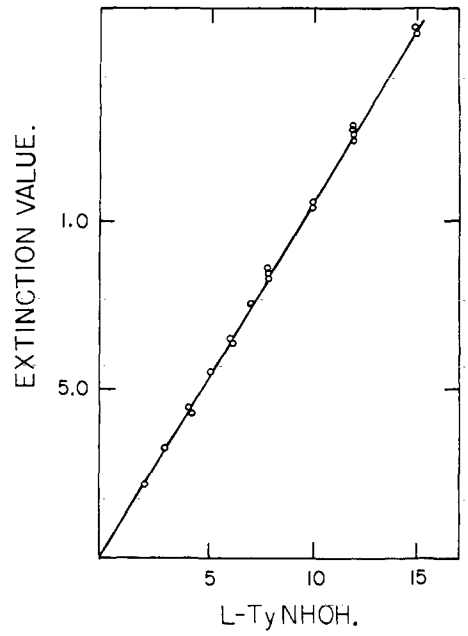

Fig. 6.—Extinction values for a 1-cm. path at 505 mμ. *vs.* concentration of L-tyrosinhydroxamide after reaction of the latter substance with the ferric chloride–hydrochloric acid reagent and subsequent dilution to 10 ml.; concentration of L-tyrosinhydroxamide in units of 10^{-3} *M* as present in the original reaction mixture.

At selected time intervals a 1.0-ml. aliquot of the reaction mixture was added to the contents of one of the above flasks, the solution made up to volume with methanol, and the optical density of the resulting solution, for a path of 1 cm. and at 505 mμ, determined in a model B Beckman spectrophotometer. A solution containing all of the components except the L-tyrosinhydroxamide, *i.e.*, the specific substrate, was used to zero the instrument. It will be seen from Fig. 6 that the dependence of the optical density upon the concentration of L-tyrosinhydroxamide was linear over the range of concentrations ordinarily used. When concentrations of L-tyrosinhydroxamide were used which were higher than those indicated on the abscissa of the plot given in Fig. 6, 2.5 ml. or 5.0-ml. aliquots of the stock solution were introduced into 25- or 50-ml. flasks and diluted to the appropriate volume after the addition of 1.0 ml. of the reaction mixture.

Enzyme Experiments.—The reaction mixtures used for the determination of the *p*H–activity relationship were either 0.1 *M* with respect to the amine component of a tris-(hydroxymethyl)-aminomethane–hydrochloric acid buffer or 0.1 *M* with respect to arsenic present as cacodylic acid in a cacodylic acid–sodium cacodylate buffer. In all of the kinetic studies the reaction mixtures were 0.2 *M* with respect to the amine component of a tris-(hydroxymethyl)-aminomethane–hydrochloric acid buffer and possessed a *p*H of 6.9 ± 0.05 at 25 ± 0.1°, the temperature at which all measurements were made. The α-chymotrypsin employed was an Armour preparation, lot no. 90402, and the enzyme concentration in all experiments was equivalent to 0.104 mg. protein-nitrogen/ml. In view of the value of K_S that has been computed for the system in question there can be no doubt that with the above enzyme concentration zone A conditions[23] have been satisfied. The values of the constants K_S and k_3 were obtained from the primary experimental data as described previously.[12]

PASADENA 4, CALIFORNIA

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF KANSAS SCHOOL OF MEDICINE]

Synthesis of Chloropyrimidines by Reaction with N-Chlorosuccinimide, and by Condensation Methods[1]

BY ROBERT A. WEST AND HAROLD W. BARRETT

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In glacial acetic acid, N-chlorosuccinimide can be used for nuclear substitution of uracil, thymine and derivatives of 2-thiouracil, and in chloroform plus benzoyl peroxide this same reagent can be used for substitution of an allylic methyl side chain. Theoretical considerations indicate that electrophilic attack should occur preponderantly at position 5 of the nucleus in 2-methylthiouracil and substances of this type. Two of the 5-chloro derivatives were obtained also by the base-catalyzed condensation of α-chloro-β-ketoesters with methylisothiourea sulfate.

