have shown that Fraction B and streptothricin demonstrate similar inhibition activities for 41 other bacteria.

With *B. subtilis* as the test organism, the ratios of activity in broth to that in an agar diffusion assay are similar for Fraction B and streptothricin. These ratios differ markedly from that of streptolin. Streptolin has less than $^1/_{13}$ the activity of Fraction B or streptothricin in the agar diffusion assay, but is more than 10 times as active against *B. subtilis* in broth medium.

The intravenous toxicity of Fraction B is similar to that of streptothricin. Streptolin, on the other hand, is 36 times as toxic as Fraction B on a weight basis, and 500 times more toxic on a unit basis.

The data indicate that the strain of Streptomyces which produces streptolin also produces a second antibiotic identical with streptothricin.

RESEARCH LABORATORIES
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KALAMAZOO, MICHIGAN RECEIVED JUNE 12, 1947

A Novel Replacement of Alkyl Groups by Chlorine

BY SIDNEY D. ROSS AND MATTHEW NAZZEWSKI

While the cleavage or replacement of functional groups by chlorine during chlorinations with gaseous chlorine has been frequently observed[1,2,3] no similar replacements are reported for chlorinations with sulfuryl chloride. Silberrad[4] has correctly reported that in the chlorination of toluene by sulfuryl chloride, catalyzed by sulfur monochloride and aluminum chloride, the side chain is neither cleaved nor attacked. We find, however, that under very similar conditions both ethyl and isopropyl groups are replaced by chlorine.

In a typical experiment one mole of ethylpentachlorobenzene was refluxed for one hundred and ten hours with three moles of sulfuryl chloride, 0.138 mole of sulfur monochloride and 6 g. of iron powder. The liquid remaining was removed in vacuo and the solid residue was crystallized from trichloroethylene to yield 82% of hexachlorobenzene of m. p. 227–229°.

Anal.[5] Calcd. for C_6Cl_6: C, 25.35; H, 0.00; Cl, 74.65. Found: C, 25.20, 25.03; H, 0.00, 0.00; Cl, 75.10, 75.01.

Under identical conditions isopropylpentachlorobenzene gave a 45% yield of hexachlorobenzene, and diethyltetrachlorobenzene, obtained from the Dow Chemical Company, gave 57% of hexachlorobenzene and 4% of ethylpentachlorobenzene. The latter was separated from hexachlorobenzene by virtue of its solubility in hot alcohol. Pentachlorotoluene did not react under these conditions.[6]

Both iron powder and sulfur monochloride are essential catalysts for the reaction and no replacement was obtained with either sulfuryl chloride and iron alone or sulfuryl chloride and sulfur monochloride alone. Anhydrous ferric chloride and sulfuryl chloride also gave no reaction. No effort was made to determine either the specific functions of the two catalysts or the form in which the alkyl group came off. The fact that the reaction is accompanied by copious evolution of hydrogen chloride suggests that the side chain may be chlorinated prior to its cleavage.

CONTRIBUTION FROM THE
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(1) Page, *Ann.*, **225**, 208 (1884).

(2) Quist and Holmberg, *C. A.*, **27**, 5726 (1933).

(3) Dvornikoff, Sheets and Zienty, THIS JOURNAL, **68**, 142 (1946).

(4) Silberrad, *J. Chem. Soc.*, **127**, 2677 (1925).

(5) Analyses are by Dr. Carl Tiedcke.

(6) In this connection it is of interest to point out that pentachlorotoluene and hexachlorobenzene have very similar physical properties and solubilities and, moreover, do not depress one another on mix-melting. In cases where there is a possibility of confusing the two compounds we have found side-chain chlorination, which converts pentachlorotoluene to pentachlorobenzal chloride and leaves hexachlorobenzene unaffected, a convenient method of differentiation.

The Metachromatic Reaction of Hexametaphosphate

BY J. M. WIAME[1]

Metachromasy denotes the property of certain dyes to undergo changes in their absorption spectrum under certain conditions other than changes in *p*H or oxido-reduction potential.[1a] Certain substances induce metachromasy when they are mixed with these dyes. Among these substances are some of biological interest and the appearance of metachromasy in histological staining makes it possible to detect these substances in cells.

