melting point was 145° with decomposition. W. Sloan Mills,[8] who first prepared iodo-acetyl lactose, found a melting point of 142°, but did not record a rotation.

One of the authors (A. K.) expresses his thanks to the International Education Board for a grant which enabled him to take part in this research.

Summary

The specific rotation of pure iodo-acetyl lactose has been measured, and the specific rotations of chloro- and of bromo-acetyl lactose have been remeasured; all have been found to agree with the theoretical requirements.

WASHINGTON, D. C.

[CONTRIBUTION FROM THE KENT CHEMICAL LABORATORY OF THE UNIVERSITY OF CHICAGO AND THE OTHO S. A. SPRAGUE MEMORIAL INSTITUTE]

THE MERCURATION OF METHYLENE BLUE[1,2]

BY LYMAN CHALKLEY, JR.

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Introduction

In the course of the work on tuberculosis which is being carried out by the Sprague Institute[3] it was early found that tubercle bacilli are readily stained in vitro by methylene blue and a few closely related compounds.[4] It was also shown that tubercle bacilli were quite sensitive to poisoning by mercury salts.[5] Since the discovery of these reactions various attempts have been made to prepare an organic mercury derivative of methylene blue.

A double salt of methylene blue and mercuric chloride is mentioned in the literature.[6] Dr. Sidney M. Cadwell[7] purified this substance and obtained it in a well crystalline form, which Dr. Louis M. Larson[7] showed by analyses to have the formula, $C_{16}H_{18}N_3ClS.HgCl_2$. Moreover, Larson showed that it was a double salt by extracting mercuric chloride with ether from its aqueous solution.[7] Cadwell tried to mercurate methylene-

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[8] W. Sloan Mills, *Chem. News*, **106**, 165 (1912).

[1] This is an abstract of a portion of a dissertation submitted by the author in part fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Chicago.

[2] Read at the New Haven meeting of the American Chemical Society, April, 1923.

[3] See the series of "Studies on the Biochemistry and Chemotherapy of Tuberculosis," *J. Infectious Diseases*. The first article is by Wells, **11**, 349 (1912).

[4] Corper, *ibid.*, **11**, 373 (1912). DeWitt, *ibid.*, **12**, 68 (1913); **13**, 378 (1913); **14**, 498 (1914). Sherman, *ibid.*, **12**, 249 (1913).

[5] DeWitt and Sherman, *ibid.*, **15**, 245 (1914).

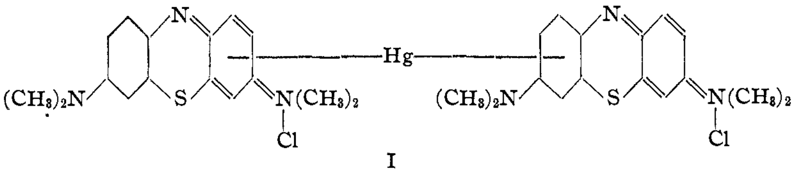
[6] Koch, *Ber.*, **12**, 594 (1879).

[7] Private reports to the Sprague Institute in the hands of Dr. DeWitt.

blue acetate with mercuric acetate using equimolecular proportions, but he could find no evidence of reaction either when the mixture was heated or when it was allowed to stand for some time at room temperature.[7] Dr. Morris Kharasch found that the leuco base of methylene blue could not be mercurated because it reduced the mercuric acetate.[8] With Dr. Piccard he then prepared the *o,o′*-dibromo compound of Bindschedler's green.[9] From this he prepared the leuco base and attempted to replace the bromine with mercury by means of sodium amalgam, but was unsuccessful.[8]

In the course of all this work the supposition developed that when methylene blue is mixed with mercuric acetate it forms so stable a complex with the mercuric ion that the concentration of the mercuric ion becomes too small to mercurate the methylene blue.