Although the 5-iodo and 5-bromo derivatives of uracil and of the 2-alkylthio analogs are readily prepared,[2,3] considerable difficulties have been encountered in preparation of the 5-chloro derivatives. Chlorination of uracil gives a mixture of 5-chloro- and 5,5-dichloro-6-hydroxyuracil in water[4] and a small yield of 5-chlorouracil in glacial acetic acid.[3] In the latter solvent, 2-methylthiouracil also gives a small amount of the 5-chloro derivative, but the chief product is a salt which decomposes in the presence of moisture to liberate methyl mercaptan.[3,4] In order to obtain derivatives of this type in sufficient quantities for physiological testing, we have investigated the behavior of N-chlorosuccinimide (NCS) whose use was suggested by the unusual nuclear, as well as allylic, substitutions produced by the analogous N-bromosuccinimide.[4-8] N-Chloroacetamide and dichloramine-T were tested also, but were found to be ineffective for the chlorination of pyrimidines.

It was found that attack by NCS could be directed at will to the C_5 nuclear position, or to a methyl group in the 5- or 6-position, depending on the reaction conditions. Thus, when 2,6-dimethylthiouracil was treated with NCS in glacial acetic acid, nuclear attack occurred to produce the 5-chloro derivative. When the same pyrimidine was dissolved in chloroform containing benzoyl peroxide, it reacted with NCS to give 6-chloromethyl-

(1) This work was supported in part by a grant G-3195 from the National Institutes of Health, Public Health Service, and in part by a grant from the General Research Fund of the University of Kansas.

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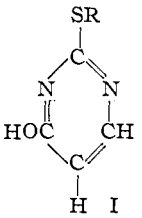
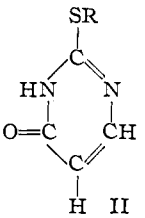
2-methylthiouracil. Thymine, with the C_5 nuclear position unavailable, failed to react in glacial acetic acid, but gave 5-chloromethyluracil in chloroform containing benzoyl peroxide. Uracil, on the other hand, failed to react in chloroform, but gave 5-chlorouracil in glacial acetic acid. Although the presence of benzoyl peroxide was essential for substitution of the allylic methyl groups, nuclear catalysts (aluminum chloride, etc.) had no measurable effect on the ring substitutions. Other solvents were tried for these reactions, but the two used proved most suitable. Thiouracil and other pyrimidines with unsubstituted sulfur could not be chlorinated with NCS.

Failure of NCS to produce certain allylic substitutions has led to the view that it is ineffective for this general reaction,[9,10] but our results with the pyrimidines show that this view must be modified. N-Bromosuccinimide also has proved ineffective for certain allylic substitutions—2-methyl-5-ethoxythiazole[7] and 6-methyl-2-pyridone[8]—even when classical free-radical conditions were used. Electrophilic attack on the pyrimidine ring, as produced here by NCS in glacial acetic acid, invariably results in C_5-substitution when this position is available. This result would be predicted by application of resonance theory. The pyrimidines used in this work exist in either two or three principal tautomeric forms depending on the C_2-substituent. For 2-methylthiouracil, the two principal forms are the Kekulé structure I, and the 4-keto structure II. From these tautomers, there may be derived six

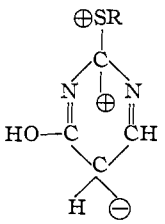


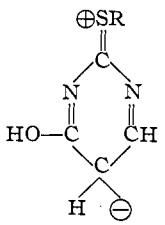
I

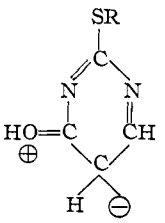


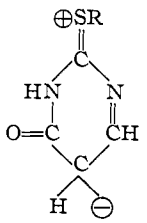
II

resonance hybrids with single-charge separation, and showing a negative charge at C_5. The following hybrids are chosen as most contributory.









