added to the residue, and the solid collected; wt. 5.9 g. (73%), m.p. 270–271° dec. The product crystallized from ethanol as colorless prisms; m.p. 279–280° dec., λ_{max} 268 mμ (E × 10^{-3} = 9.98).

Anal. Calcd. for $C_4H_3IN_2O_2$: C, 20.2; H, 1.3; N, 11.8; I, 53.3. Found: C, 20.7; H, 1.5; N, 12.4; I, 53.1.

DETROIT 1, MICH.

[CONTRIBUTION FROM THE BIOLOGICAL SCIENCES DIVISION, STANFORD RESEARCH INSTITUTE]

Potential Anticancer Agents.[1] LX. Synthesis of 5-Diazoacetyluracil and Related Compounds

LEONARD O. ROSS, EDWARD M. ACTON, W. A. SKINNER, LEON GOODMAN, AND B. R. BAKER

Received February 15, 1961

The synthesis of 5-diazoacetyluracil (XI) from 5-acetyluracil (III) was accomplished by treatment of the oximino ketone (X) of III with chloramine. In another attempted approach to XI, 5-bromoacetyluracil (V) was prepared and was converted to a number of interesting 5-substituted uracils. A new synthesis of 6-acetyluracil (XX) was developed and unsuccessful attempts were made to convert it to 6-diazoacetyluracil.

Most of the alkylating agents that have interest as anticancer compounds possess the bis(2-chloroethyl) amino moiety as the alkylating group.[2] Azaserine (*O*-diazoacetyl-L-serine)[3] and DON (6-diazo-5-oxo-L-norleucine)[4] also have interesting antitumor properties. It has been proposed[2] that these diazoacetyl compounds and the nitrogen mustards might be considered as members of a broad class of anticancer agents which consist of metabolites bearing an alkylating group that function by irreversible inhibition of the corresponding enzymes. To test this hypothesis two diazoacetyl derivatives of 3-phenylpropionic acid were prepared[5] in order to compare their antitumor activities with those of the analogous nitrogen mustards—*e.g.*, chlorambucil.[6] This manuscript describes the preparation of 5-diazoacetyluracil (XI) in order that its anticancer activity might be compared with that of the corresponding nitrogen mustard, uracil mustard.[7]

The reaction between sodium nitrite and the salt of an α-amino ketone constitutes one of the common synthetic approaches to α-diazo ketones; this was the preparative method for 6-diazo-5-oxo-L-norleucine.[8] Accordingly, the initial sequence visualized for the preparation of XI required the prior synthesis of a salt of 5-glycyluracil (VIII). The use of Johnson and Bergmann's[9] method to cyclize ureidomethylene acetoacetic ester (II),[10] which, in turn, was prepared from ethoxymethylene acetoacetic ester (I),[10] gave a reasonable yield of 5-acetyluracil (III). Bromination of the ketone (III), suspended in methanol, proceeded readily to give 5-bromoacetyluracil (V.) That side-chain bromination to give V had occurred rather than ring bromination at C-6 was demonstrated in two ways. First, treatment of V with dimethyl sulfoxide followed by the reaction of the product with phenylhydrazine gave the osazone (VII) of the glyoxal formed by oxidation of V with dimethyl sulfoxide.[11] Secondly, the reaction of V with pyridine gave the pyridinium salt which was cleaved with aqueous base to uracil-5-carboxylic acid (IV),[12] identical with an authentic sample of IV.

The bromo ketone (V) underwent ready displacement with azide ion to give the azido ketone (VI). Hydrogenation of a suspension of VI in dilute hydrochloric acid solution using a palladium catalyst afforded the hydrochloride of VIII which was best characterized as the crystalline picrate. Alternatively, the action of an acetic acid solution of hydrogen bromide[13] on VI reduced the azide group and yielded VIII, isolated as the picrate. Hydro-

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. SA-43-ph-1892. The opinions expressed in this paper are those of the authors and are not necessarily those of the Cancer Chemotherapy National Service Center. For the preceding paper in this series, see E. J. Reist, J. H. Osiecki, A. Benitez, L. Goodman, and B. R. Baker, *J. Org. Chem.*, **26**, 0000 (1961).

(2) H. F. Gram, C. W. Mosher, and B. R. Baker, *J. Am. Chem. Soc.*, **81**, 3103 (1959) and references therein.

(3) For a summary of chemical and biological data, see Cancer Chemotherapy Reports, No. 7, 65 (1960), a publication of the Cancer Chemotherapy National Service Center, National Institutes of Health.

(4) Ref. 3, page 86.

(5) W. A. Skinner, H. F. Gram, C. W. Mosher, and B. R. Baker, *J. Am. Chem. Soc.*, **81**, 4639 (1959).

(6) J. L. Everett, J. J. Roberts, and W. C. J. Ross, *J. Chem. Soc.*, 2386 (1953).

(7) D. A. Lyttle and H. G. Petering, *J. Am. Chem. Soc.*, **80**, 6459 (1958), D. A. Lyttle and H. G. Petering, *J. Natl. Cancer Inst.*, **23**, 153 (1959).

(8) H. A. DeWald and A. M. Moore, *J. Am. Chem. Soc.*, **80**, 3941 (1958).

(9) T. B. Johnson and W. Bergmann, *Ber.*, **66B**, 1492 (1933).

(10) L. Claisen, *Ann.*, **297**, 1 (1897).

(11) N. Kornblum, J. W. Powers, G. J. Anderson, W. J. Jones, H. O. Larson, O. Levand, and W. M. Weaver, *J. Am. Chem. Soc.*, **79**, 6562 (1957).

(12) L. C. King, *J. Am. Chem. Soc.*, **66**, 894 (1944).

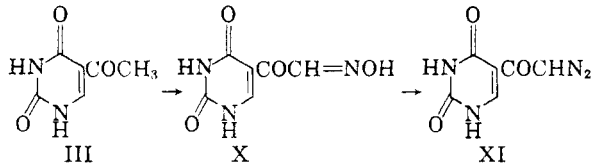
(13) P. A. S. Smith and B. B. Brown, *J. Am. Chem. Soc.*, **73**, 2438 (1951).

genation of VI suspended in acetic acid which contained acetic anhydride gave 5-(*N*-acetyl)glycyluracil (IX).

The reaction of VIII with sodium nitrite under a variety of conditions did not give 5-diazoacetyluracil (XI). When exactly equimolar amounts of the hydrochloride of VIII and sodium nitrite were allowed to react, a solid precipitate formed that was probably the free aminoketone (VIII). The infrared spectrum of the solid was well defined and showed the absence of $-NH_3^+$ absorptions. The solid could be converted to the picrate of VIII and to the hydrochloride of VIII. Attempts to neutralize aqueous solutions of the hydrochloride of VIII with sodium bicarbonate or sodium acetate gave intractable, colored materials which seemed to result from dimerization of the amino ketone (VIII).

