5. The absorption spectra of the aqueous solutions of the chlorides of copper, cobalt, and nickel and of the pyridine and carbon tetrachloride solutions of the stearates of these metals have been photographed. No fundamental difference has been noted in the absorption spectra of the aqueous and non-aqueous solutions.

The author wishes to take this opportunity of thanking Professor Kahlenberg for his valuable suggestions during the progress of this work which was undertaken at his request.

CHEMICAL LABORATORY, UNIVERSITY OF WISCONSIN,
MADISON, WIS.

[FROM THE HENRY PHIPPS INSTITUTE OF THE UNIVERSITY OF PENNSYLVANIA.]

THE PREPARATION OF COMPOUNDS OF TRYPAN-RED WITH IODINE AND OTHER SUBSTANCES.

BY ROBERT B. KRAUSS.

Received January 24, 1914.

It was found by Lewis[1] in 1912 in this laboratory that certain dyes, namely, trypan-red, trypan-blue, and isamine-blue, had a more or less selective affinity for different portions of the tuberculous tissue experimentally produced in the rabbit. Trypan-red, for example, when injected into a living animal which was suffering from tuberculosis, would be found after several days in considerable concentration in the central, more or less necrotic portions of the diseased tissues.

It was thought possible that this fact might be used as the basis for an attempt to distribute various physiologically active substances to the diseased tissue. Iodine was the active substance first selected. It was chosen because it is one of the few substances having any sustained reputation among physicians as a useful agent in the treatment of certain forms of localized tuberculosis. It is my purpose to present here, as briefly as possible, the purely chemical aspects of the work so far done. The physiological results obtained with the use of the substances whose preparation and analyses are presented in this paper will shortly be reported in an appropriate place.

The chemical problem, accordingly, was to prepare compounds allied physiologically to trypan-red and containing iodine in the molecule. We have been able to obtain three direct substitution products of trypan-red with iodine. A compound of iodoform and trypan-red has been produced. We were able to obtain compounds of the dyestuff with thymol, eucalyptol, guaiacol, phenol, the cresols, cinnamic esters and pyrrol. The compounds with these substances have been further varied by substitution with iodine.

[1] Paul A. Lewis, "The Selective Relation of Certain Vital Stains to the Tubercle," *Arch. Intern. Med.*, 10, 68 (1912).

Experimental.

Method of Analysis.—The elementary analyses mentioned in this paper were conducted according to Dennstedt's simplified method, with the modification that a platinum capillary insert was used in place of the glass capillary of the original method, in order to avoid loss of time from breakage of the latter. Also, glass tubing with rubber connections was substituted for the rubber tubing of the method where it was feasible to do so, the former combination being sufficiently flexible to serve the purpose, obviating thereby the constant absorption of moisture through the rubber into the system.

Determinations of iodine were carried out also by the method of E. C. Kendall,[1] with slight modifications. These were for the most part very kindly furnished by Kendall himself, in the form of additional notes to his paper. We have made one slight departure of our own. This consisted of heating the crucibles in a firebrick[2] furnace having an insert (Fig. 1). By this means they were heated more indirectly and evenly than possible in his method. At the same time contamination from gases was totally eliminated.

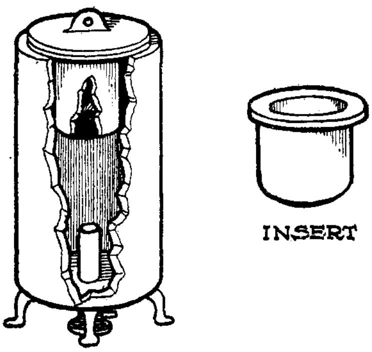
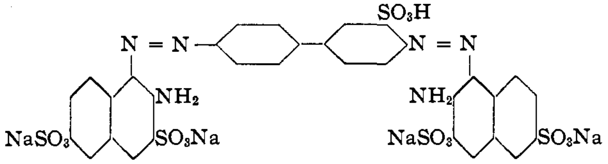


FIG. I

Trypan-red is a red dye very soluble in water, insoluble in alcohol. The manufacturers have not, to our knowledge, patented the preparation and have furnished no data as to its composition or the methods used in its manufacture. Ehrlich and Shiga first called attention to this dyestuff which they used in experiments, now classical, in the treatment of experimental infections with trypanosomes.