Lison[2] found that sulfuric esters of polymeric carbohydrates were responsible for the metachromatic staining of various biological materials. Recently it was shown[3] that yeast is able to accumulate in large amounts a metachromatic substance which contains phosphoric acid rather than sulfuric acid. This substance was isolated and found to be metaphosphate.[4] This finding led to a study of the metachromatic reaction of metaphosphate in solution. Sodium trimetaphosphate and sodium hexametaphosphate prepared according to Jones[5] were used. The dye used was toluidin blue.[6]

When a 0.1% solution of hexametaphosphate was mixed with an excess of toluidin blue (0.5%), a precipitate formed. Trimetaphosphate, pyrophosphate and orthophosphate gave no precipitate under these conditions.

When hexametaphosphate (10^{-2} to 10^{-4} M)[7] was mixed with a dilute solution of dye (10^{-4} M) a purple color appeared. This color was studied spectroscopically.[8] Solutions of hexametaphos-

(1) Fellow of the Belgian American Educational Foundation.

(1a) For general treatment and bibliography see L. Michaelis and S. Granick, THIS JOURNAL, **67**, 1212 (1945).

(2) L. Lison, "Histochimie animale," Gauthier-Villars, Paris, 1936.

(3) J. M. Wiame, *Compt. rend. soc. biol.*, **140**, 897 (1946).

(4) J. M. Wiame, *Bull. soc. chim. Biol.*, **28**, 552 (1946).

(5) L. T. Jones, *Ind. Eng. Chem., Anal. Ed.*, **14**, 536 (1942).

(6) The Coleman and Bell Co. sample; the ϵ_m of pure toluidin blue in alcohol is reported to be 63,000.[1a] Only 31,600 was obtained with the commercial product, owing to the presence of inert impurities. The molarities reported in this paper are based on spectrophotometric measurement.

(7) The molarities are always calculated as sodium metaphosphate.

(8) With the Beckman photoelectric spectrophotometer. Results given in ϵ_m, defined as $\log_{10} I_0/I = \epsilon_m Cd$, where C is the concentration in moles/liter, d the width of the vessels, I_0 and I the incident and transmitted light.

phate of different concentration were mixed with equal volumes of toluidin blue (0.5×10^{-4} *M*). Under these conditions no precipitate was formed. The results are given in Fig. 1. The absorption maximum shifted from 630 mμ, in water, to 530 mμ in hexametaphosphate.

Defining metachromasy as ε at 530 mμ/ε at 630 mμ, the maximum metachromasy was obtained at a concentration of hexametaphosphate[7] which was 8 times that of the dye. It decreased in very low and in high concentration (Table I). Trimetaphosphate gave a very slight metachromatic reaction which may have been due to some contaminating hexametaphosphate. Ortho-, pyro-, tri-phosphate and adenosine triphosphate gave no metachromasy. Nucleic acid and salts (potassium sulfate) inhibited metachromasy.

According to Michaelis and Granick[1a] metachromasy is due to a polymerization of the dye. This suggests the following explanation for the case of hexametaphosphate. When many molecules of dye are joined on the same molecule of hexametaphosphate they can be considered as polymerized, not directly but through the metaphosphate. When the concentration of metaphosphate is increased, the probability of molecules of dye to be joined on the same molecule of hexametaphosphate decreases and there is less metachromasy. When there is an excess of dye, the free molecules of dye not being metachromatic, metachromasy also decreases. That the maximum metachromasy is not obtained for equimolarity may be explained by the dissociation of the toluidin blue–hexametaphosphate compound.

