It is a dark red product, insoluble in water, quite soluble in methyl alcohol, ethyl alcohol, and acetone, giving purple solutions, but slightly soluble in ether and benzene.

Calc. for $C_{14}H_6O_4Fe$: Fe, 19.05. Found: 19.8.

The cobalt and chromium salts of alizarine as well as the copper and nickel salts of anthrapurpurine and flavopurpurine were prepared and, with the exception of the cobalt salt of alizarine, which analyzed for $C_{42}H_{21}O_{12}Co$, appear to be normal salts.

Summary.

Copper, cadmium, nickel and iron salts of alizarine are formed when alizarine is boiled with the respective salts of these metals, anhydrous sodium acetate, and nitrobenzene.

These alizarine salts, though slightly soluble in water, penetrate unmordanted wool fiber and produce colors identical with those produced by alizarine in wool fibers mordanted with the respective metallic salts. These dyes appear to be *in* the fiber and not simply *on* the fiber.

The formation of an insoluble alizarine dye in a mordanted fiber is accomplished by the combined action of physical and chemical phenomena.

BOUND BROOK, N. J.

GENTIAN VIOLET—ITS SELECTIVE BACTERICIDAL ACTION.[1]

BY M. L. CROSSLEY.

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Shortly after the United States entered the war, it was discovered that the supply of gentian violet dye used in biological work as a differentiating stain was limited. Prior to the war, this material had been imported from Germany and no particular attention had been given to its composition. Careful study of the available literature gave no information of importance and it was decided to undertake an investigation of the product in order to determine its composition. Some of the information found in the literature was later discovered to be erroneous or confusing. For example, Pappenhein[2] states that it belongs to the rosaniline group and probably contains dextrine. This fact proved to be correct. On the other hand, Muhr and Richie[3] consider it synonymous with benzyl violet and pyoktanin. This, of course, is an erroneous judgment, for benzyl violet is a mixture of benzyl-pentamethyl-*p*-rosaniline hydrochloride and hexamethyl-*p*-rosaniline hydrochloride, while pyoktanin is a trade name for a mixture of hexamethyl-*p*-rosaniline hydrochloride and pentamethyl-*p*-

[1] Presented before the Dye Section of the American Chemical Society at the Philadelphia meeting, September 2–6, 1919.

[2] *Grundriss der Farb. Chemie zum Gebrauch bei Mikroskopischen Arbeiten.*

[3] "Manual of Bacteriology," p. 101.

rosaniline hydrochloride. Pyoktanin then is another name for methyl violet B.

A preliminary study of the gentian violet obtained from the Grübler laboratory at Leipzig, furnished me by the Connecticut State laboratory, showed the product to be mixture of dextrine and dye in about equal proportions. A qualitative study of the physical and chemical properties of the dye indicated that it belonged to the triphenylmethane class and that it contained free amino hydrogen. Further investigation showed that the dye was chiefly a mixture of hexamethyl-*p*-rosaniline hydrochloride and pentamethyl-*p*-rosaniline hydrochloride.

On acetylation of the dye, two products were obtained: one which did and one which did not contain an acetyl group. The first was a violet dye which reduced to a product identical with hexamethyl-*p*-leucaniline; the other reduced to acetyl-pentamethyl-*p*-leucaniline. The limited quantity of material available for the investigation and the difficulty encountered in making a sharp separation of the two products made it impossible to get a quantitative analysis of the product. The sample investigated, however, showed that hexamethyl-*p*-rosaniline hydrochloride was present in larger proportion than the pentamethyl product. There was also present a small amount of another dye which gave an acetyl derivative not crystallizable from the acetic acid solution and which, on reduction and qualitative study, appeared to be the acetyl derivative of the tetramethyl-*p*-rosaniline hydrochloride. It would, therefore, appear that the so-called gentian violet is simply a prepared mixture or blended mixture of the above mentioned dyes.