If attempts to mercurate methylene blue had been unsuccessful because of the suppression of mercuric ion through complex formation, it seemed that one should be able to mercurate the dye by using a large excess of mercuric acetate. It was on this basis that the work reported in this paper was carried out. The basic experimental problem that such a method of attack presents is the separation of inorganic mercury combined in the form of a coördination compound from a true organic mercury derivative without breaking the mercury-carbon bond. The solution of this problem is surrounded with numerous difficulties in the case of methylene blue because of the instability and complexity of the dye molecule. As a result of the work which has so far been done, the author seriously doubts that the formation of a complex ion with mercuric salts is the only factor that renders methylene blue difficult to mercurate. In the course of the work, the separation of inorganic mercury was attempted by a number of physical methods, no one of which was successful. Recourse was finally taken to the use of a reducing agent, ferrous hydroxide, which also reduced the dye to its leuco base. When reoxidized the dye, which was then free from inorganic mercury, could be handled by ordinary methods. Small quantities of two organic mercury compounds have been isolated. One of them has not been obtained pure because of the readiness with which it goes into colloidal suspension, which makes it difficult to handle. It is thought to have Formula I.



I

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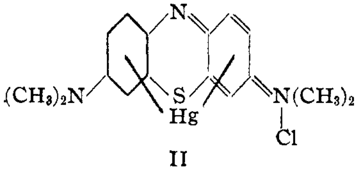
[8] Private communication.

[9] Kharasch and Piccard, This Journal, **42,** 1855 (1920).

The second compound is only very slightly soluble, and has been obtained in what seems to be a pure state. It apparently has Formula II.



II

The work upon this problem is by no means complete, but because of lack of time the author is unable to carry the work farther than is recorded in this paper. It therefore seems advisable to publish the work that has so far been done in this incomplete form. The Sprague Institute, however, expects to continue the investigation of the mercuration of methylene blue.

Experimental Part[10]

Mercuration of the Benzoyl Derivative of Leuco Methylene Blue.—After some preliminary experiments with methylene blue it was decided to study first the mercuration of a related sulfur compound that might be easier to work with than the dye. The benzoyl derivative of leuco methylene blue was chosen. Nothing was known of the reactions of this compound with mercury salts, but leuco methylene blue was known to form a complex compound with mercuric chloride.[11] The benzoyl derivative of leuco methylene blue was prepared by Cohn[12] from the zinc chloride double salt of the leuco base described by Möhlau.[13] In the present work the following modified method of preparation was used.

A solution of 50 g. of U. S. P. methylene blue in 1 liter of water is heated almost to boiling in a 4-liter beaker; 25 g. of zinc dust is added and then, during vigorous stirring, 100 cc. of pure concd. hydrochloric acid as fast as is possible without causing the solution to boil over. The solution is decolorized in the course of a minute or so, and is then immediately filtered with suction, since the double, or complex salt quickly precipitates, even when the solution is kept boiling. The filtrate is allowed to stand in a stoppered flask in the ice box overnight; it is then cooled in an ice-bath and filtered from the greenish or brownish crystals, which are well dried in a desiccator; yield, 55–60 g. For benzoylation, 225 g. of the dry zinc salt is dissolved in 450 cc. of dry pyridine, the solution treated with 103 cc. of benzoyl chloride and refluxed for one hour. The reaction mixture is poured into a large volume of water and the tarry substance that separates is triturated with sodium hydroxide until it becomes a white powder. It is then washed with water and well extracted with 10% hydrochloric acid. The acid solution is made alkaline with aqueous ammonia and the white precipitate collected on a filter and dried in a desiccator over sulfuric acid. The dry substance is recrystallized from alcohol, in which it is difficultly soluble; yield, 50–55 g. of pale, greenish needles. The substance

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[10] For numerous details for which there is not space in this paper, see the author's Dissertation in the University of Chicago Libraries.

[11] Bernthsen, *Ann.*, **230,** 150 (1885).

[12] Cohn, *Ber.*, **33,** 1567 (1900).