Two further single-charge-separated hybrids showing a positive charge at C_6 may be represented, but none showing a negative charge at C_6, or a positive charge at C_5, unless one postulates a simple heterolytic opening of the 5:6 double bond in this manner. Moreover in the transition state hybrids, the positive charge resulting from attack of Cl at C_6 is not diffusible among the remaining carbons, while such attack at C_5 allows diffusion of the positive charge among both the remaining ring carbons and the hetero atoms. Therefore, with both positions available, electrophilic attack should take place at C_5 and nucleophilic attack at C_6. The actual reactions of pyrimidines of this type, so far as they have been reported, are entirely in accord with these predictions.

As a further method of obtaining 5-chloropyrimidines, we investigated the base-catalyzed condensation of α-chloro-β-ketoesters with methylisothiourea sulfate. Although this method has been the classical procedure for obtaining other derivatives of 2-thiouracil, it evidently has not been considered as a route to the 5-halogeno derivatives. An apparent danger in this procedure is loss of chlorine, either in formation of the sodium enolate of the required ester, or in the subsequent condensation in aqueous base to form the pyrimidine. We found, however, that loss of chlorine is minimized if formation of the sodium enolate is carried out in anhydrous ether with the temperature maintained below 20°. In the resulting enolate, the chlorine is sufficiently stabilized by virtue of its vinyl position that it shows little or no tendency to undergo a Wurtz reaction, or to suffer nucleophilic displacement in cold alkaline media. Two of the 5-chloro derivatives were obtained by this procedure, but those pyrimidines with an allylic chlorine atom could not be prepared by condensation methods. Although better yields of the 5-chloro derivatives were produced more conveniently by the NCS method, it was of considerable interest to show that they could be obtained also by condensation procedures, as this method of synthesis provides final proof for the position of the 5-chloro substituent.

Experimental

Reactions with NCS. General Procedures.—For nuclear substitution, the pyrimidine was dissolved in glacial acetic acid containing 2% acetic anhydride, and heated to 80° for a few minutes to remove any moisture. A 25% molar excess of NCS was added to the solution at 50–55° and the solution held at this temperature until only a weak test for NCS was obtained with starch–iodide paper. The solution was then poured into ice-water to precipitate the product. Since NCS forms hypochlorous acid in water, a small amount of phenol or sodium bisulfite was dissolved in the water to prevent oxidative attack on those compounds containing sulfur. For the side chain chlorination, the pyrimidine was dissolved in anhydrous chloroform containing a 15% molar amount of benzoyl peroxide, and treated with a 10% molar excess of NCS. The solution was maintained at reflux temperature, and completion of the reaction again judged by the starch–iodide test. The product was filtered off after partial evaporation of the solvent. All compounds were recrystallized first from alcohol, then from water, with the exception of 6-chloromethyl-2-methylthiouracil which was recrystallized only from alcohol. Melting points were determined on a Fisher block. The derivatives obtained by these procedures are listed in the table along with their constants.

As indicated by the analysis of 6-chloromethyl-2-methylthiouracil, we were unable to obtain a completely chlorinated derivative from 2,6-dimethylthiouracil. Recrystallization of the product from water resulted in decreased chlo-

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TABLE I

	Yield, %	M.p., °C.	Formula	Analyses, % Chlorine Calcd.	Chlorine Found	Nitrogen Calcd.	Nitrogen Found
5-Chlorouracil	52	324–325 dec.	$C_4H_3O_2N_2Cl$	24.1	24.2	21.46	21.33
5-Chloromethyluracil	50	222–224	$C_5H_5O_2N_2Cl$	22.08	21.9	19.38	19.47
5-Chloro-2-methylthiouracil	63	259–260	$C_5H_5N_2OSCl$	20.08	19.90	15.86	15.94
5-Chloro-2,6-dimethylthiouracil	64	270 dec.	$C_6H_7N_2OSCl$	18.85	18.34	14.69	14.77
6-Chloromethyl-2-methylthiouracil	30	230–235	$C_6H_7N_2OSCl$	18.85	16.65	14.69	16.22