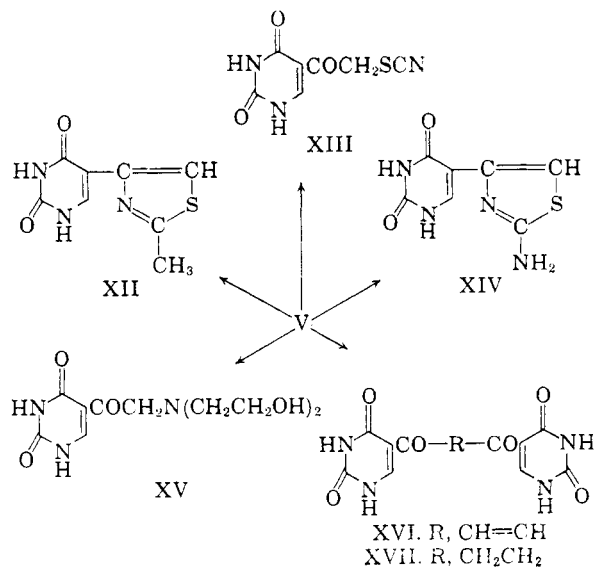
The reaction of the hydrochloride of VIII with excess sodium nitrite in aqueous solution led to the recovery of a fair yield of uracil-5-carboxylic acid (IV) as the only isolated product. Recently Baumgarten and Anderson[14] described the conversion of several α-amino ketones to the parent acids—*e.g.*, ω-aminoacetophenone to benzoic acid—and the conversion of VIII to IV represents another example of such a reaction.

Two other methods of preparation of α-diazo ketones were investigated. An attempt was made to prepare the mono-*p*-tolylsulfonylhydrazone from the reaction product of V and dimethyl sulfoxide. The decomposition with alkali of the mono-*p*-tolylsulfonylhydrazone of the aldehyde function of the uracil-5-glyoxal intermediate would be expected to yield the diazo ketone (XI).[15] The desired derivative could not be isolated, however. Alternatively, 5-acetyluracil (III) was converted in good yield to the oximino-



ketone (X) with a stoichiometric amount of butyl nitrite and hydrochloric acid in *N*,*N*-dimethylformamide.[16] The reaction of X with chloramine prepared *in situ*, as described by Cava, *et al.*[15] gave a non-homogeneous sodium salt of XI which was characterized by a strong infrared diazo band at 4.75 μ. Acidification of an aqueous solution of the salt of XI with acetic acid or with carbon dioxide yielded the insoluble diazo ketone (XI) that gave good elemental analyses. As a definitive structure proof for XI, the salt of XI was treated with aqueous hydrobromic acid to afford a fair yield of the bromo ketone (V).

In an effort to exploit the α-bromo ketone (V) as the source of a number of new, substituted uracil derivatives, the ketone (V) was treated with a variety of nucleophilic reagents. When strong nucleophiles were used, clean-cut displacement reactions were observed and, in general, good yields of the expected products were isolated. Thus, the reactions of V with thiocyanate ion, with thioacetamide, with thiourea, and with 2,2′-iminodiethanol gave the α-thiocyano ketone (XIII), the 2-methylthiazole (XII), the 2-aminothiazole (XIV), and the α-amino ketone (XV),[17] respectively. The conversion of V to the α-azido ketone (VI) represents another example of this class of reaction. However, the reaction of V with cyanide ion, acetate ion, potassium phthalimide, or hydroxide ion gave a very insoluble solid as

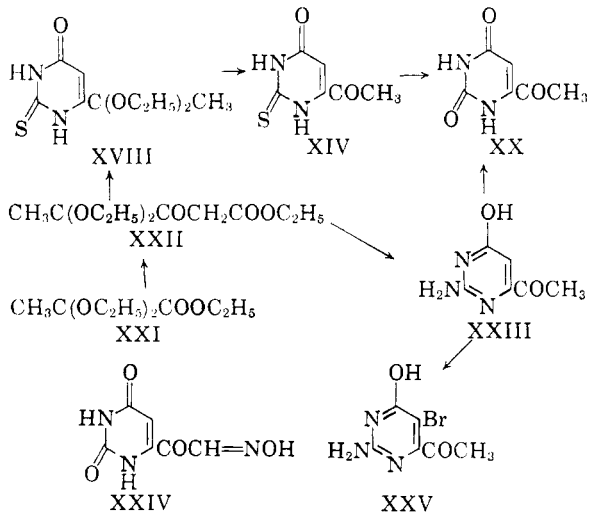


XVI, R, CH=CH
XVII, R, CH_2CH_2

(14) H. E. Baumgarten and C. H. Anderson, *Abstracts American Chemical Society 137th Meeting*, Cleveland, Ohio, p. 15-O.

the common product of these reactions. The identity of these products was shown by infrared spectral and paper chromatographic comparison. Although the material could not be adequately purified, its structure seems best represented either as the diketoethylene derivative (XVI) or the diketoethane derivative (XVII). The conversion of V to XVI would be similar to the conversion of ω-bromoacetophenone to 1,2-dibenzoylethylene by the action of basic reagents.[18,19]

The successful synthesis of 5-diazoacetyluracil (XI) was the stimulus to an investigation of the synthesis of 6-diazoacetyluracil by way of 6-acetyluracil (XX) and its derivatives. The preparation of XX was first described by Langley[20] using the reaction between 2,4-diethoxy-6-pyrimidinyllithium and acetaldehyde, followed by oxidation, then hydrolysis. The description of the method made it unlikely that this route could be utilized for the large amount of XX that was required. A synthesis of XX was devised that was patterned after that used by Johnson and Schroeder to prepare uracil-6-carboxaldehyde.[21] The diethylketal of ethyl pyruvate (XXI)[22] was condensed with ethyl



acetate with the aid of sodium hydride to afford a 63% yield of the 4-diethylketal of ethyl 3,4-dioxovalerate (XXII). Condensation of XXII with guanidine and hydrolysis of the intermediate product gave a 54% yield of the pure aminopyrimidine (XXIII). The reaction of XXIII with sodium nitrite in an aqueous mixture of sulfuric and hydrochloric acids then gave a 64% yield of 6-acetyluracil (XX).

Alternatively, the condensation of XXII and thiourea afforded the crystalline 2-thiouracil ketal (XVIII). Stepwise hydrolysis of XVIII yielded first 6-acetyl-2-thiouracil (XIX) and finally 6-acetyluracil (XX); preparatively, it was more convenient not to isolate XIX. Both XVIII and XIX were sharply melting, crystalline compounds.

All efforts to convert XX to 6-bromoacetyluracil were unsuccessful. Although discrete compounds could not be isolated, the lack of reactivity of the brominated products to nucleophilic agents strongly suggested that bromination of the ring at C-5 was the predominant reaction.

The bromination of the aminopyrimidine (XXIII) in acid solution was investigated with the hope that the protonated form of XXIII might resist substitution at C-5 by analogy with the behavior of aniline and permit the formation of an α-bromo ketone. A solid compound was isolated which had the correct analysis for a monobromo derivative of XXIII. The bromine atom, however, could not be displaced by nucleophiles such as azide ion or thiourea, and the infrared spectrum indicated the absence of the olefinic C—H absorption which was identifiable in XXIII, XVIII, XIX and XX; the solid was therefore assigned structure XXV.

The reaction of the ketone (XX) with butyl nitrite, using the conditions that were successful for the preparation of the 5-oximino ketone (X), did not yield the desired oximino ketone (XXIV). A new product resulted from the reaction as shown by paper chromatography. Its analysis was not in agreement with that of structure XXIV nor did it afford a diazo ketone by reaction with chloramine.

