$: Hg, 58.7. Found: 57.4.

The use of the disodium salt instead of the monosodium salt seems to interfere with the reaction. This is shown by the following experiment: 3.5 g. of phenolsulfon-phthalein, 20 cc. of sodium hydroxide solution, 250 cc. of water and 5 g. of mercuric oxide were boiled for 9 hours. After clarification of the solution by centrifuging, the dye was precipitated by dil. sulfuric acid and the precipitate was washed free from sulfate by centrifuging, and then dried.

Subs., 0.2000: HgS, 0.1028.
Calc. for $C_{19}H_{12}O_5S(HgOH)_2$: Hg, 50.9. Found: 44.3.

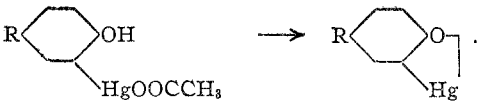
In this case, even long boiling with an excess of the oxide did not cause the reaction to proceed completely to the second stage, although it passed the first stage, which requires a mercury content of 35.1%.

Action of Mercuric Acetate on Phenolphthalein.—Three g. of the phthalein was dissolved in 50 cc. of alcohol and mixed with a filtered solution of 25 g. (8 mols) of mercuric acetate in 50 cc. of water and 50 cc. of glacial acetic acid. At room temperature overnight no reaction occurred beyond the formation of a small amount of mercurous acetate. The color of a sample when heated with alkali was that of the unchanged phthalein. The mercurous acetate was removed and the filtrate heated on the steam-bath. In about 2 hours rosets of stout bladed crystals began to form rapidly. The heating was continued for one hour longer and the mixture was allowed to stand overnight. The crystal paste was filtered, washed with alcohol to remove unchanged phenolphthalein, then with water acidulated with acetic acid and finally with water to remove the excess of mercury salt. The crystals were dried in the air for several days and at 100° for one hour. The analysis indicates tri-acetoxy-mercury-phenolphthalein

Subs., 0.2000: HgS, 0.1292.
Distn. with H_3PO_4: Subs., 0.5000: 14.4 cc. 0.1 *N* NaOH.
Subs., 0.8648: Loss 0.0420, dried to constant weight at 120°.
Calc. for $C_{20}H_{11}O_4(HgOOCCH_3)_3$: Hg, 55.0; CH_3COOH, 16.4; loss at 120, 5.4. Found: Hg, 55.6; CH_3COOH, 17.1; loss, 4.8.

In view of the slightly high value found for acetic acid and the loss at 120°, it was thought that the substance in hand might possibly be a tetramercury derivative containing acetic acid of crystallization. It was found, however, that these values do not agree nearly so well with the formula for any such substance as they do with that of the trimercury derivative given above, which contains no solvent of crystallization. The loss at 120° is to be ascribed to the elimination of one molecule of acetic acid, formed by one of the acetate groups and the hydrogen of an hydroxyl, thus:[1]

[1] In unpublished work, the writer has noticed a similar elimination of acetic acid in the case of mercury derivatives of certain simpler phenols.



Tri-acetoxy-mercury-phenolphthalein is insoluble in all the usual solvents, but is readily and completely soluble in a solution of sodium hydroxide with the production of a deep purple color. This shows that the bladed crystals are not mercurous acetate and do not contain any appreciable amount of it. The alkaline solution is decolorized by carbon dioxide, and solutions of the salt on standing some time deposit a small amount of gray substance, probably metallic mercury.

A product which gives the same analysis and shows the same properties, but consists of minute granules, is obtained if only 4 equivalents of mercuric acetate are used. This material is easily soluble in hot glacial acetic acid, from a mixture of which, with water and alcohol, it can be crystallized, in the form of bladed crystals, which are almost insoluble in acetic acid.

Action of Mercuric Acetate on Fluorescein.—Three and three-tenths g. of fluorescein was dissolved in 200 cc. of 0.1 *N* sodium hydroxide solution and acidified with 5 cc. of glacial acetic acid. To this was added the filtered solution of 25 g. (about 8 mols) of mercuric acetate in 200 cc. of water slightly acidulated with acetic acid to prevent hydrolysis. The mixture was boiled for 10 hours, while water was added to keep the volume about constant, washed free from mercury by the centrifuge and dried. There was some mechanical loss by the washing, as a portion formed a fine suspension which could not be clarified. The yield of product was about 10 g.

Subs., 0.2000, 0.2000: HgS, 0.1450, 0.1447.
Distn. with H_3PO_4: Subs., 0.5000: 5.5 cc. 0.1 *N* NaOH.