According to Ehrlich and Shiga[3] the composition of trypan-red is: Tetrazobenzidine monosulfonic acid (1 mol) coupled to β-naphthylamine disulfonate of sodium, R salt (2 mols). Structural formula:



These authors furnish no analyses of the substance and we are familiar with no published evidence for the formula given. Of the dyestuffs, fur-

---

[1] E. C. Kendall, This Journal, 34, 894 (1912).

[2] Crucible furnaces of this design made of fire clay in three pieces have been recently made to our order at a reasonable cost.

[3] Ehrlich and Shiga, *Berl. klin. Wochenschrift*, 41, 13 (1904).

nished commercially, even the purest does not correspond to this formula. In the various specimens analyzed wide variation in the sulfonic acid radical content was observed, as well as foreign organic substances, such as benzidine sulfone-sulfonic acid, benzidine di- and trisulfonic acids coupled variously with aminonaphthol sulfonic acids, Neville and Whinther's acid, amino G acid, amino R acid and amido H acid.

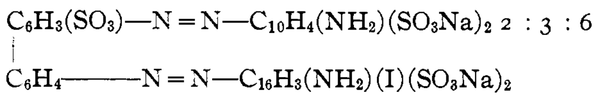
In the purification of the trypan-red considerable difficulty was accordingly encountered. It was deemed advisable to avoid separations with saline solutions owing to the difficulty of completely removing inorganic salts from the purified dyestuff. The purification was accomplished by a rather tedious fractional precipitation, using alcoholic solutions of widely variable concentration in conjunction with acetic and hydrochloric acids. The analysis of the purified material gave values approximately called for by the formula ($C_{32}H_{20}N_6S_5Na_4O_{15}$) of Ehrlich. The following is an example:

Calc.: C, 39.18; H, 2.04; N, 8.57; S, 16.33; Na, 9.39
Found: C, 39.20, 39.24; H, 2.14, 2.10; N, 8.59, 8.63; S, 16.40, 16.52; Na, 9.30, 9.34

In the interpretation of such results as we have obtained, we have assumed the substantial correctness of the formula. We have made certain attempts to prepare trypan-red from our own materials. When we have worked with purified components we have never succeeded in making the dye. On the other hand, as the result of the combination of impure benzidine monosulfonic acid as a rough sulfonation product and an impure R salt we have obtained a mixture of substances, from which we have been able to separate a large proportion of a dye having the chemical and biological characteristics of trypan-red.

We mention this matter because of its bearing on our failure to get some results which might have been expected from the formula. Briefly, benzidine monosulfonic acid can be combined with iodine in at least five different proportions. We have never, however, been able to combine the iodized benzidine with pure R salt in such a way as to give any substance having a resemblance biologically to trypan-red. In view of our failure to prepare trypan-red from pure components we do not consider it certain that iodized trypan-red with iodine substituted benzidine cannot be prepared. As above stated, we have been able to make a number of preparations containing iodine in the molecule and more or less closely resembling trypan-red in their biological properties. The methods which we have used, the analyses of the preparations and their probable formulas are briefly presented in the following paragraphs:

Compound $C_{32}H_{19}O_{15}N_6S_5Na_4I$, *Monoiodo Trypan-red*



$$\begin{array}{l} C_6H_3(SO_3)\text{—}N=N\text{—}C_{10}H_4(NH_2)(SO_3Na)_2\ 2:3:6 \\ | \\ C_6H_4\text{———}N=N\text{—}C_{16}H_3(NH_2)(I)(SO_3Na)_2 \end{array}$$