EXPERIMENTAL[23]

5-Acetyluracil (III) was prepared by cyclization of ureidomethylene acetoacetic ester[10] (II) in aqueous alkali using the procedure of Johnson and Bergmann.[9] The recrystallized product was obtained in 26% yield and had m.p. 293–295° dec. (lit.[9] reported 25% yield and m.p. 294° dec.); $\lambda_{max(\mu)}^{KBr}$

(15) M. P. Cava, R. L. Litle, and D. R. Napier, *J. Am. Chem. Soc.*, **80**, 2257 (1958).

(16) The use of excess butyl nitrite gave rise to a compound, $C_6H_5N_3O_5$, which was an oxidation product of X, its structure was not determined.

(17) It is possible that compound XV exists as the hemiketal since its ultraviolet spectrum at *p*H 1 differs considerably from that of 5-glycyluracil (VIII). Two analogous bis(2-hydroxyethyl)amines have been shown to exist as hemi-ketals by B. M. Mikhailov and A. N. Makarova, [*J. Gen. Chem. U. S. S. R.* (*Eng. Transl.*), **28**, 149 (1958)] and W. A. Skinner, H. F. Gram, and B. R. Baker, *J. Org. Chem.*, **25**, 953 (1960).

(18) M. S. Kharasch, W. Nudenberg, and E. K. Fields, *J. Am. Chem. Soc.*, **66**, 1276 (1944).

(19) B. M. Bogoslovskiĭ, *J. Gen. Chem.* (*U. S. S. R.*), **14**, 993 (1944).

(20) B. W. Langley, *J. Am. Chem. Soc.*, **78**, 2136 (1956).

(21) T. B. Johnson and E. F. Schroeder, *J. Am. Chem. Soc.*, **53**, 1989 (1931).

(22) C. L. Stevens and A. E. Sherr, *J. Org. Chem.*, **17**, 1228 (1952).

(23) Melting points and boiling points are uncorrected, the latter were obtained with the Fisher-Johns apparatus. Paper chromatography was done by the descending technique on Whatman No. 1 paper and the spots were detected by visual examination under ultraviolet light. Adenine was used as a standard and the spots were located relative to R_{Ad} 1.00. The solvent systems used were, A, 1-butanol-acetic acid-water (5/2/3), B, water-saturated 1-butanol, C, 2-propanol-2*N* aqueous hydrochloric acid (65/35), D, 2-methoxyethanol-water (9/1), and E, 5% disodium hydrogen phosphate (no organic phase).

3.25 (NH), 5.80–5.97 (uracil and ketone C=O), 6.20 (uracil C=C), 6.65 (uracil ring); $\lambda_{max(m\mu)}^{C_2H_5OH}$ 224 (ε 10,000) and 283 (ε 10,600). Compound III moved as a single spot on paper chromatography in solvent A with R_{Ad} 1.01.

5-Acetyluracil-p-tolylsulfonylhydrazone. A mixture of 1.0 g. (6.5 mmoles) of 5-acetyluracil (III), 1.3 g. (7.0 mmoles) of *p*-tolylsulfonylhydrazine, 30 ml. of ethanol, 10 ml. of water, and 0.10 ml. of glacial acetic acid was heated on the steam bath for 30 min. during which time all the solid dissolved. The solution was chilled and the flask scratched, causing the precipitation of 1.8 g. (86%) of a crystalline solid, m.p. 229–231° dec. The solid was recrystallized from 140 ml. of hot water to yield 1.4 g. (69%) of material, m.p. 230–232° dec.; $\lambda_{max(\mu)}^{Nujol}$ 3.08 and 3.24 (NH), 5.62, 5.79, and 5.92 (uracil C=O), 7.41 and 8.60 (SO_2N), 12.61 (*p*-disubstituted phenyl). On paper chromatography in solvent A, the compound moved as a single spot with R_{Ad} 1.04.

Anal. Calcd. for $C_{13}H_{14}N_4O_4S$: C, 48.4; H, 4.37; S, 9.94. Found: C, 48.3; 48.1; H, 4.81, 4.52; S, 9.84.

5-Bromoacetyluracil (V). Bromine (2.2 ml., 42.4 mmoles) was added dropwise to a stirred suspension of 5.0 g. (32.2 mmoles) of 5-acetyluracil (III) in 500 ml. of reagent methanol at room temperature. After the addition, stirring was continued until the solid had dissolved (about 30 min.) and then the solution was evaporated to dryness *in vacuo*. The residue was dissolved in 300 ml. of boiling methanol, the hot solution was treated with Norit and filtered. The chilled filtrate deposited 4.5 g. (60%) of product, m.p. 240–245° dec.; $\lambda_{max(\mu)}^{Nujol}$ 3.12 and 3.24 (NH), 5.62, 5.83, 5.94–6.02 (uracil and ketone C=O); 6.21 (uracil C=C), 6.57 (uracil ring); $\lambda_{max(m\mu)}^{C_2H_5OH}$ 227 (ε 6,600), 289 (ε 10,200). The compound move as a single spot on paper chromatography in solvent A with R_{Ad} 1.25.

Anal. Calcd. for $C_6H_5BrN_2O_3$: C, 31.0; H, 2.17; Br, 34.4. Found: C, 31.4; H, 2.36; Br, 33.8.

Phenylosazone of uracil-5-glyoxal (VII). A mixture of 0.25 g. (1.07 mmoles) of 5-bromoacetyluracil (V) and 5 ml. of dimethyl sulfoxide[11] was allowed to stand at 10–15° for 18 hr. Water (40 ml.) was added to the yellow solution which was concentrated *in vacuo* at 60–70° to leave a viscous, yellow liquid. A portion of the residue was added to a solution of 0.50 g. (4.7 mmoles) of phenylhydrazine in 10 ml. of ethanol which contained 1 drop of glacial acetic acid, and the solution was heated at 60–70° for 10 min. Hot water (10 ml.) was added to the solution, causing the precipitation of a yellow solid, m.p. 233–238° dec. The solid was recrystallized by solution in 25 ml. of hot methanol, filtration of the solution, and addition of 20 ml. of hot water to the filtrate. The chilled solution precipitated the yellow, crystalline solid, m.p. 236–238° dec.; $\lambda_{max(\mu)}^{Nujol}$ 3.15, 6.43 and 6.60 (NH), 5.77, 5.92, and 5.97 (uracil C=O), 6.25 (aryl and C=N), 13.50 and 14.60 (monosubstituted phenyl).

Anal. Calcd. for $C_{18}H_{16}N_6O_2$: C, 62.1; H, 4.63; N, 24.4. Found: C, 61.7; H, 4.86; N, 23.6.

Conversion of 5-bromoacetyluracil (V) *to uracil 5-carboxylic acid* (IV). A suspension of 0.50 g. (2.15 mmoles) of the bromo ketone (V) in 10 ml. of dry pyridine[12] was heated, with stirring, for 30 min. at 70–80°. During this period the solid completely dissolved and a new tan precipitate then appeared. The mixture was chilled (0°) and filtered to isolate the solid, 0.50 g., which failed to melt at 300° and was probably the quaternary pyridinium salt.