[13] Möhlau, *Ber.*, **16,** 2728 (1883).

thus prepared usually contains a trace of ammonium chloride from which it may be easily purified by solution in 10 times its weight of boiling benzene, filtration from the chloride, and reprecipitation with 3 volumes of medium-boiling ligroin. Less than 2% is lost in this process. The author has found that the melting point of the benzoyl derivative of leuco methylene blue is 196–198° (corr.) instead of 185–187° as given by Cohn.

For mercuration, the benzoyl compound was dissolved in about 50 parts of alcohol, the solution treated with mercuric acetate dissolved in water, and refluxed. When the benzoyl compound is boiled for 20 minutes with 2 molecular equivalents of mercuric acetate and the mixture is poured into water, a white precipitate is obtained. Analysis shows that this contains nearly two atoms of mercury for each molecule of benzoyl leuco base. However, as will be shown later, only one mercury atom is attached to carbon.

Preliminary experiments on the separation of the inorganic mercury by means of the usual bridging agents, sulfides, iodides and thiosulfates, were not very satisfactory; but it was found that when the crude mercurated product was suspended in water and the suspension extracted with benzene, the inorganic mercury salt remained in the water, while the organic mercury compound and the unmercurated starting product dissolved in the benzene. Upon addition of ligroin to the benzene extract a monomercury compound of the benzoyl derivative of leuco methylene blue precipitated. When more ligroin was added unmercurated benzoyl compound precipitated.

It is thus seen that the benzoyl compound of leuco methylene blue is partially, but not completely, mercurated by boiling for 20 minutes with 2 molecular equivalents of mercuric acetate. The mercuration is not incomplete because of the short time of heating the reaction mixture, for when it is boiled for two hours the yield of monomercury compound is not increased and 30% of unmercurated benzoyl compound may be recovered. The possibility is suggested that the organic mercury compound in solution may be in equilibrium with an unmercurated double salt of the benzoyl derivative of leuco methylene blue and mercuric acetate. This does not appear to be true, however, for an aqueous-alcoholic solution of the pure, freshly prepared mercury compound to which a few drops of acetic acid have been added may be boiled for one and a half hours without the formation of an inorganic mercury salt, as indicated by the ammonium sulfide test.

The method adopted for the preparation of the acetoxymercuri-benzoyl compound of leuco methylene blue was as follows.

A solution of 5 g. of the pure benzoyl derivative of leuco methylene blue in 300 cc. of alcohol was treated with a solution of 13.7 g. (3 molecular equivalents) of 90% mercuric acetate in 15 cc. of water and 7 cc. of glacial acetic acid. The solution became deep blue in the course of a minute. It was boiled for 20 minutes, allowed to cool and filtered. The precipitate, white crystals weighing 3.8 g., appeared to be largely mercurous acetate. The filtrate was evaporated to dryness in a vacuum. This was easier than the isolation of a crude product by precipitation with water, although the latter could be done. The residue was suspended in 100 cc. of water and extracted with about 600 cc. of benzene. The benzene extract was dried and allowed to evaporate at room temperature to a small volume, then made up to 50 cc. and the substance precipitated with 150 cc. of medium-boiling ligroin. The precipitate was twice redissolved in benzene and reprecipitated with ligroin. After the final precipitation the substance was washed on the filter with low-boiling ligroin in order to remove any high-boiling hydrocarbons that may have been in the medium-boiling ligroin; yield, 4.3 g., or 52%.

The substance was a white powder of no definite melting point. It was quite soluble in benzene and moderately soluble in alcohol. From solutions of the compound hydrogen sulfide precipitated a fairly stable yellow sulfide that could be collected and dried. When the compound was placed in an evacuated desiccator over sulfuric acid, it came to constant weight only very slowly, requiring about a month. During this time the compound underwent some decomposition. When it was dissolved in alcohol it left a white residue and a little metallic mercury; also, it was no longer completely soluble in benzene. Lack of time prevented a careful study of this decomposition or the preparation of undecomposed dry samples for analysis. The following analyses were made on a sample dried in a vacuum over sulfuric acid.