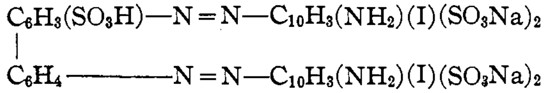
Tetrazobenzidine monosulfonic acid coupled to monoiodo-β-naphthylamine disulfonic salt R and β-naphthylamine disulfonic R salt. This was prepared by treating 25 g. trypan-red in a flask with 16 g. of potassium iodide and 125 cc. water, then adding 25 cc. conc. sulfuric acid. The flask is attached to a reflux condenser and heated to boiling for an hour. After neutralizing with sodium carbonate solution the dyestuff is filtered, washed with dilute alcohol, absolute alcohol and dried at 110°. It is a crimson powder, soluble in water to a red solution. It is insoluble in alcohol, ether and benzol. Analysis:

Calc. for $C_{32}H_{19}N_6S_5Na_4IO_{15}$: C, 34.72; H, 1.42; N, 7.59; S, 14.47; Na, 8.31; I, 11.48
Found: C, 34.76, 34.78; H, 1.70, 1.74; N, 7.58, 7.60; S, 14.48, 14.50; Na, 8.32, 8.34; I, 11.47, 11.47

When treated with KOH solution the dye turns to a violet color; with concentrated H_2SO_4, blue; on heating, iodine is given off.

Determination of the Location of the Iodine.—The dyestuff was dissolved in a little water and the solution made strongly alkaline with ammonia; zinc dust was then added and the solution heated to boiling, whereupon the the color disappeared. On acidifying, cooling, and filtering, the benzidine monosulfonic acid remains with the zinc dust on the filter, while the second components are in the filtrate as *o*-diamino sulfonic salts. The benzidine monosulfonic acid, when dissolved from the zinc, evaporated to dryness, and treated with concentrated sulfuric acid and warmed, does not give off any iodine. The filtrate from the zinc containing the naphthylamine fraction of the dye shows distinctly this reaction, therefore: All the iodine present is located in the β-naphthylamine disulfonic salt, and since one atom of iodine is present, only one molecule of the second component has been substituted.

Compound $C_{32}H_{18}N_6S_5O_{15}Na_4I_2$, *Di-iodo Trypan-red*



$$\begin{array}{l} C_6H_3(SO_3H)\text{—}N=N\text{—}C_{10}H_3(NH_2)(I)(SO_3Na)_2 \\ | \\ C_6H_4\text{————}N=N\text{—}C_{10}H_3(NH_2)(I)(SO_3Na)_2 \end{array}$$

Tetrazobenzidine monosulfonic acid coupled to monoiodonaphthylamine disulfonic acid R and monoiodonaphthylamine disulfonic acid R. This was obtained by treating 25 g. of trypan-red in a flask with 100 cc. of 50% alcohol and 50 cc. of saturated alcoholic solution of iodine in the presence of mercuric oxide. The flask is boiled under reflux for three hours. After cooling, the dye is filtered off and washed on the filter with alcohol, dissolved from the filter in hot water, the solution made acid with hydrochloric acid, saturated with hydrogen sulfide to remove any mercury present, filtered, boiled to expel hydrogen sulfide, filtered and evaporated to dryness. A red dyestuff is obtained which dissolves in water to a deep red solution. Reduction and examination, as before, showed the iodine to be present only in the β-naphthylamine salt.

Calc. for $C_{32}H_{18}N_6S_5Na_4I_2O_{15}$: C, 31.20; H, 1.46; N, 6.82; S, 12.98; Na, 7.47; I, 20.61.
Found: C, 31.11, 31.00; H, 1.46, 1.48; N, 6.81, 6.76; S, 12.96, 12.88; Na, 7.48, 7.50; I, 20.59, 20.60.

On treating the dye with concentrated potassium hydrate solution the color becomes violet; with concentrated sulfuric acid, blue, and on heating iodine is given off.

Iodoform Trypan-red.—On heating trypan-red with a large excess of iodoform in alcoholic solution under reflux for six hours, a dark brown compound of iodoform and trypan-red results. The dye is washed with alcohol until no more iodoform can be detected in the washings and then dried at 130°. This insures the removal of any remaining free iodoform.