The solid was dissolved in a solution of 0.50 g. of sodium hydroxide in 25 ml. of water and the solution was heated for 1 hr. at 70–80°, treated with Norit and filtered. The filtrate was chilled in ice bath and adjusted to *p*H 1 with 6*M* hydrochloric acid. A precipitate formed, which was separated by filtration and washed with 30 ml. of water, yielding 0.08 g. (24%) of product which did not melt below 300°. The solid was recrystallized from 10 ml. of water to give 0.06 g. (18%) of material which failed to melt at 300°. It showed a single spot with R_{Ad} 0.77 in solvent A, behaving identically with a commercial sample of 5-carboxyuracil (IV) hemihydrate in both paper chromatography and infrared spectrum; $\lambda_{max(\mu)}^{Nujol}$ 2.90 (OH), 3.20 and 3.29 (NH), 4.10, 4.38–4.46, and 5.18 (carboxyl OH), 5.81 and 5.99 (uracil and carboxyl C=O), 6.19 (C=C), 6.59 (uracil ring), 6.98, 7.46, and 8.41 (COOH).

Anal. Calcd. for $C_5H_4N_2O_4 \cdot {}^1/_2H_2O$: C, 36.3; H, 3.05; N, 16.3. Found: C, 35.5; H, 3.38; N, 16.3.

5-Azidoacetyluracil (VI). A solution of 0.56 g. (8.7 mmoles) of sodium azide in 10 ml. of water was added to a solution of 1.0 g. (4.3 mmoles) of the bromoketone (V) in 30 ml. of 2-methoxyethanol. After the resulting solution had stood at room temperature for 2 hr., it was chilled at 0°. The precipitate, 0.25 g. (30%), was separated and recrystallized from 10 ml. of boiling methanol to yield 0.12 g. (15%) of solid which partially decomposed near 200° but did not melt on heating to 300°; $\lambda_{max(\mu)}^{Nujol}$ 3.21 (NH), 4.75 (N_3), 5.80, 5.92 and 6.05 (uracil and ketone C=O), 6.26 (C=C), 6.57 (uracil ring); $\lambda_{max(m\mu)}^{C_2H_5OH}$ 224 (ε 9,300), 287 (ε 11,300). On paper chromatography in solvent A, the product moved as a single spot with R_{Ad} 1.21.

Anal. Calcd. for $C_6H_5N_5O_3$: C, 36.9; H, 2.58; N, 35.8. Found: C, 37.1; H, 2.78; N, 35.3.

On a preparative scale, 15.0 g. of the bromo ketone (V) afforded 10.5 g. (85%) of azide (VI) that was suitable for conversion to the amine (VIII).

5-Glycyluracil (VIII) *picrate.* A. *By hydrogenation of the azido ketone* (VI). A suspension of 1.0 g. (5.1 mmoles) of the azido ketone (VI) and 1.0 g. of 5% palladium-on-charcoal catalyst in 100 ml. of 50% aqueous ethanol containing 4.0 ml. of 6*M* hydrochloric acid was stirred under 1 atm. of hydrogen for 8 hr. at room temperature, during which time there was no apparent hydrogen uptake. The suspension was filtered and the filtrate evaporated to dryness *in vacuo*, leaving 0.60 g. of amorphous solid which failed to melt at 300°.

A portion of the solid (0.20 g.) was added to a hot (70–80°) solution of 0.25 g. (1.09 mmoles) of picric acid in 25 ml. of water. The yellow solution was chilled (0°) and a yellow precipitate slowly formed. The precipitate (0.30 g., m.p. 189–191° dec.) was dissolved in 10 ml. of hot water, the solution treated with Norit, filtered and the filtrate chilled to yield 0.15 g. (23% from VI) of pure picrate, m.p. 198–200° dec.; $\lambda_{max(\mu)}^{KBr}$ 2.5–3.7 (NH_3^+), 5.75 and 5.85 (uracil and ketone C=O), 6.20 (aryl and uracil C=C), 6.56–6.66 (uracil ring), 6.40 and 7.50 (NO_2).

Anal. Calcd. for $C_{12}H_{10}N_6O_{10}$: C, 36.2; H, 2.53; N, 21.1. Found: C, 36.2, 36.3; H, 2.92, 2.64; N, 20.5.

When a supercooled solution of the above picrate in water was seeded with the second form of the picrate, m.p. 224–225° dec., (*cf.* below), the solution deposited the second form of the picrate, m.p. 223–225° dec., which possessed an infrared spectrum identical with that of the second form and markedly different than that of the 198–200°-melting picrate.

B. *By treatment of the azido ketone* (VI) *with hydrobromic acid.* A suspension of 0.50 g. (2.56 mmoles) of the azido ketone (VI) in 7.0 ml. of a 32% solution of hydrogen bromide in glacial acetic acid was stirred at room temperature for 20 min. during which time gas evolution was noted and the solid gradually dissolved. When the solution had stood about 10 min. more, a yellow precipitate formed. Ether (40 ml.) was added to precipitate completely the solid, which was then separated by filtration and added to 10 ml. of water. The aqueous mixture was filtered to remove an unidentified solid and to the hot (60–70°) filtrate was added a hot solution of 0.58 g. (2.56 mmoles) of picric acid in 40 ml. of water. The yellow solution was chilled and seeded with the 198–200°-melting picrate (see Method A, above) causing the precipitation of 0.20 g. (20%) of yellow solid, m.p. 200–204° dec. This solid was recrystallized from 5 ml. of hot water to give 0.10 g. (10%) of the analytical sample, m.p. 224–225° dec.; $\lambda_{max(\mu)}^{KBr}$ 3.10–3.18 (NH), 3.3–3.7 (NH_3^+), 5.75–5.92 (uracil and ketone C=O), 6.15 (aryl and uracil C=C), 6.60 (uracil ring), 6.35 and 7.50 (NO_2).

Anal. Calcd. for $C_{12}H_{10}N_6O_6$: C, 36.2; H, 2.53; N, 21.1. Found: C, 36.4; H, 2.82; N, 21.1.

5-Glycyluracil (VIII) *hydrochloride.* A suspension of 3.0 g. (7.5 mmoles) of 5-glycyluracil (VIII) picrate, 150 ml. of

benzene, 60 ml. of water, and 7.8 ml. of concd. hydrochloric acid was stirred vigorously for 1 hr. at room temperature. The aqueous layer was separated and was extracted with two 50-ml. portions of benzene. On standing at room temperature the aqueous solution deposited 0.50 g. of the amine hydrochloride which did not melt below 300°; $\lambda_{max(\mu)}^{Nujol}$ 3.16 (NH), 3.59, 3.86, 4.91, and 5.22 (NH_3^+), 5.60, 5.76, 5.90, 6.10 (uracil and ketone C=O), 6.27 and 6.36 (C=C and NH_3^+), 6.70 (uracil ring and NH_3^+); $\lambda_{max(m\mu)}^{pH\ 1}$ 227 (ε 10,200), 285 (ε 12,300); $\lambda_{max(m\mu)}^{pH\ 7}$ 227 (ε 10,300), 285 (ε 12,500). The material streaked badly in several paper chromatographic solvent systems.

Anal. Calcd. for $C_6H_8ClN_3O_3$: C, 35.0; H, 3.92; Cl, 17.2. Found: C, 35.0; H, 3.90; Cl, 17.%.