Anal. Subs., 0.2231: 12.6 cc. of dry N_2 (22°, 727.2 mm.). Subs., 0.4266: HgS, 0.125. Calcd. for $C_{25}H_{25}O_3N_3SHg$: N, 6.49; Hg, 30.96. Found: N, 6.28; Hg, 32.8.

The substance was burned with lead chromate and any oxygen removed from the nitrogen by sodium hydrosulfite. The substance is difficult to decompose with nitric acid. For the Carius determination of mercury, 75 drops of fuming nitric acid (Kahlbaum's) was used, and the tube was heated at 320–330° for eight hours.

The Mercuration of Methylene Blue.—Methylene-blue chloride in solution was converted into the acetate by means of mercurous acetate. The methylene-blue acetate was not isolated from solution. The solution of the acetate gives no precipitate with mercuric acetate solution, but the addition of a chloride to a mixture of methylene-blue and mercuric acetates produces an immediate precipitate of the double salt. The separation of mercury from a mixture of mercuric and methylene-blue acetates cannot be conveniently effected by means of the usual bridging agents—thiosulfates, sulfides, alkaline reducing agents and iodides, because these all react with methylene blue as well as with mercuric ion. With iodides a precipitate is obtained which contains mercury as well as the dye. It was found, however, that methylene blue could be easily and completely salted out of solution by means of sodium chlorate, while the mercury remained in solution. The methylene-blue chlorate so obtained readily dissolves in water and may be dissolved and reprecipitated as often as desired, or it may be converted into the chloride by precipitation with sodium chloride. One molecule of methylene blue is well separated from 6 molecules of mercuric acetate by 3 successive precipitations with chlorate, or from 15 molecules by 4 precipitations. The precipitation of methylene blue by chlorate is not prevented by the presence of acetic acid, and unless the acid is very strong and the solution allowed to stand for some time the methylene blue is not oxidized in such solutions.

In order to study the mercuration of methylene blue, aqueous solutions of the acetate were heated for five to six hours on the steam-bath with 1 to 15 molecular equivalents of mercuric acetate. During the heating some mercurous acetate is formed. The quantity of this is roughly proportional to the amount of mercuric acetate used and the time of heating. After the solutions had been heated they were treated with an excess of sodium chlorate solution. The methylene blue which had been heated with 1 or 2 molecular equivalents of mercuric acetate was completely precipitated by the chlorate, and contained no mercury. However, the dye which was heated with 5 or more molecular equivalents of mercuric acetate was precipitated only very slowly and never completely. In these slow precipitations the dye formed large, green crystals.

After many unsuccessful experiments the following procedure was tried. A solution of 70 g. of U. S. P. methylene-blue chloride in 1800 cc. of water was shaken and allowed to stand some hours with 100 g. of mercurous acetate. It was then filtered from the mercurous acetate and chloride, and the precipitate washed until it was only light blue. The filtrate was then mixed with a solution of 480 g. of mercuric acetate in 1100 cc. of water and 50 cc. of glacial acetic acid. The resulting solution was heated on the water-bath for ten hours; then allowed to cool and filtered from 37 g. of mercurous acetate. The solution had a volume of 3 liters. It was reduced in lots of 1 liter each, since the process from the beginning of the reduction to the isolation of the chlorate must be carried out in one day. One liter of this solution was treated with the suspension of ferrous hydroxide prepared by precipitation of a solution of 350 g. of pure ferrous sulfate heptahydrate in 700 cc. of water with 290 cc. of 30% ammonium hydroxide. The mixture was shaken and kept cool in an ice-bath for an hour. It was then centrifuged. The gelatinous precipitate was collected and treated with 260 cc. of glacial acetic acid, which gave it a total volume of about 800 cc. This mixture, which was intensely blue, was mechanically stirred for an hour and then centrifuged well. The liquid was filtered and the filtrate was treated with 200 cc. of a 50% solution of sodium chlorate, allowed to stand for two hours and centrifuged. The precipitate was suspended in 2 liters of water and boiled gently for half an hour during mechanical stirring. The hot solution was then filtered. When it did not filter easily it was refiltered until it ran through readily without clogging the filter. The filtrate was treated with a solution of 200 g. of sodium chloride in 600 cc. of water. After 24 hours the solution was filtered. The precipitate was extracted with 2 liters of boiling water and the solution filtered. The filtrate was treated with 300 g. of sodium chloride in 900 cc. of water and allowed to stand for some days before it was filtered. The filtration was slow and difficult because of the colloidal nature of the precipitate. When the substance was finally obtained on the filter it could not be washed without running through the paper. The following analysis was made on a sample that had been washed with 250 cc. of water, but still seemed to be impure. The yield is about 1/20 of the weight of the methylene-blue chloride used. Only one analysis was obtained on this substance.