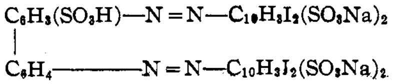
On standing, iodoform is slowly liberated only after considerable time. Heating with conc. H_2SO_4 turns the dye blue and iodoform is given off; with caustic potash the color change is to red-violet. The dried red-brown dye is completely soluble in water to bright red solution.

Calc. for $C_{32}H_{20}N_6S_5Na_4O_{15}.CHI_3$: I, 28.60. Found: I, 28.59, 28.60, 28.61, 28.54

Compound, $C_{32}H_{16}N_4O_{15}S_5Na_4I_4$, *Tetraiodo Trypan-red.*—By means of the Sandmeyer reaction, trypan-red yields an iodine derivative of dark brown color, which dissolves in water to a deep red solution with a violet tinge. Analysis showed the iodine to be present in the naphthalene disulfonic salt, and corresponding to four atoms iodine.

Calc. for $C_{32}H_{16}N_4S_5Na_4I_4O_{16}$: C, 26.37; H, 1.09; S, 10.98; N, 3.84; Na, 6.31; I, 34.89.
Found: C, 26.37, 26.20; H, 1.07, 1.04; S, 10.85, 10.78; N, 3.79, 3.80; Na, 6.18, 6.22; I, 34.87, 34.79, 34.88.

Formula:

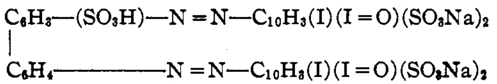


$$\begin{array}{l} C_6H_3(SO_3H)\text{—}N=N\text{—}C_{10}H_3I_2(SO_3Na)_2 \\ | \\ C_6H_4\text{————}N=N\text{—}C_{10}H_3I_2(SO_3Na)_2. \end{array}$$

Concentrated H_2SO_4 turns the dye dark blue and liberates iodine on warming; caustic potash gives a dark red color.

Compound, $C_{32}H_{16}N_4O_{17}S_5Na_4I_4$, *Diiodo-diiodoso Trypan-red.*—The preceding preparation dissolved in 20% sodium hydroxide was treated with a current of chlorine gas. The temperature of the solution was kept constant at 40°. A diiodo-diiodoso trypan-red was formed, having an active oxygen content of 2.09%. It oxidizes hydriodic acid, liberating iodine. This may be separated by shaking with chloroform and then can be titrated with thiosulfate.

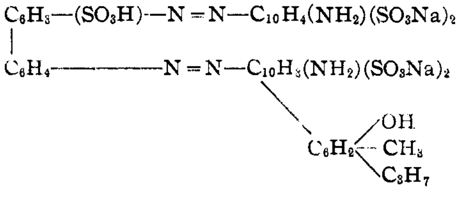
Calc. for $C_{32}H_{16}N_4S_5Na_4I_4O_{17}$: C, 25.80; H, 1.08; N, 3.76; S, 10.75; Na, 6.04; I, 34.14. Active O, 2.09.
Found: C, 25.81, 25.63; H, 1.07, 1.05; N, 3.70, 3.78; S, 10.70, 10.65; Na, 6.06, 6.10; I, 34.08, 33.91; Active O, 2.09. 2.07.



$$\begin{array}{l} C_6H_3\text{—}(SO_3H)\text{—}N=N\text{—}C_{10}H_3(I)(I=O)(SO_3Na)_2 \\ | \\ C_6H_4\text{————}N=N\text{—}C_{10}H_3(I)(I=O)(SO_3Na)_2 \end{array}$$

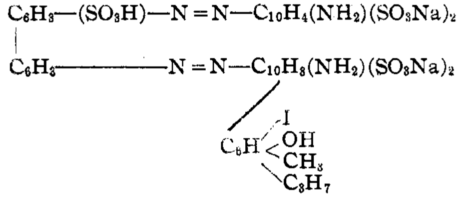
Compound, $C_{42}H_{32}N_6O_{16}S_5Na_4$, *Thymol Trypan-red.*—This dyestuff is formed when 25 g. trypan-red, 300 cc. 50% alcohol and 15 g. thymol are boiled together in a flask under reflux for 24 hours. It is a crimson dye of the above composition, soluble in water to a crimson solution and insoluble in alcohol. The dye turns dark blue with concentrated sulfuric acid, liberating thymol on heating. With caustic potash the color is yellow-red.