The mother liquors from the analytical sample were evaporated *in vacuo* and left 1.0 g. of solid which did not melt below 300° and had an infrared spectrum identical with that of the analytical sample. This material was readily recrystallized from 6*M* hydrochloric acid. The total yield of hydrochloride from the picrate was 1.5 g. (97%).

Conversion of 5-glycyluracil (VIII) *to uracil-5-carboxylic acid* (IV). A solution of 0.515 g. (7.26 mmoles) of sodium nitrite in 5 ml. of water was added to a stirred solution of 0.50 g. (2.42 mmoles) of 5-glycyluracil (VIII) hydrochloride in 10 ml. of water. The solution was stirred for 3 hr. at room temperature, during which time a solid, 0.15 g. (40%) and m.p. 285–287° dec., precipitated. The solid was recrystallized twice from water to give 0.05 g. (13%) of product, m.p. >300°. It was identical in paper chromatographic behavior and in infrared spectrum with a commercial sample of the hemihydrate of uracil-5-carboxylic acid (IV).

5-(N-Acetyl)glycyluracil (IX). A suspension of 0.25 g. (1.28 mmoles) of the azido ketone (VI), 10 ml. of glacial acetic acid, 0.10 g. of 5% palladium-on-charcoal, and 0.27 g. (2.56 mmoles) of acetic anhydride was stirred with hydrogen at room temperature for 6 hr., during which time there was no visible uptake of hydrogen. Methanol (20 ml.) was added to the mixture, which was then heated to boiling on the steam bath and the mixture filtered. The filtrate was evaporated *in vacuo* at 40–50°, leaving a solid residue, m.p. 290–295°. The product was recrystallized from 30 ml. of hot water, yielding 0.20 g. (74%) of a solid which did not melt below 300°; $\lambda_{max(\mu)}^{Nujol}$ 3.02, 3.18, 3.28 (NH), 5.81, 5.95, and 6.22 (uracil and ketone C=O, amide C=O, and C=C); there was a broad, unassigned band at 3.70–3.82 μ. On paper chromatography in solvents A and B the compound was homogeneous with R_{Ad} 1.10 and 0.44, respectively.

Anal. Calcd. for $C_8H_9N_3O_4$: C, 45.4; H, 4.29; N, 19.8. Found: C, 45.5; H, 4.51; N, 20.1.

2,4-Dioxo-1,2,3,4-tetrahydro-5-pyrimidineglyoxyl aldehyde aldoxime (X). To a stirred, chilled (0–3°) solution of 1.0 g. (6.4 mmoles) of 5-acetyluracil (III), 0.72 ml. (6.4 mmoles) of *n*-butyl nitrite,[16] and 60 ml. of *N,N*-dimethylformamide was added 0.52 ml. (6.4 mmoles) of concd. hydrochloric acid over a period of about 2 min. The mixture was stirred at room temperature for 18 hr. and was concentrated *in vacuo* at 35–40° with the aid of an oil pump to about 10–12 ml., then poured over 40–50 g. of ice. The precipitated solid (0.80 g., 68%) failed to melt below 300°. The product was recrystallized from 30 ml. of hot water to give 0.50 g. (43%) of material which did not melt below 300°. From a previous preparation, an analytical sample was obtained which had $\lambda_{max(\mu)}^{Nujol}$ 2.83, 2.95, and 3.05 (NH, OH), 5.81 and 5.98 (uracil and ketone C=O), 6.25 (C=N and pyrimidine ring), 6.82 and 6.99 (pyrimidine ring); $\lambda_{max(m\mu)}^{pH\ 1}$ 239 (broad) (ε 8,500), 290 (ε 10,200); $\lambda_{max(m\mu)}^{pH\ 7}$ 239 (ε 8,500), 313 (broad) (ε 19,000); $\lambda_{max(m\mu)}^{pH\ 13}$ 334 (ε 15,200). On paper chromatography in solvents A and D, the product moved as a single spot with R_{Ad} 0.84 and 1.30, respectively.

Anal. Calcd. for $C_6H_5N_3O_4 \cdot H_2O$: C, 35.8; H, 3.50; N, 20.8. Found: C, 36.2, 36.2; H, 3.36, 3.28; N, 21.0, 20.8.

5-Diazoacetyluracil (XI). To a chilled (0–3°), stirred solution of 2.0 g. (10.9 mmoles) of the oximino ketone (X), 0.88 g. (21.8 mmoles) of sodium hydroxide, 45 ml. of concd. ammonium hydroxide, and 120 ml. of water was added dropwise 20 ml. (10.2 mmoles) of 4.8% aqueous sodium hypochlorite over a period of 20 min. After the addition, the mixture was stirred at 0–3° for 1 hr., at room temperature for 3 hr., then evaporated *in vacuo* to 15–20 ml.[24] while maintaining the bath temperature below 30°. The solution was chilled to afford 0.60 g. of a yellow solid which failed to melt below 300°. The infrared spectra of the products from a number of runs were identical: $\lambda_{max(\mu)}^{Nujol}$ 2.76 and 2.98 (NH), 4.72 (—$\overset{+}{N}\equiv\overset{-}{N}$), 5.82 (C=O), 6.05, 6.17, 6.22 and 6.40 (C=C and pyrimidine ring); $\lambda_{max(m\mu)}^{H_2O}$ 309; $\lambda_{max(m\mu)}^{pH\ 13}$ 335.

The sodium salt prepared as above (6.0 g.) from several runs was dissolved in 150 ml. of water at room temperature, the solution filtered, and the filtrate chilled to 0°. Glacial acetic acid was added dropwise to the cold solution to adjust the *p*H to 7, at which point the product precipitated. The solid, 3.0 g. (15% from X), was collected and washed well with cold water. It did not melt below 300° and had $\lambda_{max(\mu)}^{Nujol}$ 3.18, 3.22–3.29, and 6.50 (NH), 4.60, 4.70, 4.74, and 4.82 (—$\overset{+}{N}\equiv\overset{-}{N}$), 5.83–5.94 (ketone and uracil C=O), 6.16 (C=C).

Anal. Calcd. for $C_6H_4N_4O_3$: C, 40.0; H, 2.23; N, 31.1. Found: C, 40.0; H, 2.64; N, 30.9.

Conversion of the sodium salt of XI *to the bromo ketone* (V). To a stirred solution of 1.0 g. of the crude sodium salt of XI (see previous experiment) in 20 ml. of water, 25 ml. of 48% aqueous hydrobromic acid was added over a period of 10 min. The solution was allowed to stand at room temperature for 1 hr., then was evaporated to dryness *in vacuo* with the bath temperature maintained at 30–40°. The residue was triturated with 10 ml. of absolute ethanol which was decanted and the undissolved solid was washed with 25 ml. of cold water, leaving 0.50 g. of a solid which partially decomposed near 210° and whose infrared spectrum and paper chromatographic behavior agreed well with those of authentic V. A portion of this residue, 0.100 g., was recrystallized from 7 ml. of methanol to afford 0.020 g. of solid, m.p. 230–234° dec., whose infrared spectrum and paper chromatographic behavior in solvents B, C, and D were the same as those of authentic V.

Anal. Calcd. for $C_6H_3BrN_2O_3$: C, 31.0; H, 2.17; Br, 34.4. Found: C, 31.5; H, 2.59; Br, 34.4.