TABLE I

Toluidin blue 0.25×10^{-4} *M* in (*p*H between 6.5 and 7)	Metachromasy ε 530 mμ / ε 630 mμ
Water	0.23
2×10^{-5} *M* hexametaphosphate	.46
4×10^{-5} *M* hexametaphosphate	1.24
1×10^{-4} *M* hexametaphosphate	3.52
2×10^{-4} *M* hexametaphosphate	3.59
4×10^{-4} *M* hexametaphosphate	3.31
8×10^{-4} *M* hexametaphosphate	2.97
4×10^{-3} *M* hexametaphosphate	2.17
2×10^{-2} *M* hexametaphosphate	1.16
5×10^{-2} *M* hexametaphosphate	0.73
1×10^{-1} *M* hexametaphosphate	.50
2×10^{-4} *M* hexa. + 1.25×10^{-4} *M* K_2SO_4	2.00
2×10^{-4} *M* hexa. + 1.25×10^{-3} *M* K_2SO_4	1.56
2×10^{-4} *M* hexa. + 1.25×10^{-2} *M* K_2SO_4	0.84
2×10^{-4} *M* hexa. + 1.25×10^{-1} *M* K_2SO_4	.26
2.5×10^{-5} *M* trimetaphosphate	.22
2.5×10^{-4} *M* trimetaphosphate	.26
2.5×10^{-3} *M* trimetaphosphate	.31
0.5×10^{-3} *M* orthophosphate	.21
$.5 \times 10^{-3}$ *M* pyrophosphate	.21
$.5 \times 10^{-3}$ *M* triphosphate	.24
$.5 \times 10^{-4}$ *M* triphosphate	.23
$.5 \times 10^{-5}$ *M* adenosine triphosphate	.22

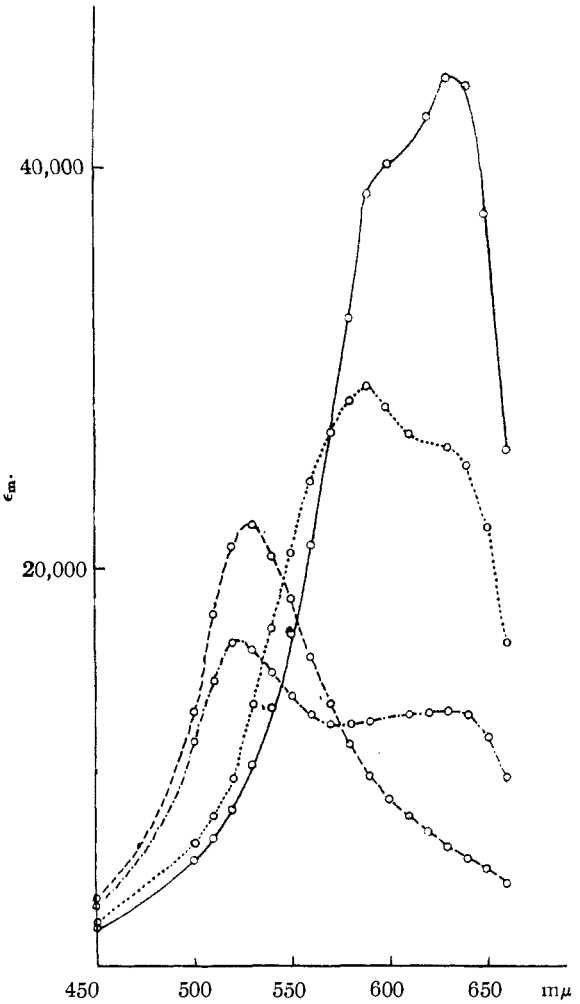

Fig. 1.—Toluidin blue 0.25×10^{-4} *M*: ——, in water; — · — · —, 0.4 × 10^{-4} hexametph.; — — — —, 2×10^{-4} hexametph.; ----------, 1×10^{-1} hexametph.

As shown by the data, the metachromatic reaction in this series of phosphoric acid compounds is specific and very sensitive for hexametaphosphate.

DEPARTMENT OF BIOLOGICAL CHEMISTRY
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NEW COMPOUNDS

4-Nitro-6,9-dichloroacridine[1]

5-Chloro-2′-nitrodiphenylamine-2-carboxylic Acid.—The Ullmann[2] method of diphenylamine synthesis was

(1) The work described herein was carried out under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Illinois.

(2) Ullmann, *Ber.*, **36**, 2383 (1903).