Calc. for $C_{42}H_{32}N_6Na_4S_5O_{16}$: C, 44.68; H, 2.83; N, 7.44; Na, 8.15; S, 14.18.
Found: C, 44.72, 44.82; H, 2.88, 2.90; N, 7.48, 7.54; Na, 8.16, 8.25; S, 14.20, 14.28.



Compound, $C_{42}H_{31}O_{16}N_6S_5Na_4I$, *Iodothymol Trypan-red.*—Thymol trypan-red (25 g.) was treated with 30 g. sodium hydroxide, 200 cc. water, and 10 g. iodine and then warmed to 80°. As soon as the reaction is completed the solution is neutralized with hydrochloric acid, the dyestuff separating out. It is washed on the filter with alcohol and dried. The scarlet powder is soluble in water to a scarlet solution. On warming the dye with concentrated H_2SO_4 iodine is liberated.

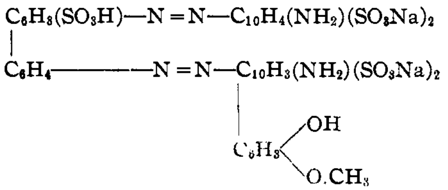
Calc. for $C_{42}H_{31}N_6Na_4S_5I.O_{16}$: C, 40.18; H, 2.47; N, 6.69; S, 12.75; Na, 7.34; I, 10.12.
Found: C, 40.18, 40.12; H, 2.50, 2.62; N, 6.65, 6.68; S, 12.80, 12.72; Na, 7.32, 7.19; I, 10.08, 10.16.



Compound, Eucalyptol Trypan-red.—This was formed when 15 g. trypan-red, 5 g. eucalyptol, and 50 cc. of 80% alcohol are heated under reflux to boiling for three hours. The insoluble eucalyptol trypan-red is filtered off and washed with alcohol. It is a red-brown dye of varied composition, soluble in water to a red solution. An iodine derivative of this dyestuff was also prepared.

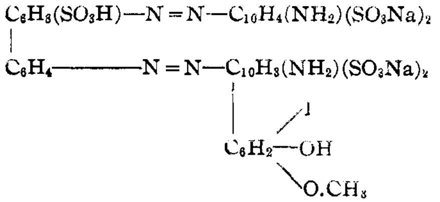
Compound, $C_{39}H_{26}N_6O_{17}S_5Na_4$, *Guaiacol Trypan-red.*—Finely divided trypan-red (25 g.) is heated with 10 g. guaiacol and 300 cc. 50% alcohol, for three hours under reflux. On cooling, the dyestuff settles out, is filtered, and washed with alcohol and dried at 110°. It is a red-brown powder soluble in water to a red solution with a yellow tinge.

Calc. for $C_{39}H_{26}N_6S_5Na_4O_{17}$: C, 42.39; H, 2.62; N, 7.60; S, 14.48; Na, 8.34.
Found: C, 42.39, 42.27; H, 2.61, 2.66; N, 7.61, 7.64; S, 14.49, 14.20; Na, 8.33, 8.41.



Compound, $C_{39}H_{25}N_6O_{17}S_5Na_4I$, *Iodoguaiacol Trypan-red.*—Prepared by heating 15 g. trypan-red, 6 g. iodoguaiacol and 150 cc. 50% alcohol as under the preceding. The dark brown dye which results is soluble in water to a red solution with a slight yellow tinge. Sulfuric acid liberates iodine from the dye on heating. Caustic potash gives a yellow-red color.