1,2,3,4-Tetrahydro-2,4-dioxo-5-pyrimidinylcarbonylmethyl thiocyanate (XIII). To a warm (60°) solution of 0.53 g. (2.28 mmoles) of the bromo ketone (V) in 10 ml. of 2-methoxyethanol was added 0.30 g. (3.10 mmoles) of potassium thiocyanate and the solution was maintained at 50–60° for 5 min., during which time a brown color developed. The solution was evaporated *in vacuo* with the temperature maintained below 50°. Water (10 ml.) was added to the brown residue and the mixture was filtered, yielding 0.45 g. (94%) of a solid which darkened near 200° but failed to melt below 300° and whose infrared spectrum was almost identical with that of the analytical sample. The material was recrystallized from methanol (80 ml./g.) to yield 0.30 g. (63%) of the analytical sample, m.p. >300°; $\lambda_{max(\mu)}^{Nujol}$ 3.17, 3.25, and 3.28 (NH), 4.65 (SCN), 5.77 and 5.96 (uracil and ketone C=O), 6.25 (C=C). On paper chromatography in water, the compound moved as a single spot with R_{Ad} 1.87.

Anal. Calcd. for $C_7H_5N_3O_3S$: C, 39.8; H, 2.39; N, 19.9. Found: C, 40.0; H, 2.58; N, 19.5.

5-(2-Methyl-4-thiazolyl)uracil (XII). To a warm (50°) solution of 0.23 g. (1.0 mmole) of the bromo ketone (V) in 3.5 ml. of 2-methoxyethanol was added 0.09 g. (1.2 mmoles) of thioacetamide. Within 1 min. the solution began to deposit a crystalline, white precipitate. After it had stood at room temperature for 1 hr., the mixture was chilled and filtered, yielding 0.18 g. (87%) of solid which failed to melt below 300° and whose infrared spectrum was identical with that of the analytical sample. The material was recrystallized

(24) If the solution was allowed to evaporate to dryness, the infrared diazo band was weak or missing in the resultant solid.

by dissolving it in 20 ml. of hot (90°) *N,N*-dimethylformamide, diluting the solution with 7 ml. of water, and chilling, to yield the analytical sample, m.p. >300°; $\lambda_{max(\mu)}^{Nujol}$ 3.01, 3.18, 3.22 (NH), 5.82 and 5.95 (uracil C=O), 6.05 (C=N), 6.22 (C=C); there were strong, unassigned bands at 8.09 and 8.22 μ and unexplained, broad absorptions at 3.6–4.2 μ; $\lambda_{max(m\mu)}^{pH\ 1}$ 233 (ϵ 9,700), 274 (ϵ 11,500); $\lambda_{max(m\mu)}^{pH\ 7}$ 249 (ϵ 11,800), 265 (ϵ 8,700), 294 (shoulder, ϵ 7,500); $\lambda_{max(m\mu)}^{pH\ 13}$ 249 (broad, ϵ 11,700), 307 (broad, ϵ 9,700). On paper chromatography in solvents A and C, the product moved as a single spot with R_{Ad} 1.28 and 1.08, respectively.

Anal. Calcd. for $C_8H_7N_3O_2S$: C, 45.9; H, 3.38; N, 20.1. Found: C, 46.1; H, 3.56; N, 19.5.

5-(2-Amino-4-thiazolyl)uracil (XIV) *and the hydrobromide and picrate salts.* A mixture of 0.27 g. (1.20 mmoles) of 5-bromoacetyluracil (V), 0.92 g. (12.0 mmoles) of thiourea, and 10 ml. of 2-methoxyethanol was heated at 70–80° for 1 hr., during which time a heavy solid formed. This was separated by filtration and washed with cold water, leaving 0.30 g. (87%) of the hydrobromide salt of XIV which did not melt below 300°. The solid was recrystallized from 300 ml. of hot water, yielding 0.27 g. (78%) of product, m.p. >300°; $\lambda_{max(\mu)}^{Nujol}$ 3.00–3.22 (NH), 3.6–4.2 (broad absorptions, possibly NH_3^+), 5.81 and 5.93 (uracil C=O), 6.14 (C=C and C=N or C=N$^+$); $\lambda_{max(m\mu)}^{pH\ 1}$ 242 (ϵ 12,800); $\lambda_{max(m\mu)}^{pH\ 7}$ 243 (ϵ 12,000); $\lambda_{max(m\mu)}^{pH\ 13}$ 241 (broad, ϵ 13,400), 316 (ϵ 8,000). On paper chromatography in solvent C, the product moved as a single spot with R_{Ad} 0.83.

Anal. Calcd. for $C_7H_6N_4O_2S\cdot HBr$: C, 28.9; H, 2.43; S, 11.0. Found: C, 28.8, 29.2; H, 3.02, 3.02; S, 10.9, 10.7.

A solution of 0.200 g. of the hydrobromide salt of XIV in 25 ml. of hot water was adjusted to *p*H 7 with solid sodium bicarbonate, causing the precipitation of 0.125 g. (91%) of the free base (XII) as a solid which did not melt below 300°. The solid was recrystallized from 40 ml. of hot water to yield 0.075 g. (55%) of product, m.p. >300°; $\lambda_{max(\mu)}^{Nujol}$ 2.92, 3.02 and 3.12 (NH), 5.81 and 5.92 (uracil C=O), 6.14 (C=N and C=C); there was a strong, unassigned band at 8.30 μ. On paper chromatography in solvents A and C, the compound moved as a single spot with R_{Ad} 0.81 and 0.81, respectively.

Anal. Calcd. for $C_7H_6N_4O_2\cdot 1/2\ H_2O$: C, 38.4; H, 3.22; N, 25.6. Found: C, 39.0, 39.2; H, 3.41, 3.40; N, 25.2.

To a hot solution of 0.137 g. (0.600 mmole) of picric acid in 40 ml. of methanol was added 0.105 g. (0.500 mmole) of the free base (XIV). The hot solution was filtered and the filtrate was chilled overnight to cause precipitation of the picrate. The yellow solid was recrystallized twice from hot methanol to give a crystalline solid which decomposed without melting near 260°; $\lambda_{max(\mu)}^{Nujol}$ 2.84, 2.98, 3.13 (NH), 3.22 (NH and aryl), 5.76 and 5.86 (uracil C=O), 6.06 (aryl and C=N$^+$), 6.44, 7.46 and 7.56 (NO_2).

Anal. Calcd. for $C_{13}H_9N_7O_9S$: C, 35.5; H, 2.06; N, 22.3. Found: C, 36.0; H, 2.59; N, 22.6.