	Hg.	CH_3COOH.
Calc. for $C_{20}H_9O_5(HgOH)_3$	61.3	0
$C_{20}H_8O_5(HgOH)_4$	66.9	0
$C_{20}H_9O_5(HgOOCCH_3)_3$	54.3	16.2
$C_{20}H_8O_5(HgOOCCH_3)_4$	58.7	17.5
Found	62.5, 62.3	6.6

These figures indicate that the product is a mixture of tetra-hydroxy-mercury-fluorescein and tetra-acetoxymercury-fluorescein, the former predominating and probably being formed from the latter by hydrolysis. Calculation indicates that the latter is present to the extent of 6.6/17.5, or 37.7%. If the remainder, or 62.2%, is the tetrahydroxy derivative, the mercury content of the mixture should be:

$$\frac{37.7 \times 58.7 + 62.2 \times 66.9}{100}, \text{ or } 63.7\%.$$

The calculated value is so close to that found that the composition of the mixture seems fairly exactly fixed.

The substance is insoluble in the usual solvents, soluble in alkali with a color similar to that of dihydroxy-mercury-fluorescein. On treatment of the alkaline solution with iodine the brilliant color of erythrosin is produced, and the product stains the skin a bright rose color, whereas the mercury compound has only feeble staining power.

Action of Mercuric Acetate on *o*-Cresol-phthalein.—Three g. of the phthalein in 50 cc. of alcohol was treated with 25. of mercuric acetate in 50 cc. water and 50 cc. glacial acetic acid. Since no precipitate formed when this mixture was heated for 2 hours on the steam-bath, the heating was continued overnight. A pasty precipitate, representing most of the material, was produced. It contained much mercurous acetate, in addition to the mercuration product, and could not be purified. Alcohol added to the solution precipitated a further small amount of mercurous acetate in fine crystals. After filtering these off, the filtrate was diluted with a large amount of water and after standing for some time the yellowish granular material was filtered off. To remove any unchanged cresol-phthalein it was ground up and washed thoroughly with alcohol, in which it was only slightly soluble. The residue was dissolved in hot 50% acetic acid, from which on standing a slightly yellow, granular precipitate settled. It could not be crystallized; so it was pressed between paper, dried at 105° and analyzed. The yield was very small.

Subs., 0.1000: HgS, 0.0550.

Calc. for $C_{22}H_{16}O_4(HgOOCCH_3)_2$: Hg, 46.6. Found: 47.4.

This substance dissolves in alkali with a brilliant purple color. The shade is much deeper, *i. e.*, bluer, than that of the analogous derivative of phenolphthalein.

Action of Mercuric Acetate on Dibromo-fluorescein.—Forty-nine g. of dibromo-fluorescein was dissolved in 8 g. of sodium hydroxide in 50 cc. of water, and diluted to 200 cc. This solution was stirred and treated with 12.5 cc. of glacial acetic acid. An even, pasty precipitate was formed if the stirring was vigorous. A solution of 22.5 g.[1] of mercuric oxide in 25 cc. of glacial acetic acid and 50 cc. of water, diluted after solution to 100 cc., previously prepared and filtered, was added to the dye suspension and the whole was diluted to a volume of about 500 cc. The mixture was boiled until a portion of filtered solution when treated with ammonium sulfide gave no test for mercury. In various preparations the time required was 4.5 to 6 hours. As the solution boiled, the precipitate became darker in color and somewhat more granular. The filtration, however, was difficult, so the material was washed by centrifuging to remove acetic acid and sodium acetate, and dried at 110°. The yield was almost quantitative.

[1] The theoretical amount for one equivalent is 21.7 g. The excess used takes care of the amount lost as mercurous acetate, which is always formed in small amount when commercial yellow oxide of mercury is dissolved in acetic acid.

Subs., 0.2000: HgS, 0.0657.

Distn. with H_3PO_4: Subs., 0.5000: 0.5 cc. 0.1 *N* NaOH.

Calc. for $C_{20}H_9O_5Br_2(HgOOCCH_3)$: Hg, 26.8; CH_3COOH, 8.0. Calc. for $C_{20}H_9O_5Br_2(HgOH)$: Hg, 28.4; CH_3COOH, 0.0. Found: Hg, 28.3; CH_3COOH, 0.6.

These results indicate that the product is dibromo-hydroxymercury-fluorescein. The hydrolysis of the acetoxy-mercury compound, which is probably formed as an intermediate step, appears to be almost if not quite complete. It is possible that the small titration value found for the distillate from the phosphoric acid is due to a small amount of sodium acetate that was not removed in the washing.