Calc. for $C_{39}H_{25}N_6S_5Na_4IO_{17}$: C, 38.08; H, 2.11; N, 6.84; S, 13.02; Na, 7.49; I, 10.34. Found: C, 38.17, 38.07; H, 2.12, 2.14; N, 6.82, 6.76; S, 13.01, 13.06; Na, 7.47, 7.50; I, 10.33, 10.28.



Compound, $C_{36}H_{23}N_7O_{15}S_5Na_4$, *Pyrrol Trypan-red.*—When 15 g. trypan-red, 5 g. pyrrol and 100 cc. of 50% alcohol are heated under reflux for three hours, a condensation of pyrrol and trypan-red takes place and a red-brown dye of the above composition is formed. It is soluble in water to a yellow-red solution and insoluble in alcohol and ether. The dye does not give a reaction for pyrrol with isatin and sulfuric acid or phenanthraquinone.

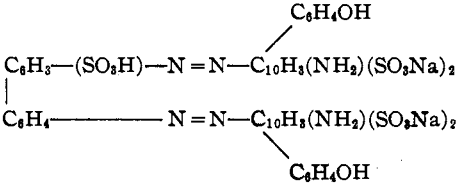
Calc. for $C_{36}H_{23}N_7Na_4S_5O_{15}$: C, 41.27; H, 2.39; N, 9.35; Na, 8.79; S, 15.28.
Found: C, 41.26, 41.20; H, 2.39, 2.35; N, 9.34, 9.38; Na, 8.78, 8.82; S, 15.30, 15.27.

Compound, $C_{36}H_{19}N_7O_{15}S_5Na_4I_4$, *Tetraiodopyrrol Trypan-red.*—This dye is formed when tetraiodopyrrol (from pyrrol and iodine in alkaline solution) is treated with trypan-red as in the preparation of pyrrol trypan-red. The tetraiodo dye is a dark brown salt soluble in water to a red solution. On addition of concentrated H_2SO_4 the dye turns blue and iodine is liberated on warming.

Calc. for $C_{36}H_{19}N_7I_4Na_4S_5O_{15}$: C, 27.88; H, 1.23; N, 6.32; I, 32.79; Na, 5.94; S, 10.32.
Found: C, 27.85, 27.80; H, 1.28, 1.35; N, 6.31, 6.28; I, 32.75, 32.70; Na, 5.96, 5.93; S, 10.31, 10.42.

Compound, $C_{44}H_{28}N_6O_{17}S_5Na_4$, *Phenol Trypan-red.*—Obtained by heating trypan-red under reflux with an excess of phenol in alcoholic solution for three hours. It is a crimson dye, soluble in water to a yellow-red solution. This dye does not give tests for phenol. On heating with concentrated sulfuric acid the phenol is split off.

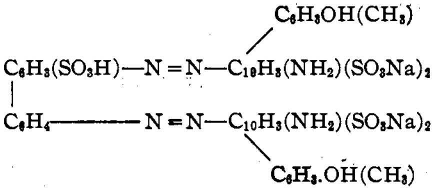
Calc. for $C_{44}H_{28}N_6Na_4S_5O_{17}$: C, 44.82; H, 2.37; N, 8.31; Na, 7.81; S, 13.58.
Found: C, 44.95, 44.90; H, 2.39, 2.57; N, 8.28, 8.26; Na, 7.68, 7.70; S, 13.51, 13.62.



$$\begin{array}{l} \qquad\qquad\qquad\qquad\qquad C_6H_4OH \\ C_6H_3—(SO_3H)—N=N—C_{10}H_3(NH_2)(SO_3Na)_2 \\ | \\ C_6H_4—N=N—C_{10}H_3(NH_2)(SO_3Na)_2 \\ \qquad\qquad\qquad\qquad\qquad C_6H_4OH \end{array}$$

Compound, $C_{46}H_{32}N_6O_{17}S_5Na_4$, *Di-o-cresol Trypan-red.*—On heating trypan-red with an excess of alcoholic *o*-cresol under reflux for three hours, a red-brown dye is formed which is soluble in water to a red solution. The dye is not broken down by heating the solution, nor can a test for cresol be obtained. Boiling with conc. sulfuric acid turns the dye blue and splits off cresol.