5-[Bis(2-hydroxyethyl)aminoacetyl]uracil (XV). To a warm (55–60°) solution of 1.50 g. (6.44 mmoles) of 5-bromoacetyluracil (V) in 150 ml. of methanol was added 1.68 g. (16.1 mmoles) of 2,2′-iminodiethanol. The solution was warmed at 55–60° for 5 min. and was then evaporated *in vacuo*, leaving a brown sirup. Water (30 ml.) was added to the residue and the mixture was heated at 65–70° for 15 min. The solution was chilled and the flask scratched, causing the precipitation of 1.15 g. (70%) of crystalline solid, m.p. 135–137°. The solid was recrystallized from 25 ml. of hot water, yielding 1.0 g. (60%) of product, m.p. 145–147° with darkening around 135°. From a previous run, a sample was obtained, after recrystallization from water, which had m.p. 144–146° with prior darkening; $\lambda_{max(\mu)}^{Nujol}$ 2.93, 2.99, and 3.11 (OH, NH), 3.24 (NH), 6.00, 6.08, 6.20 (uracil and ketone C=O and C=C), 9.12, 9.27 and 9.43 (C—OH); $\lambda_{max(m\mu)}^{pH\ 1}$ 258 (ϵ 7,700), 287 (shoulder, ϵ 3,500); $\lambda_{max(m\mu)}^{pH\ 7}$ 250 (ϵ 6,430), 302 (ϵ 5,570); $\lambda_{max(m\mu)}^{pH\ 13}$ 244 (ϵ 8,270), 302 (ϵ 15,900). On paper chromatography in solvents A and D, the compound moved as a single spot with R_{Ad} 0.40 and 1.10, respectively.

Anal. Calcd. for $C_{10}H_{15}N_3O_5\cdot H_2O$: C, 43.6; H, 6.23; N, 15.3. Found: C, 43.9; H, 6.55; N, 14.9.

Ethyl 3,4-dioxovalerate 4-diethylketal (XXII). In a three neck, 100-ml. flask equipped with a condenser, dropping funnel, stirrer, and drying tube was placed 15.0 g. (78.9 mmoles) of ethylpyruvate diethyl acetal (XXI),[22] 6.93 g. (0.158 mole) of sodium hydride (as a 54.5% suspension in mineral oil), and 10.3 g. (0.118 mole) of ethyl acetate and the mixture was stirred at 20–25° (water-bath temperature) for 1 hr. Ethyl acetate (10.3 g., 0.118 mole) was added dropwise with stirring over a 20-min. period, then the resulting solution was stirred at room temperature for 3 hr. The flask contents were poured over 40 g. of ice and the resulting mixture was adjusted to *p*H 4 with cold 6*M* hydrochloric acid and extracted with three 50-ml. portions of ether. The extracts were combined, washed with two 25-ml. portions of water, dried over magnesium sulfate, and distilled from a short Vigreux column, after being filtered. After removal of the ether at atmospheric pressure, a forerun of ethyl acetoacetate was collected at 45–50° (4–6 mm.), followed by 11.5 g. (63%) of the β-keto ester (XXII) collected at 81–83° (3 mm.), n_D^{20} 1.4331. From an earlier run, a similar fraction was collected, n_D^{20} 1.4376 and $\lambda_{max(\mu)}^{film}$ 3.55–3.75 (broad, enolic OH), 5.72–5.83 (ester and ketone C=O), 6.05–6.13 (chelated C=O), 6.36 (C=C), 8.35 and 8.60–8.85 (ester C—O—C), 9.60 (ester and ketal C—O—C), 12.30 (olefinic H).

Anal. Calcd. for $C_{11}H_{20}O_5$: C, 56.9; H, 8.68. Found: C, 57.0; H, 8.49.

2-Amino-6-acetyl-4(3H)-pyrimidinone (XXIII). A mixture of 3.00 g. (12.9 mmoles) of the β-keto ester (XXII), 2.82 g. (15.7 mmoles) of guanidine carbonate, and 40 ml. of absolute ethanol was stirred under reflux for 14 hr. The ethanol was evaporated *in vacuo* and to the residue was added 40 ml. of water. The solution was adjusted to *p*H 1 with 6*M* hydrochloric acid, heated with stirring at 90–100° for 15 min., cooled to 10–15°, and neutralized (*p*H 7) with 10% aqueous sodium bicarbonate. A brown solid, 2.00 g. (83%), separated and was dissolved in a solution of 5 ml. of 6*M* hydrochloric acid and 50 ml. of water and the solution treated with Norit. After filtration the filtrate was adjusted to *p*H 7 with 10% aqueous sodium bicarbonate solution, yielding a tan solid, 1.30 g. (54%), m.p. >300°; $\lambda_{max(\mu)}^{Nujol}$ 3.01 and 3.22 (NH_2, NH), 5.82 and 5.95–6.10 (ring and ketone C=O, NH_2, C=C), 6.48–6.70 (pyrimidine ring), 11.60 (olefinic H). On paper chromatography in solvents A, C, and D, the compound moved as a single spot with R_{Ad} 1.05, 1.06, and 1.22, respectively. The anhydrous solid was obtained by dissolving in *N,N*-dimethylformamide and reprecipitating with 2-methoxyethanol.

Anal. Calcd. for $C_6H_7N_3O_2$: C, 47.0; H, 4.60; N, 27.4. Found: C, 47.1; H, 4.80; N, 26.7.

2-Amino-5-bromo-6-acetyl-4(3H)-pyrimidinone (XXV). To a stirred suspension of 3.00 g. (19.2 mmoles) of 2-amino-6-acetyl-4(3H)-pyrimidinone (XXIII) in 80 ml. of glacial acetic acid was added dropwise 1.00 ml. (19.6 mmoles) of bromine over a period of 5 min. The solution was stirred at room temperature for 4.5 hr. and was evaporated *in vacuo* at 40–50°. Ice water (80 ml.) was added to the solid residue and the solution was adjusted to *p*H 8 with 10% aqueous sodium bicarbonate. The solid product, 3.00 g. (70%), decomposed without melting at 215–217° and was recrystallized from 200 ml. of absolute ethanol to yield 2.50 (60%) of product with the same decomposition behavior as the crude product; $\lambda_{max(\mu)}^{Nujol}$ 3.02 and 3.19 (NH_2, NH), 5.80, 5.94, 6.06, 6.22 (ring and ketone C=O, NH_2, C=C), 6.41 (pyrimidine ring); there was no olefinic CH in the 11.5–11.8 μ region; $\lambda_{max(m\mu)}^{pH\ 1}$ 226 (ϵ 9,900), 309 (ϵ 5,200); $\lambda_{max(m\mu)}^{pH\ 7}$ 225 (ϵ 10,700), 304 (ϵ 4,900); $\lambda_{max(m\mu)}^{pH\ 13}$ 296 (ϵ 3,500). On paper chromatography in solvent A, the compound moved as a single spot with R_{Ad} 1.30.

Anal. Calcd. for $C_6H_6N_3O_2Br$: C, 31.1; H, 2.60; Br, 34.4. Found: C, 31.7; H, 2.87; Br, 34.0, 34.2.

6-(1,1-Diethoxyethyl)-2-thiouracil (XVIII). A mixture of

2.00 g. (8.61 mmoles) of the β-keto ester (XXII), 0.49 g. (9.00 mmoles) of sodium methoxide, 0.76 g. (10.0 mmoles) of thiourea, and 10 ml. of absolute ethanol was heated under reflux with stirring for 7 hr. The mixture was evaporated *in vacuo* at 50–60°, the red residue was dissolved in 15 ml. of water and the solution heated at 70–80° for 10 min. After treatment with Norit the hot solution was filtered, the filtrate chilled and adjusted to *p*H 4 with 6*M* hydrochloric acid. A gummy material precipitated which crystallized on standing to yield 0.90 g. (43%) of crystalline solid, m.p. 144–147°; $\lambda_{max(\mu)}^{Nujol}$ 3.20–3.27 (NH), 5.93 (C=O), 6.12 (C=C), 6.42 (pyrimidine ring), 8.41 (C=S), 9.50 and 9.62 (C—O—C), 11.77 (olefinic CH). On paper chromatography in solvent B, the compound moved as a single spot with R_{Ad} 1.65.