In view of the fact that Dr. Churchman had shown gentian violet to possess selective bactericidal action, and since it now appeared that gentian violet was not an individual dye, it, therefore, seemed pertinent to learn whether or not the selective activity of gentian violet was an inherent property of the mixture called gentian violet or of one or both of its components. Using the method suggested by Dr. Churchman,[1] it was found that the organisms which are destroyed by gentian violet in the dilution of 1 : 100,000 parts, were also destroyed by hexamethyl-*p*-rosaniline hydrochloride, pentamethyl-*p*-rosaniline hydrochloride and rosaniline in the same dilution, and those that were negative to gentian violet were also negative to the other dyes mentioned. For example, *B. Prodigiosus*, *B. fluorescens* (liquefying and non-liquefying), *B. mucosus capsulatus* or pneumobacillus of Friedländer, *Streptococcus pyogenes* and *B. coli* (*communis*), grew equally well on agar agar medium containing gentian violet, hexamethyl-*p*-rosaniline hydrochloride, pentamethyl-*p*-rosaniline hydrochloride and rosaniline in the dilution of 1 : 100,000, while *B. subtilis*, *B. cereus*, *Sarcina aurantiacea*, *Sarcina lutea*, *Staphylococcus*

[1] *J. Exp. Med.*, 16, 228–248 (1912); 18, 579–583 (1913).

pyogenes, *B. violaceae* and *B. mycoides* were killed by all 4 compounds in the dilution of 1 : 100,000. It is, therefore, evident that the selective bactericidal action of gentian violet was a property of its components. That this selective action is related to the constitution of the dye, I have no doubt. It seems that the effectiveness of any one of the dyes mentioned in killing organisms is governed by two factors; first, the composition of the dye, and second, the nature of the organism. Both chemical and physical phenomena are undoubtedly concerned in the reaction.

Malachite green will kill *B. subtilis* and *B. mycoides* in the dilution of 1 : 100,000, but not in the dilution of 1 : 200,000. In fact, no one of the dyes mentioned is effective for *B. subtilis* in dilutions greater than 1 : 100,000. *B. mycoides*, however, is destroyed by malachite green in the dilution of 1 : 100,000, rosaniline in dilution of 1 : 200,000, and by gentian violet, hexamethyl-*p*-rosaniline hydrochloride, and pentamethyl-*p*-rosaniline hydrochloride in the dilution of 1 : 300,000. The constitution of the dye is, therefore, and important factor.

It would seem from the evidence at hand, that this selective bactericidal action of the dyes mentioned is a property of the triphenylmethane dyes and is particularly related to the basic nitrogen groups. An increase in the number of these groups and substitution of methyl radicals for amino hydrogen seem to intensify the bactericidal power. This suggests an extremely interesting and important line of research, that is, the relation of chemical constitution to selective bactericidal action. I feel, however, that this problem cannot be successfully attacked from the chemical point of view only. In order to understand the chemistry involved, we must know more about the composition of bacteria. I am inclined to believe that the part played by the bacteria in this selective action is of prime importance.

Of the group of organisms studied, those that were gram positive, that is, those that were stained by the dye, were also gentian positive, that is, killed by gentian violet; while those that were gram negative were not inhibited in their growth by gentian violet. This same observation was true with respect to the other dyes mentioned. This, it seems to me, is evidence of a chemical reaction between some component or components of the bacteria cell and the dye. It has already been shown[1] that malachite green combines with sodium nucleate to form a black, porous nucleate of the dye. This selective bactericidal action seems then to be caused by the presence of compounds with reactive groups in the structure of the bacteria capable of readily combining with the dyes mentioned through certain reactive groups in their structure. Wherever this combination results in the formation of compounds which are detrimental to the life

[1] *Biochem. Z.*, **42**, 440–469 (1912).

functions of the organisms, either limitation or complete destruction of their life activities result.