Calc. for $C_{46}H_{32}N_6Na_4S_5O_{17}$: C, 46.30; H, 2.68; N, 7.04; Na, 7.71; S, 13.42.
Found: C, 46.26, 46.42; H, 2.74, 2.78; N, 7.08, 7.11; Na, 7.89, 7.73; S, 13.38, 13.30.



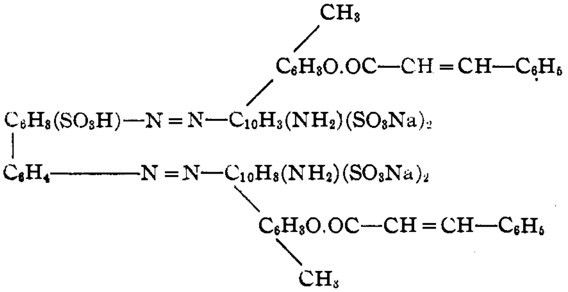
$$\begin{array}{l} \qquad\qquad\qquad\qquad\qquad C_6H_3OH(CH_3) \\ C_6H_3(SO_3H)—N=N—C_{10}H_3(NH_2)(SO_3Na)_2 \\ | \\ C_6H_4—N=N—C_{10}H_3(NH_2)(SO_3Na)_2 \\ \qquad\qquad\qquad\qquad\qquad C_6H_3.OH(CH_3) \end{array}$$

In the same manner di-*m*-cresol trypan-red, a dark red-brown dye, and di-*p*-cresol trypan-red, a dark red-brown dye, were prepared. With sulfuric acid the *m*-derivative gives a violet-blue color and the *p*-derivative a dark blue color. By treating these three isomers with iodine in caustic soda solution, and then neutralizing with acid, their iodine substitution products are obtained. In this way were made di-*o*-triiodocresol trypan-red, $C_{46}H_{26}N_6O_{17}S_5Na_4I_6$; di-*m*-triiodocresol trypan-red, $C_{46}H_{26}N_6O_{17}S_5Na_4I_6$; di-*p*-triiodo cresol trypan-red, $C_{46}H_{26}N_6O_{17}S_5Na_4I_6$.

Calc. for 6I, 39.69%.
Found for *o*-deriv. 39.64, 39.64; *m*-deriv., 39.62, 39.65; *p*-deriv. 39.67, 39.70.

Compound, $C_{64}H_{44}N_6O_{19}Na_4S_5$, *Dicinnamyl-di-o-cresol Trypan-red.*—Prepared by treating, in a flask, 146 pts. di-*o*-cresol trypan-red, 18 pts. sodium cinnamate, and 100 pts. benzene in the cold with 9 pts. phosporous pentachloride and then heated under reflux until the evolution of hydrochloric acid has ceased. The benzene is then poured off and the residue neutralized with sodium carbonate. It is a red dye soluble in water to a red solution. Sulfuric acid turns it blue and caustic soda to a yellow-red solution.

Calc. for $C_{64}H_{44}N_6Na_4S_5O_{19}$: C, 52.89; H, 3.03; N, 5.76; Na, 6.33, S, 11.01.
Found: C, 55.71, 52.76; H, 3.06, 3.14; N, 5.78, 5.81; Na, 6.31, 6.39; S, 10.97, 11.08.

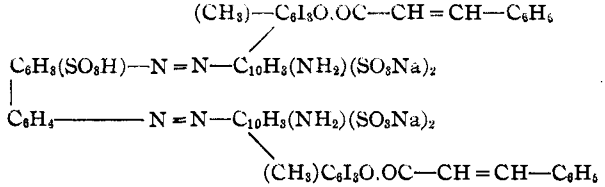


Dicinnamyl-di-*m*-cresol Trypan-red. Color scarlet.

Dicinnamyl-di-*p*-cresol Trypan-red. Color dark red.

