The investigation discussed here was undertaken by the Forest Products Laboratory to obtain a more complete knowledge of the pyrolysis of lignin from wood, specifically from Douglas fir by the Madison wood-sugar process. Elucidation of the chemistry of Douglas fir lignin is highly pertinent because great quantities may remain from saccharification. The literature reports no systematic destructive distillation of lignin from any species isolated by this method.

For comparison with previous work and to lay groundwork for the ramification of lignin pyrolysis investigations, six batches of Madison wood-sugar lignin totaling 199.2 pounds were distilled at atmospheric pressure at a final retort temperature of 375 to 392°.[4] The yield of charcoal (55 to 66%) decreased with increasing amounts of residual cellulose, and decreased an extra amount in each of the three runs where the lignin had been washed free of the approximately 1.3% sulfuric acid remaining in it.

The yield of aqueous distillate (13 to 21%) and settled tar (5.2 to 8.9%) increased with increasing amounts of residual cellulose, and increased an extra amount in the distillations of acid-free lignin. Conventional methods show the presence in the aqueous distillate of 0.3 to 0.5% methyl alcohol, 0.15 to 0.25% acetone, 0.14% formic acid, and 0.2 to 0.4% acetic acid on the basis of dry acid-free lignin. There is reason to believe, however, that these methods give only approximations.

Since some of the settled tar appeared to be crystalline, the effect of various solvents was observed. It was found that 82% of the tar, including the solid material, was extractable with petroleum ether. Evaporation of the solvent, cooling and filtration led to recovery of some white crystalline material (about 3.5% of the settled tar), which appears to be a mixture of long chain aliphatic acids in the C_{18}-C_{22} range. The extracted oil was then separated into phenolic (35.5% of the settled tar), neutral (32%) and acid fractions (7 to 8%). The residual tar was also separated into various fractions, including a basic mixture.

Each of the foregoing mixtures was further separated by steam-distillation. Thus far the presence of the following phenols has been demonstrated in the steam-volatile fraction after vacuum distillation through a 4-foot Fenske-type column: phenol, *o*-cresol, *p*-cresol, guaiacol, 2,4-xylenol, 4-methylguaiacol, and 4-ethylguaiacol. The 4-methylguaiacol was identified as its aryloxyacetic acid, m. p. 117–118.5°; known compound, m. p. 117–118°; mixed m. p. 117.5–118.5°. This agrees with J. H. Fletcher and Tarbell,[5] m. p. 115–116°. Bridger[2] reported the melting point of this aryloxyacetic acid as 86°, but reported no mixed melting point.

Further work is being done on the phenols, and the other fractions are being investigated.

(4) The distillations were carried out by E. Beglinger and L. D. Pennington.

(5) THIS JOURNAL, **65**, 1431 (1943).

U. S. FOREST PRODUCTS LABORATORY
MADISON 5, WISCONSIN RECEIVED JUNE 19, 1947

Evidence for the Presence of Streptothricin in Streptolin Culture Filtrates

BY D. H. PETERSON, D. R. COLINGSWORTH, L. M. REINEKE AND C. DEBOER

An antibiotic fraction similar to streptothricin has been isolated from a culture filtrate of a *Streptomyces sp.*[1] which produces streptolin.[2] The culture was grown according to the procedure described by Rivett and Peterson.[2] A high potency streptolin (Fraction A) was prepared by the streptomycin procedure of Vander Brook, *et al.*,[3] which involved adsorption of the active material on 1% Darco G-60 at *p*H 6.5–7.0. However, in order to prepare the streptothricin antibiotic (Fraction B) relatively free of streptolin, it was first necessary to remove the streptolin by adsorption on 2% Darco G-60 at *p*H 6.5–7.0. This step also removed some of fraction B from the culture filtrate. The *p*H of the filtrate was adjusted to 8.0 and the remainder of the second antibiotic (Fraction B) adsorbed on 1% Darco G-60. The steps which followed were essentially those for the preparation of streptomycin,[3] *viz.*, the carbon was eluted with dilute acetone at *p*H 2.5, the acetone concentration of the eluate increased to 75% to precipitate the active fraction, the precipitate leached with water, the *p*H adjusted to 7.2 and the aqueous solution lyophilized to give a powder. Two hundred and sixty liters of culture filtrate yielded 4.8 g. of crude Fraction B assaying 95 units per mg. (see footnote *a*, Table I). Aluminum oxide chromatography increased the activity to 325 units per mg.

TABLE I

	Streptothricin-like (Fraction B)	Streptothricin	Streptolin (Fraction A)	Streptomycin
Agar plate assay, units per mg.[a]	325	437	24	400
Broth Dilution Activities[b]				
B. subtilis (Illinois)	8700	10,600	113,000	3500
E. coli (ATCC 26)	1650	1,480	5,200	760
S. marcescens (ATCC 60)	2800	2,600	8,000	7100
S. albus (ATCC 151)	3000	2,600	57,000	2250
S. aureus (FDA 209)	6450	10,100	42,000	7700
B. cereus (ATCC 9139)	62	82	1,200	2100
P. vulgaris (ATCC 8427)	4750	7,500	20,000	1900
S. schottmuelleri (ATCC 9149)	2800	3,400	9,900	490
PCI-3[c]	1100	1,060	2,000	10
T. R.-160[d]	45	41	3,800	210
Toxicity intravenously, LD-50 per 20 g. mouse				
Mg./mouse	4	7.5	0.11	9
Units/mouse	1300	3,280	2.6	3600

[a] Agar plate method of Loo, Skell, Thornberry, Ehrlich, McGuire, Savage and Sylvester, *J. Bact.*, **50**, 701 (1945). The unit employed is based on the activity of 1 microgram of streptomycin. [b] Figures represent highest dilutions of 1 mg. which inhibited test organisms in peptone yeast extract medium at *p*H 7.25. [c] Culture obtained from F.D.A. [d] *B. subtilis* (Illinois) made resistant to streptothricin.

The results in Table I indicate that Fraction B is similar to streptothricin but differs from streptolin and streptomycin. The inhibition of growth of the ten bacteria in broth medium by Fraction B is practically identical with that shown by streptothricin, but differs from the inhibition by streptolin and streptomycin. Additional spectra studies

(1) The Streptomyces culture was received from Prof. W. H. Peterson of the University of Wisconsin.

(2) Rivett and Peterson, THIS JOURNAL, **69**, 3006 (1947).

(3) Vander Brook, Wick, DeVries, Harris and Cartland, *J. Biol. Chem.*, **165**, 643 (1946).

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
THE UPJOHN COMPANY
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
RESEARCH LABORATORIES OF THE
SPRAGUE ELECTRIC COMPANY
NORTH ADAMS, MASSACHUSETTS RECEIVED JULY 30, 1947

(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.