strated by isolation and synthesis by alternative routes the nature of the coupling products in several instances and established the structural requirements for the occurrence of the reaction. Polonovski and Pesson have usefully extended these observations, but have failed to mention the considerable use to which the reaction already has been put in synthetic work.[4,5,6,7,8]

Experimental

2-(*p*-Chlorophenylthiol)-4-hydroxypyrimidine.—Five grams of *p*-chloroaniline in 100 ml. of *N* hydrochloric acid was diazotized with 3.0 g. of sodium nitrite at 0°. Seven and one-half grams of sodium bicarbonate was added to the solution and then the whole was added rapidly with stirring to a solution of 5 g. of 2-thiol-4-hydroxypyrimidine in 4 l. of water. When the addition of the diazonium solution was complete, 10 ml. of a 15% solution of sodium carbonate was added. The solution turned pinkish in color, some gas was evolved and a solid began to form. After standing two hours the solution was neutralized with acetic acid and the solid filtered off. The pinkish powder (5.4 g.), after recrystallization from alcohol containing a little pyridine gave colorless octahedra, m. p. 243–245°.

Anal. Calcd. for $C_{10}H_7ON_2SCl$: C, 50.4; H, 3.0; N, 11.7. Found: C, 50.4; H, 2.9; N, 11.5.

2-(*p*-Chlorophenylthio)-4-hydroxy-6-methylpyrimidine.—To a solution of 5 g. of 2-thiol-4-hydroxy-6-methylpyrimidine in 3 l. of water was added a diazonium solution prepared from 8.9 g. *p*-chloroaniline (2 moles), followed by 16.6 ml. of a 15% solution of sodium carbonate. After standing for two hours, the solution was neutralized with acetic acid and the reddish solid was filtered off (8.3 g.). After washing with warm ethanol to remove some red material the substance was recrystallized from boiling ethanol. It formed colorless rectangular prisms, m. p. 223.5°.

Anal. Calcd. for $C_{11}H_9ON_2SCl$: N, 11.1. Found: N, 11.3.

Hydrolysis of *p*-Chlorophenylthioethers with Hydrochloric Acid.—One gram of each of the above compounds was refluxed with 25 ml. of 6 *N* hydrochloric acid for three hours. Steam distillation gave *p*-chlorothiophenol in about 75% yield, m. p. 53–54°; benzoyl derivative, m. p. 74–74.5°.[9] The acid solution on evaporation gave uracil or 6-methyluracil, according to the starting material, in about 80% yield. The compounds were identified by melting points, ultraviolet absorption spectra and analyses.

Anal. Calcd. for $C_4H_4N_2O_2$: C, 42.8; H, 3.6. Found: C, 42.5; H, 3.6. Calcd. for $C_5H_6N_2O_2$: C, 47.7; H, 4.8. Found: C, 47.6; H, 4.4.

The authors are indebted to Samuel W. Blackman for the microanalyses reported here.

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The Surface Area of Vermiculite

BY B. L. HARRIS

The surface area of vermiculite before and after exfoliation was measured by adsorption of nitrogen at −195° by the method of Brunauer, Emmett and Teller[1] in order to ascertain whether the area was large enough that the material might be used as a catalyst support. The area of the unexfoliated sample was measured by nitrogen adsorption and found to be 0.52 sq. m./g., giving a calculated average thickness of the platelets of 1.8 microns when the theoretical density of 2.13 is assumed. This indicates that the material was thoroughly fissured before exfoliation.

A sample of the vermiculite was exfoliated by heating for five minutes at 950°, resulting in a silvery-white accordion-like structure. The loss in weight on exfoliation was 19.6%. The surface area of this sample was 10.35 sq. m./g., giving a calculated platelet thickness of 0.091 micron. The structure of vermiculite consists of sheets of $(OH)_4Mg_6(Si, Al)_8O_{20}$ of 9.26 Å. thickness with alternate layers of 8 H_2O spaced the order of 4.8 Å.[2] It was hoped that the platelet spacing might approximate this order of magnitude, resulting in a very large surface area. The smaller area, corresponding to a thickness some 200 times as great, agrees with the conclusion of Gruner that the structure collapses above 750° and that exfoliation is mechanical, due to the formation of steam.

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DEPARTMENT OF CHEMICAL ENGINEERING
JOHNS HOPKINS UNIVERSITY
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The Interaction of Purified Antibody with Homologous Hapten. Antibody Valence and Binding Constant

BY HERMAN N. EISEN[1] AND FRED KARUSH[2]

As part of a study of the relation between protein structure and specificity we are investigating the properties of soluble complexes formed between purified antibody and homologous haptens. Rabbits were immunized with sheep serum coupled with diazotized *p*-arsanilic acid. Purified antibody, homologous to *p*-azophenylarsonic acid (R), was obtained[3] by treating pooled antisera with human erythrocyte stromata coupled with diazotized *p*-arsanilic acid (R-stromata). After several washings with 0.16 *M* sodium chloride, the antibody was eluted from R-stromata by acidification (*p*H 3.8) with acetic acid. The R-stromata was removed by centrifugation and the supernate, which contained the antibody, was neutralized. In the antibody solutions thus prepared at least 90% of the protein was specifically precipitable with R-stromata, in agreement

(1) Research Fellow, National Institute of Health.
(2) Investigation conducted during tenure of a Fellowship in Cancer Research of the American Cancer Society, recommended by the Committee on Growth of the National Research Council.
(3) K. Landsteiner and J. van der Scheer, *J. Exp. Med.*, **63**, 325 (1936).

with the observations of Campbell, *et al.*,[4] who also prepared purified antibody by exposure to acid *p*H (3.5). Controls involving the treatment with R-stromata of normal rabbit serum, a mixture of bovine γ-globulin and purified antibody, and bovine γ-globulin alone, showed no appreciable non-specific precipitation. Protein concentrations were determined by ultraviolet absorption[5]; the validity of this procedure was assured by low R-stromata blanks, and by the characteristic shape of the absorption curves.