Dibromo-hydroxymercury-fluorescein is a red powder which, when ground up, shows marked electric properties; it sticks to glass or paper even when perfectly dry. It is insoluble in the usual solvents, but dissolves in 2 equivalents of sodium hydroxide, to give a deep cherry-red solution. This solution is fairly stable, but on long standing a small amount of metallic mercury or mercurous oxide may settle out. This decomposition seems to be retarded if the solution is kept in dark bottles. The free acid is also completely soluble in ammonium sulfide; from this solution mercuric sulfide is precipitated only slowly.[1]

Action of Mercuric Acetate on Phenolsulfon-phthalein. — Three and a half g. of the dye was dissolved in 200 cc. of 0.1 *N* solution of sodium hydroxide, acidified with 10 cc. of glacial acetic acid, which did not re-precipitate the phthalein, and treated with the filtered solution of 15 g. of mercuric acetate in 100 cc. of water. The solution was boiled for 3 hours, at the end of which time a somewhat crusty precipitate had formed. The precipitate was washed free of salts by centrifuging and dried. It formed a dark brown powder, insoluble in the usual solvents except methyl alcohol, with which it gave a slight color, and boiling glacial acetic acid, with which it gave an intense purple-red solution. This, however, contained only a small amount of dissolved material, and a crystalline product was not obtained. The substance dissolved in a dilute solution of sodium hydroxide with a bluish-purple color, much deeper than that of the parent substance.

Subs., 0.2000: HgS, 0.1442.

Distn. with H_3PO_4: Subs., 0.5000: 5.2 cc. 0.1 *N* NaOH.

Calc. for $C_{19}H_{10}O_5S(HgOH)_4$: Hg, 65.8; CH_3COOH, 0. Calc. for $C_{19}H_{10}O_5S(HgOOC\,CH_3)_4$: Hg, 57.8; CH_3COOH, 17.2. Found: Hg, 62.1; CH_3COOH, 6.2.

These figures indicate that a mixture of the hydroxymercury- and acetoxymercury-derivatives was formed, as in the case of fluorescein. If the

[1] The disodium salt of dibromo-hydroxymercury-fluorescein, "Mercurochrome-220," has found some use as therapeutic agent and is being prepared on a commercial scale. The product has been found to contain about 23% of mercury and about 8% of moisture. The mercury content calculated to a dry basis varies from 24 to 25% instead of the theoretical value of 26.8% for a pure salt. Probably the difference is due to the presence of a small amount of sodium acetate.

content of acetoxymercury-derivative is 6.2/17.2 or 36.0%, the mercury content of the mixture should be $\frac{36.0 \times 57.8 + 64.0 \times 65.8}{100}$, or 63.0%, a figure in close agreement with that found.

Some of the product was boiled a long time with a large amount of water in an attempt to complete the hydrolysis. No change in the material appeared to take place and the mercury content was not increased, as would have been the case had further hydrolysis taken place. An attempt was made to secure a product free from combined acetic acid by dissolving the material in a 10% solution of sodium hydroxide and precipitating it with sulfuric acid, but this led to considerable decomposition.

Summary.

Two processes of mercuration are described which are applicable to phthaleins. The substitution of mercury is analogous to sulfonation, halogenation or nitration, and the metal enters the phenolic group of the phthalein molecule, in the *ortho* position to the hydroxyl or the quinone oxygen. The number of atoms of mercury entering the molecule is limited by the number of such free *ortho* positions, and if all 4 are occupied no substitution of mercury takes place.

Mercury derivatives containing from one to 4 atoms of the metal have been prepared from the following phthaleins; phenolphthalein, *o*-cresolphthalein, fluorescein, di-bromo-fluorescein and phenolsulfon-phthalein. The methods of substitution differ from those used in the earlier literature on mercurated phthaleins.

It is pointed out that chemical composition alone does not fix the identity of biological behavior or therapeutic effect of compounds of this class, and that samples cannot be considered identical unless they are made in precisely the same way and have the same composition.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF CORNELL UNIVERSITY.]

TETRACHLORO-PHENOLPHTHALEIN.

BY E. T. WHITING.

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As this derivative of phenolphthalein had never been made and as it was desirable to measure its ultra-violet and visible absorption,[1] its preparation was undertaken by the author at the suggestion of Professor W. R. Orndorff and under the direction of Dr. S. A. Mahood.

Numerous attempts to chlorinate phenolphthalein in alkaline solution and in carbon tetrachloride resulted in failure. Tetrachloro-phenolphthalein was finally made by the following method. 50 g. of *pure* phenol-

[1] See *Phys. Rev.* N. S., 10, 779 (1917) for the absorption curves.