I believe that careful investigation will show that there is variation of the susceptibility of bacteria to different chemical destructive agents and that no one chemical substance is capable of destroying all species of bacteria. There is then much work to be done in this field, for the whole system of sanitation rests upon the assumption that a disinfectant capable of killing certain pathogenic organisms will generally kill others. I trust that some biological chemist will take up this problem and continue the investigation, since I am no longer in a position to do so.

Experimental Part.

1. **Alcohol-insoluble Portion of Gentian Violet.**—2.2285 g. of the Grübler gentian violet was extracted with absolute alcohol and filtered. The residue was washed with alcohol until the filtrate was colorless. It was then dried and weighed. 1.1124 g. was obtained. This represented 49.92% of the dye. A second analysis gave 49.54% of alcohol-insoluble material. This product was soluble in water, gave the iodine test for dextrine, gave a brown solution with sulfuric acid which charred on heating and gave the characteristic dextrine odor. A water solution of the product reacted with an ammoniacal solution of lead acetate to give a heavy, white precipitate. The water solution had the characteristic dextrine odor. This evidence proved the alcohol-insoluble substance to be dextrine.

2. **Ash.**—0.6140 g. of dye was ignited in a platinum crucible and 0.0070 g. of ash, equal to 1.14% obtained.

3. **Identification of the Dye.**—The alcohol solution of the dye was carefully evaporated to dryness and dried to a constant weight. It was soluble in water and the solution gave the following reactions: It reacted with a solution of picric acid to give an insoluble, dark violet picrate; it gave a dark brown precipitate when treated with sodium hydroxide; it reduced to a colorless solution when treated with ammonium hydroxide and zinc dust, and gave an insoluble red-violet precipitate when treated with potassium dichromate solution. The dye dissolved in conc. sulfuric acid giving an orange colored solution which on dilution with water changed to red-green, then to green, to blue, and finally to violet, with increasing dilution, color phenomena characteristic of the alkylated *p*-rosanilines. When a portion of the dye was boiled with anhydrous sodium acetate and an excess of acetic anhydride, a solution was obtained which gave a violet spot with a green ring indicating the presence of free amino hydrogen in the dye. This evidence indicated that the dye belonged to the *p*-rosaniline group and confirmed Pappenheim's opinion. This was proven as follows: A portion of the dye was mixed with an excess of acetic anhydride and anhydrous sodium acetate and boiled several hours. The reaction product

was dissolved in water and salted with sodium chloride and zinc chloride. A violet precipitate formed leaving a green solution. This was filtered and the violet dye again dissolved in water and again salted with sodium chloride. The resulting dye was filtered and the filtrate added to that obtained from the previous filtration. The violet dye was then reduced and gave a product melting at 173°, proving it to be hexamethyl-*p*-leucaniline which showed that the violet dye was hexamethyl-*p*-rosaniline hydrochloride. The green solution was reduced with zinc dust and gave a precipitate of a leuco compound which crystallized from pure alcohol as colorless, concentric, needle crystal aggregates, melting at 142–3°. It analyzed for $C_{26}H_{31}N_3O$ and was identical with acetyl-pentamethyl-*p*-leucaniline.

Calc. for $C_{26}H_{31}N_3O$: N, 10.47. Found: 10.32.

This was treated with hydrochloric acid and converted into a substance crystallizing from dilute alcohol in colorless needles. From benzene the substance crystallized in beautiful, colorless coalescent spears, melting at 115 to 116°. It analyzed for $C_{24}H_{29}N_3$ and was pentamethyl-*p*-leucaniline.

Calc. for $C_{24}H_{29}N_3$: N, 11.7. Found: 11.83.