Compound, $C_{64}H_{38}O_{19}N_6S_5Na_4I_6$, *Dicinnamyl-di-o-triiodocresol Trypan-red.*—Prepared according to the method indicated in the preceding paragraph, using di-*o*-triiodo trypan-red as a starting point. The dyestuff obtained is a crimson powder easily soluble in water to a red solution, giving with concentrated sulfuric acid a blue color, and on heating this iodine is split off.

Calculated for $C_{64}H_{38}N_6Na_4S_5I_6O_{19}$: C, 34.78; H, 1.72; N, 3.80; Na, 4.16; S, 7.24; I, 34.51. Found: C, 34.76, 34.70; H, 1.68, 1.94; N, 3.79, 3.65; Na, 4.11, 4.23; S, 7.23, 7.21; I, 34.47, 34.43.



Dicinnamyl-di-*m*-triiodocresol Trypan-red. A crimson powder.

Dicinnamyl-di-*p*-triiodocresol Trypan-red. A red powder.

Concentrated sulfuric acid turns both of these dyes dark blue and iodine is then split off on heating.

The cinnamyl esters of the remaining phenolic condensations mentioned are being prepared in this laboratory.

Summary.

1. Iodine derivatives of trypan-red of similar color, solubility and biological qualities were prepared.
2. An iodoform addition product of trypan-red of definite composition was prepared.
3. Condensation products of trypan-red and phenol, the cresols, thymol, guaiacol, pyrrol and their iodine substitution products of definite compositions were prepared.
4. Condensation products of trypan-red with eucalyptol and its iodine derivative of indefinite composition were prepared.

5. Cinnamic esters of the cresol trypan-reds and of the iodocresol trypan reds of definite composition were prepared.

In conclusion the author wishes to express his gratitude to Paul A. Lewis, who suggested the present investigation.

PHILADELPHIA, PA.

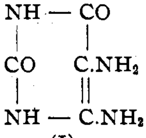
[CONTRIBUTIONS FROM THE SHEFFIELD CHEMICAL LABORATORY OF YALE UNIVERSITY.]

RESEARCHES ON PYRIMIDINES. LXIX. ON A COLOR TEST FOR 5-AMINOPYRIMIDINES.

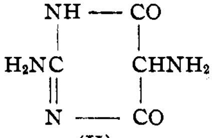
BY TREAT B. JOHNSON AND CARL O. JOHNS.

Received February 25, 1914.

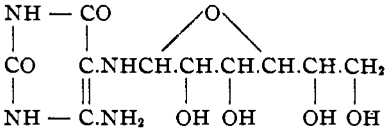
In the last paper from this laboratory on pyrimidines entitled, "The Structure of Ritthausen's Divicine,"[1] the writers emphasized the significance of the interesting observation made by Ritthausen,[2] that this base interacts with phosphomolybdic acid, in aqueous solution, giving an intense blue color. We contributed the interesting data that 4,5-diaminouracil (I) and 2,5-diamino- 4,6-dioxypyrimidine (II) likewise interact with this reagent, giving apparently the same blue color as *divicine*. Phosphotungstic acid also gave a characteristic blue color with the 2,5-diamino-4,6-dioxypyrimidine (II), but, on the other hand, the isomeric 4,5-diaminouracil interacted with formation of an amorphous green precipitate. This behavior is in accordance with that of divicine towards phosphotungstic acid. In fact, all the known experimental evidence seems to indicate that Ritthausen was actually dealing with this pyrimidine and, consequently, that his *vicine*, which he isolated from vetch seeds, is a glucoside of this interesting base as represented by formula (III).[3] Whether these assumptions are correct will be determined by an examination of the natural products. This work is now in progress.



(I)



(II)



(III)

Attention was also called, in our paper, to the fact that both of the diaminopyrimidines contain an amino group in position 5 of the pyrimidine

---

[1] Johnson and Johns, THIS JOURNAL, 36, 545 (1914).

[2] *J. prakt. Chem.*, 59, 482.

[3] The sugar might also be linked to the 4-amino group of the pyrimidine base.