Anal. Calcd. for $C_{10}H_{16}N_2O_3S$: C, 49.1; H, 6.59; S, 13.1. Found: C, 49.7; H, 6.89; S, 12.6.

6-Acetyl-2-thiouracil (XIX). A mixture of 18.0 g. (77.5 mmoles) of the β-keto ester (XXII), 9.90 g. (0.183 mole) of sodium methoxide, 11.8 g. (0.155 mole) of thiourea, and 90 ml. of absolute ethanol was stirred for 14 hr. at room temperature. The solution was heated under reflux for 6 hr. and evaporated *in vacuo*. Water (90 ml.) was added to the residue, the solution filtered and the filtrate adjusted to *p*H 3 with 6*M* hydrochloric acid. The acid solution was heated on the steam bath for 30 min., during which time crystallization began. After chilling the mixture, 8.50 g (51%) of product was obtained with m.p. 274–276° dec.; this product was identical with that prepared from XVIII.

Previously, by heating a mixture of 0.20 g. (0.82 mmole) of the diethylketal (XVIII), 5 drops of 6*M* hydrochloric acid, and 15 ml. of water at 70–80 for 30 min., a yellow, crystalline product, 0.14 g. (97%), m.p. 278–280° dec., had been obtained. This was recrystallized from 35 ml. of boiling water with the aid of Norit to yield 0.060 g. (58%) of solid, m.p. 278–280° dec.; $\lambda_{max(\mu)}^{Nujol}$ 3.18 and 3.22 (NH), 5.91 (thiouracil and ketone C=O), 6.15 (C=C), 6.40–6.50 (pyrimidine ring), 8.57 (C=S), 11.70 (olefinic CH); $\lambda_{max(m\mu)}^{pH\ 1}$ 272 (ε 20,200; $\lambda_{max(m\mu)}^{pH\ 7}$ 260 (ε 13,400); $\lambda_{max(m\mu)}^{pH\ 13}$ 267 (ε 14,700). On paper chromatography in solvents A and B, the product moved as a single spot with R_{Ad} 1.14 and 1.46, respectively.

Anal. Calcd. for $C_6H_6N_2O_2S$: C, 42.3; H, 3.54; S, 18.8. Found: C, 42.5; H, 3.64; S, 18.9.

6-Acetyluracil (XX). A. *From 6-(1,1-diethoxyethyl)-2-thiouracil* (XVIII). A stirred suspension of 0.22 g. (0.90 mmole) of the diethylketal (XVIII), 0.43 g. (4.5 mmoles) of chloroacetic acid, and 5 ml. of water was heated under reflux for 6 hr. The solution was chilled, causing the precipitation of 0.094 g. (68%) of product, m.p. 259–260° dec. This was recrystallized from 5 ml. of hot water, yielding 0.080 g. (58%) of solid, m.p. 265–266° dec. (lit.[20] m.p 255–260° dec.); $\lambda_{max(\mu)}^{Nujol}$ 3.00 and 3.19 (NH); 5.82 and 5.93 (uracil and ketone C=O), 6.10 (C=C), 11.50 (olefinic CH); $\lambda_{max(m\mu)}^{pH\ 1}$ 295 (ε 6,600); $\lambda_{max(m\mu)}^{pH\ 13}$ 295 (ε 5,400). On paper chromatography in solvents A and B, the compound moved as a single spot with R_{Ad} 0.97 and 0.92, respectively.

Anal. Calcd. for $C_6H_6N_2O_3$: C, 46.6; H, 3.92. Found: C, 46.5; H, 4.05.

In the same manner, the treatment of 7.0 g. of 6-acetyl-2-thiouracil (XIX) with a solution of 13.6 g. of chloroacetic acid in 130 ml. of water heated at reflux for 5 hr. gave 4.0 g. (63%) of 6-acetyluracil (XX), m.p. 264–265° dec., with infrared spectrum and paper chromatographic behavior identical to those of the analytical sample.

B. *From 2-amino-6-acetyl-4(3H)-pyrimidinone* (XXIII). To a stirred mixture of 2.00 g. (13.0 mmoles) of the aminopyrimidinone (XXIII), 10 ml. of 6*M* hydrochloric acid, 5 ml. of concd. sulfuric acid, and 15 ml. of water at room temperature was added dropwise a solution of 3.58 g. (52 mmoles) of sodium nitrite in 10 ml. of water over a period of 10 min. The mixture was stirred at room temperature 1.5 hr. and chilled to yield 1.40 g. (69%) of crystalline 6-acetyluracil (XX), m.p. 257–260° dec., which was identical with the analytical sample in infrared spectrum and paper chromatographic behavior.

Acknowledgment. The authors are indebted to Dr. Peter Lim for interpretation of the infrared spectra and to his staff for the paper chromatography. They also wish to thank Mr. O. P. Crews and his staff for the large-scale preparation of certain intermediates.

MENLO PARK, CALIF.

[CONTRIBUTION FROM THE DIVISIONS OF NUCLEOPROTEIN CHEMISTRY AND EXPERIMENTAL CHEMOTHERAPY, SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH; SLOAN-KETTERING DIVISION OF CORNELL UNIVERSITY MEDICAL COLLEGE]

Thiation of Nucleosides. III. Oxidation of 6-Mercaptopurines[1]

IRIS L. DOERR, I. WEMPEN, DONALD A. CLARKE, AND JACK J. FOX

Received January 5, 1961

Syntheses of the disulfides of 6-mercaptopurine, 6-thioguanine, 6-thioinosine, and 6-thioguanosine from the corresponding mercaptans are described. Cleavage of the S—S bond in the disulfide of 6-mercaptopurine and 6-thioguanine by aqueous alkali yields purine-6-sulfinate and 2-aminopurine-6-sulfinate respectively. These sulfinates also result from oxidation of the mercaptopurines with alkaline iodine solution, while the corresponding sulfonates result from oxidation of the parent mercaptans or sulfinates with alkaline permanganate. The sulfonates are also prepared by the direct replacement of the chlorine atom in 6-chloropurine and 2-amino-6-chloropurine respectively by means of sodium sulfite. The sulfinates are useful in various syntheses since the 6-sulfinate can be replaced by a chloro, hydrogen, or hydroxyl with relative ease. The shifts in the ultraviolet spectra of the bis(6-purinyl) disulfides and their nucleosides and the influence of the sulfinates and sulfonates on the pK_a values of the imidazole dissociation, are discussed. A preliminary report of the effects of the disulfides, sulfinates, and sulfonates on transplantable mouse tumor, Sarcoma 180, is given.

In bacterial and mammalian systems, 6-mercaptopurine (6-MP) has been shown to inhibit *de novo* synthesis of nucleic acid, presumably through blocking the conversion of inosinic acid into other purine ribonucleotides.[2] Similarly, recent investigations into the mechanism of the action of 6-mercaptopurine, using microbiological systems[3] and enzyme preparations,[4] have indicated that in the form of its ribonucleotide, 6-mercaptopurine inhibits the normal enzymic conversions of inosinic acid. The complete metabolic mechanism in mammals of the 6-mercaptopurines (6-mercapto-