

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 streptolisin. Streptolisin 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. Streptolisin, 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 streptolisin also produces a second antibiotic identical with streptothricin.

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### A Novel Replacement of Alkyl Groups by Chlorine

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While the cleavage or replacement of functional groups by chlorine during chlorinations with gaseous chlorine has been frequently observed<sup>1,2,3</sup> no similar replacements are reported for chlorinations with sulfonyl chloride. Silberrad<sup>4</sup> has correctly reported that in the chlorination of toluene by sulfonyl 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 sulfonyl 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.<sup>5</sup> 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.<sup>6</sup>

(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.

Both iron powder and sulfur monochloride are essential catalysts for the reaction and no replacement was obtained with either sulfonyl chloride and iron alone or sulfonyl chloride and sulfur monochloride alone. Anhydrous ferric chloride and sulfonyl 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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### The Metachromatic Reaction of Hexametaphosphate

By J. M. WIAME<sup>1</sup>

Metachromasy denotes the property of certain dyes to undergo changes in their absorption spectrum under certain conditions other than changes in pH or oxido-reduction potential.<sup>1a</sup> 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<sup>2</sup> found that sulfuric esters of polymeric carbohydrates were responsible for the metachromatic staining of various biological materials. Recently it was shown<sup>3</sup> 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.<sup>4</sup> This finding led to a study of the metachromatic reaction of metaphosphate in solution. Sodium trimetaphosphate and sodium hexametaphosphate prepared according to Jones<sup>5</sup> were used. The dye used was toluidin blue.<sup>6</sup>

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)<sup>7</sup> was mixed with a dilute solution of dye ( $10^{-4}$  M) a purple color appeared. This color was studied spectroscopically.<sup>8</sup> 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, "Histochemie 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  $\epsilon_m$  of pure toluidin blue in alcohol is reported to be 63,000.<sup>1a</sup> 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  $\epsilon_m$ , defined as  $\log_{10} I_0/I = \epsilon_m C d$ , where  $C$  is the concentration in moles/liter,  $d$  the width of the vessels,  $I_0$  and  $I$  the incident and transmitted light.