The binding of homologous haptenic dye *p*-(*p*-hydroxyphenylazo)-phenylarsonic acid by purified antibody was determined by equilibrium dialysis. One ml. aliquots of an antibody solution of known protein concentration (0.15%) containing buffer and salt (*p*H 7.4, 0.01 *M* PO_4, 0.16 *M* NaCl) were placed inside 0.25-in. dialysis bags. These were equilibrated against equal volumes of hapten in buffered saline whose initial dye concentrations were accurately known. The free dye concentrations in equilibrium with the bound dye were determined by measuring the spectral absorption, at 440 mμ, of the outside solutions, after suitable dilution and adjustment to strongly alkaline *p*H. The concentrations of bound dye were calculated from the values for the free equilibrium and initial dye concentrations, the former ranging from 5×10^{-6} *M* to 8×10^{-5} *M*. This calculation required a correction, due to adsorption of dye on the dialysis bag, amounting to 11% of the free dye concentration. The following (which corresponds to the third point in Fig. 1) represents a typical calculation: initial dye concentration = 3.96×10^{-5} *M*, free equilibrium dye concentration = 1.18×10^{-5} *M*, concentration of bound dye = 1.35×10^{-5} *M*, moles hapten bound per mole protein[6] (r) = 1.45.

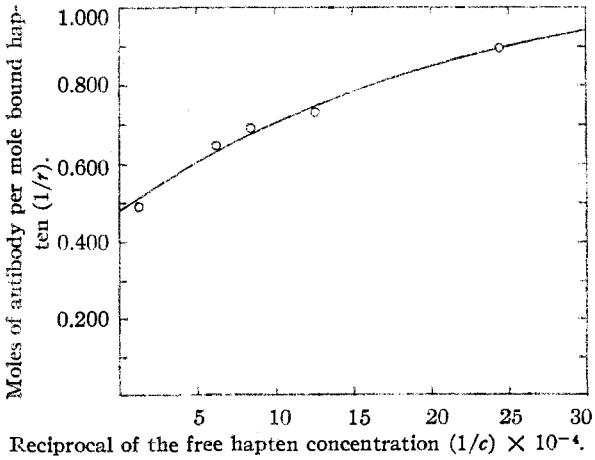

Fig. 1.

By plotting the reciprocal of moles hapten bound per mole antibody ($1/r$) against the reciprocal of free hapten concentration ($1/c$) the binding capacity is obtained from the extrapolated value of $1/r$.[7] As could be anticipated from the known heterogeneity of antibodies,[3,8] the curve obtained was not linear. In such a situation the average intrinsic association constant can be shown to be equal to the value of $1/c$ at which one-half the binding sites are occupied, if it is assumed that the variation in the free energy of binding among the various sites can be described by a Gaussian distribution function.[9] Such an assumption has been made previously by Pauling, *et al.*,[8] to describe hapten inhibition data.

The binding data obtained at room temperature (29°) with the hapten indicated above are summarized in Fig. 1. Extrapolation yields a value of 2 for the binding capacity ("valence") of the antibody, within an accuracy of 10%. The value of the intrinsic association constant is 3.5×10^5, corresponding to $\Delta F^0 = -7.7$ kcal. per mole hapten.

Antisera prepared as in these experiments by Campbell, *et al.*,[4] contained about 1.0% antibody for R-group, whereas our yields of purified antibody correspond to an initial antibody concentration in antiserum of about 0.04%. Either our antisera were far less potent than Campbell's, or the antibody removed by our purification procedure comprised only a small fraction of the total antibody present in the antiserum, in which case the above association constant would measure the average binding energy of only the most reactive antibody molecules.

Acknowledgment.—We are indebted to Professor R. Keith Cannan for the laboratory facilities so generously made available to us during the conduct of this investigation.

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DEPARTMENTS OF CHEMISTRY AND MEDICINE
NEW YORK UNIVERSITY COLLEGE OF MEDICINE
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Preparation and Properties of Several Cyclohexyl-alkyl-substituted Ketene Dimers

BY CARL M. HILL AND GILBERT W. SENTER

It has been demonstrated that tertiary aliphatic amines dehydrohalogenate acid halides with the formation of ketene monomers and dimers.[1] Sauer[2] has reported the dehydrohalogenation of several fatty acid halides by tertiary aliphatic amines to yield ketene dimers.

This paper describes the dehydrohalogenation of five omega-cyclohexyl-substituted acid chlorides (of type $C_6H_{11}\cdot(CH_2)_n\cdot COCl$) by triethylamine.

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