By oxidation of this a beautiful violet dye was obtained which was evidently pentamethyl-*p*-rosaniline. The filtrate from the acetyl-pentamethyl-*p*-leucaniline gave on standing a small quantity of a product which appeared to be an acetyl-tetramethyl-*p*-leucaniline. The small quantity available made a thorough study of it impossible. It would, therefore, seem that gentian violet is chiefly a mixture of hexamethyl-*p*-rosaniline and pentamethyl-*p*-rosaniline hydrochlorides with probably a small quantity of the tetramethyl product.

4. The Selective Bactericidal Action of Gentian Violet and its Compounds.—Several organisms which had previously been shown to be gentian positive and gentian negative were selected and the method of Churchman adopted for a study of their behavior with gentian violet free from dextrine, and C. P. samples of the dyes found in gentian violet. In order to see if the alkyl groups in these dyes had any important influence on their bactericidal properties, rosaniline was selected for comparative study. Malachite green was also used to study the effect of the number of basic nitrogen groups. The investigation was only preliminary and by no means exhaustive.

Divided plates of agar agar medium, one-half dyed with dye solution, were made as suggested by Churchman and the organisms under investigation planted on the undyed portion. Series A represents gentian violet free from dextrine, dilution 1 : 100,000; series B, hexamethyl-*p*-rosaniline hydrochloride, dilution 1 : 100,000; series C, pentamethyl-*p*-rosaniline hydrochloride, dilution 1 : 100,000; series D, rosaniline, and series E,

the above mentioned substances and malachite green in different dilutions. The organisms reported in the corresponding tables as gentian positive did not grow across into the dyed portion of the plates, while those indicated as negative did. It will be observed that in dilutions of 1 : 100,000 there was no difference in the effectiveness of the dyes for the organisms selected. With greater dilutions the bactericidal power of the dyes for two gentian positive organisms diminished with the decreasing number of the methyl groups and the basic nitrogen groups. It will be seen from Table E that rosaniline was more effective for *B. mycoides* than malachite green. In other words 3 unsubstituted amino groups were more effective than 2 in which the 4 hydrogens had been substituted by methyl radicals. Comparing the effectiveness for *B. mycoides* of the 4 substances up to a dilution of 1 : 300,000 it will be seen that hexa- and pentamethyl-*p*-rosaniline appear equal in strength and that they are the most effective of the group.

TABLE I.—SERIES A.

Gentian Violet free from Dextrine. Dilution 1:100,000.

Gram + = retains stain. Gentian + = inhibited.
Gram — = does not retain stain. Gentian — = not inhibited.

Organism.	Gram + or —.	Gentian + or —.
B. Prodigiosus	—	—
B. Subtilis	+	+
B. Cereus	+	+
B. Fluorescens (liq.)	—	—
Sarcina Aurantiaca	+	+
B. Mucosus Capsulatus or Pneumobacillus of Friedländer	—	—
Sarcina Lutea	+	+
Streptococcus pyogenes	—	—
Staphylococcus pyogenes	+	+
B. Coli (communis)	—	—
B. Violaceae	+	+
B. Fluorescens (non-liq.)	—	—
B. Mycoides	+	+

TABLE II.—SERIES B.

Hexamethyl-*p*-rosaniline Hydrochloride. Dilution 1:100.000.

Organism.	Gram + or —.	Gentian + or —.
Sarcina Lutea	+	+
B. Fluorescens (liq.)	—	—
Sarcina Aurantiaca	+	+
B. Violaceae	+	+
B. Mucosus Capsulatus	—	—
B. Prodigiosus	—	—
B. Cereus	+	+
B. Mycoides	+	+
B. Fluorescens (non-liq.)	—	—
B. Subtilis	+	+

TABLE III.—SERIES C.

Pentamethyl-*p*-rosaniline Hydrochloride. Dilution 1:100000.

Organism.	Gram + or —.	Gentian + or —.
Sarcina Lutea	+	+
B. Fluorescens (liq.)	—	—
Sarcina Aurantiaca	+	+
B. Violaceae	+	+
B. Mucosus Capsulatus	—	—
B. Prodigiosus	—	—
B. Cereus	+	+
B. Mycoides	+	+
B. Fluorescens (non-liq.)	—	—

TABLE IV.—SERIES D.