rine content. As the original substitution was calculated to be about 90% of the theoretical, this product was considered suitable for biological testing, and for use as an intermediate. The position of the chlorine was shown by hydrolyzing a sample of the derivative with concentrated HCl for four hours at 100°, to give about an 80% yield of 6-chloromethyluracil, m.p. 207–209°. This compound has been described by Johnson and Chernoff[11] as 4-chloromethyluracil according to the older system of ring numbering. A sample of 5-chloro-2-methylthiouracil was treated in the same way to give an 88% yield of 5-chlorouracil.

The position of the chlorine in 5-chloromethyluracil was shown by heating a small sample (0.2 g.) with silver carbonate in water. The distillate from this solution gave a strong test for formaldehyde (Tollens test), which is produced when thyminyl alcohol (5-hydroxymethyluracil) is boiled in water.[12] A small amount of thyminyl alcohol, m.p. 195–200°,[12] was isolated from the solution. The infrared spectrum of this chloro derivative[13] showed complete obliteration of the band assigned to the stretching vibration of the 4-keto group at 1750 cm.$^{-1}$.[14] This result has been tentatively ascribed to formation of a hydrogen bond of the –Cl···H–N– type, thus stabilizing the molecule in the 4-enol form.

Condensation Methods. 5-Chloro-2-methylthiouracil.—The sodium enolate of ethyl formylchloroacetate was prepared by adding dropwise a mixture of 73 g. (0.60 mole) of ethyl chloroacetate and 46 g. (0.62 mole) of ethyl formate to 300 ml. of anhydrous ether containing 14 g. of sodium chips. Complete addition of the esters required 7 hours, during which time the mixture was slowly stirred, and the temperature maintained below 20° by an ice-bath. The reaction mass was allowed to stand overnight and a few ml. of ethanol was added to discharge any sodium remaining, then allowed to air dry on a porous plate. The slightly yellow product weighed 90 g. and was assumed to be about 70% pure. Twenty-three grams of this enolate and 27.8 g. (0.1 mole) of finely ground methylisothiourea sulfate were dissolved in 125 ml. of water, and the solution kept basic (*p*H 10–12) for a period of 24 hours by adding sodium hydroxide as required. At the end of this time, crystals began to deposit. The mixture was then heated to 60° for a half hour to complete the reaction, then chilled in ice-water and acidified with glacial acetic acid. The precipitate was filtered off, and extracted with 15 ml. of hot water to remove any unreacted material. The remaining precipitate was dissolved in hot alcohol and treated with charcoal; the filtrate yielded 6.7 g. (38%) of 5-chloro-2-methylthiouracil identical with the product obtained in the NCS method for nuclear chlorination.

5-Chloro-2,6-dimethylthiouracil.—The intermediate chloro ester was formed from dry acetoacetic ester and sulfuryl chloride essentially as described by Dey[15] and the sodium enolate formed by dropping the ester into dry ether containing sodium chips, and proceeding as previously described. The pyrimidine was obtained as before from 10 g. of the enolate and 16.7 g. (0.06 mole) of methylisothiourea sulfate in 80 ml. of water. A yield of 1.8 g. (32%) of 5-chloro-2,6-dimethylthiouracil was isolated which proved to be identical with the product obtained by the NCS method.

These condensations were also carried out in absolute ethanol, but the yields were reduced. Thiourea itself could not be condensed with the sodium enolates of these chloro esters to give any appreciable yields.

Acknowledgments.—We wish to express our gratitude to the Arapahoe Chemical Company, of Boulder, Colorado, for supplying the NCS used in this work, and to Mrs. Marie Evans for the chlorine analyses reported here.

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(12) T. B. Johnson and A. Litzinger, *ibid.*, **58**, 1940 (1936).

(13) Obtained through the courtesy of Mr. S. H. Wilen of this University, Department of Chemistry.

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