Anal. Subs., 0.7298: HgS, 0.1543. Calcd. for $C_{32}H_{34}N_6ClS_2Hg$: Hg, 23.5. Found: 18.23.

The sample was dried in a vacuum over phosphorus pentoxide at room temperature. In the Carius analysis the sample was heated with 75 drops of fuming nitric acid for seven hours at 325–335°.

The substance is a reversible colloid. When 1 g. of it is heated on the water-bath with 25 cc. of water, a sirupy liquid is formed. When cooled a little below 100° this liquid sets to a semi-solid jelly. When 25 cc. more of water is added to the jelly and the mixture heated on the water-bath until it is homogeneous and then cooled in an ice-bath, a sirupy liquid is again obtained. This can be filtered slowly, but completely, and it leaves nothing on the filter. The compound has not been obtained in a crystalline form.

When the various precipitates discarded in the preceding preparation subsequent to the chlorate precipitation are collected and extracted with large volumes of boiling water, a second mercury compound can be salted out of the extracts by sodium chloride. The yield is extremely small, about 0.5% or less, of the weight of methylene blue used. This substance can be washed on the filter free from chlorides and unmercurated methylene blue. The compound gives a strong qualitative test for mercury. Only enough for two analyses has been obtained.

Anal. Subs., 0.3230: CO_2, 0.4069; H_2O, 0.0986. Subs., 0.2325: 16.2 cc. of dry N_2 (25°, 721 mm.). Calcd. for $C_{16}H_{16}N_3SClHg$: C, 37.06; H, 3.11; N, 8.11. Calcd. for $C_{16}H_{16}N_3SClHg.2H_2O$: C, 34.63; H, 3.63; N, 7.58. Found: C, 34.64; H, 3.44; N, 7.47.

The combustions were carried out with lead chromate; any oxygen was removed from the nitrogen by means of sodium hydrosulfite. The samples were dried over phosphorus pentoxide at room temperature.

The formulas suggested for these compounds in the introduction are no more than possibilities. The work so far done is inadequate to establish formulas, and the problem is, moreover, complicated by the ease with which methylene blue can be converted into other dyes. In the course of the work small quantities of mercury derivatives of dyes other than methylene blue were, in fact, isolated. One of these had the color and reactions of a methylene-azure derivative.

I wish here to thank Dr. Stieglitz for his kindness to me and for his encouragement during this work.

Summary

1. The work upon the mercuration of methylene blue prior to that described in this paper is reviewed.

2. The mercuration of the benzoyl derivative of leuco methylene blue is described, and it is shown that this reaction, in contrast to most mercurations, does not go to completion except in the presence of a considerable excess of mercuric acetate.

3. An account is given of the preparation of small quantities of two mercury derivatives from methylene blue.

Chicago, Illinois

NOTES

Liberation of Hydrogen from Carbon Compounds.—Under this heading Fry, Schulze and Weitkamp[1] have recently published a paper in which they describe the action of fused alkali upon acetylene, methyl alcohol and formaldehyde, and further propose to investigate the action of alkali on various carbon compounds from the point of view of the more or less general reaction in which, under the influence of fused alkali, hydrogen is liberated and the carbon compound oxidized.

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[1] Fry, Schulze and Weitkamp, This Journal, **46**, 2268 (1924).