Rosaniline. Dilution 1:100000.

Organism.	Gentian + or —.
Sarcina Lutea	+
B. Fluorescens (liq.)	—
Sarcina Aurantiaca	+
B. Violaceae	+
B. Mucosus Capsulatus	—
B. Prodigiosus	—
B. Subtilis	+
B. Fluorescens (non-liq.)	—
B. Mycoides	+

TABLE V.—SERIES E.

Different Concentrations of Dyes.

	Dyes.					
Organism.	G. V.	Hexa.	Penta.	Rosa.	Mala.	Dilution.
B. Subtilis	+	+	+	+	+	1:100000
B. Subtilis	—	—	—	—	—	1:200000
B. Mycoides	+	+	+	+	+	1:100000
B. Mycoides	+	+	+	+	+	1:200000
B. Mycoides	+	+	+	—	—	1:300000
B. Mycoides	—	—	—	—	—	1:400000

Each of the organisms was also studied with respect to its behavior with the gram stain and the result in each case was the same. All the gram positive organisms were gentian positive.

Summary.

1. Gentian violet is a mixture of dextrine and dye in about equal proportions. The dye is a mixture chiefly of hexamethyl-*p*-rosaniline hydrochloride and pentamethyl-*p*-rosaniline hydrochloride with small quantities of lower homologs, particularly the tetramethyl compound.

2. The selective bactericidal action of gentian violet is no greater than that of its component dyes and gentian violet has no advantage over these in selective or differentiating power.

3. The organisms found to be gentian positive were also gram positive, indicating that both the structure of the dye and that of the organism

are important factors in the selective action. The organisms that are positive fix the dye because they possess compounds in their structure capable of reacting with the dye to form new complexes which limit or destroy the cell activity of the organism. The concentration of the dye is an important factor in determining its power to kill the organism, or in other words, the survival of the organism depends upon the degree to which the destructive reaction between its components and the dye has taken place and this in turn is dependent on the amount of dye which the cell has absorbed.

4. The effectiveness of the dyes used was the same in dilutions of 1 : 100,000 but different in greater dilutions.

5. No one of the dyes was effective for *B. subtilis* in dilutions greater than 1 : 100,000. *B. mycoides* was killed, however, by malachite green in dilution of 1 : 100,000, by rosaniline in dilution of 1 : 200,000, and by gentian violet, pentamethyl- and hexamethyl-*p*-rosaniline hydrochloride in a dilution of 1 : 300,000.

6. The basic nitrogen groups appear to be the reactive groups and their action is intensified by substitution of methyl for amino hydrogen.

I am greatly indebted to Mr. John Burisch, formerly a student at Wesleyan University, for assistance in the bacteriological part of this investigation, and hereby express my deep appreciation of his valuable work.

BOUND BROOK, N. J.

[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH.]

SYNTHESES IN THE CINCHONA SERIES. II. QUATERNARY SALTS.

BY WALTER A. JACOBS AND MICHAEL HEIDELBERGER.

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In a recent communication a plan was outlined for the synthesis of aromatic arsenic compounds for biological study.[1] The present paper and that following it represent an attempt to apply a similar method of treatment to the field of the cinchona alkaloids, whose possibilities as material for chemotherapeutic study in one direction have been shown by the remarkable specificity for the pneumococcus displayed by ethylhydrocupreine.[2]

Without entering into any extended discussion it may be stated that previous synthetic studies in the cinchona group have consisted mainly in the preparation of ethers of the phenolic hydroxyl group at (1) in the formula (ethylhydrocupreine being the ethyl ether), and in the formation

[1] THIS JOURNAL, 41, 1581 (1919).

[2] Morgenroth and Levy, *Berl. klin. Wochschr.*, 48, 1560, 1979 (1911).