Anal. Calcd. for $C_5H_9O_3NS$ (VIIa or b): C, 36.80; H, 5.52; N, 8.59; S, 19.65; mol. wt., 163. Found: C, 36.92; H, 5.56; N, 8.58; S, 19.36; mol. wt., 158 (ebullioscopic).

Succinimide (III). Method A.—Sodium β-cyanopropionate was prepared in 75 to 78% yield by treating sodium cyanide with β-propiolactone in 50% aqueous ethanol, a variation of the procedure of Gresham, Jansen, Shaver, *et al.*[10] Recrystallized sodium β-cyanopropionate monohydrate (98 g., 0.705 mole) was mixed with 50% sulfuric acid (79 g., 0.805 mole). The mixture was heated until all of the water had been driven off and the temperature of the reaction mixture reached 200°. The flask was then fitted for distillation and heated at 200–230°, the pressure of the system being gradually reduced to about 200 mm. The succinimide sublimed and was collected in a chilled collection vessel. The sublimation was complete in 15 to 20 minutes. The sublimed material was recrystallized from 140 cc. of ethanol to give 54 g. (77%) of succinimide, melting at 124–125°. The mixture melting point of the reaction product with an authentic sample of succinimide was not depressed.

Methyl β-Chlorosulfonylpropionate (VIII).—Methyl acrylate (215 g., 2.5 moles) was treated with a 45% aqueous solution of ammonium bisulfite (550 g., 2.5 moles) adding the acrylate to the bisulfite solution at less than 50° with vigorous stirring. The mixture was then warmed at 80° for 30 minutes. Evaporation of the water and recrystallization of the product from ethanol gave 407.7 g. of ammonium β-carbomethoxyethanesulfonate melting at 203–204°. A portion of this salt (370 g.) was thoroughly mixed with phosphorus pentachloride (417 g., 2.0 moles), an exothermic reaction resulted and the mixture became fluid. The reaction mixture was heated and distilled, 203 g. (48%) of VIII distilling at 137–144° at 18 mm.

Anal. Calcd. for $C_4H_7O_4SCl$: C, 25.75; H, 3.76. Found: C, 25.75; H, 3.81.

Methyl β-Sulfamylpropionate (IX).—Methyl β-chlorosulfonylpropionate (55 g., 0.295 mole) was dissolved in 300 cc. of benzene and the solution was saturated with dry ammonia. The benzene solution was then evaporated to dryness and the residue was recrystallized from chloroform. The extract yielded 31.2 g. (63%) of IX as white plates melting at 69°.

Anal. Calcd. for $C_4H_9O_4NS$: C, 28.75; H, 5.39; N, 8.38. Found: C, 28.75; H, 5.74; N, 8.35.

β-Sulfopropionimide (IV).—Methyl β-sulfamylpropionate (6.16 g., 0.037 mole) was mixed with sodium hydroxide (1.47 g., 0.037 mole) in 50 cc. of water. The mixture was warmed on a steam-bath for one hour and neutralized with concentrated hydrochloric acid. The water solution was evaporated to dryness by warming on a steam-bath under aspirator vacuum. The residue was extracted with ethanol and the ethanol extract was distilled, after drying over anhydrous sodium sulfate. After the solvent was removed, and a milky forerun was discarded, the remainder of the material distilled at 230–250° (1.0 mm.) and solidified in the receiver. This solid was recrystallized from a small quantity of ethanol, cooling in Dry Ice and was then extracted with ether, from which was obtained 0.5 g. of white needles melting at 119° (IV).

The infrared spectrum of β-sulfopropionimide was compared with that of saccharin. The spectra are extremely similar and peaks are present to account for each functional group; the major differences are absorptions at 6.25, 6.88 and 13.16 μ for saccharin, attributed to the benzene ring, and a maximum at 7.02 μ for β-sulfopropionimide, this peak being attributed to the methylene group.

Anal. Calcd. for $C_3H_5O_3NS$: C, 26.70; H, 3.70; N, 10.37. Found: C, 26.69; H, 3.79; N, 10.35.

Acknowledgment.—The author wishes to thank A. K. Kuder and J. R. Kubik for the elemental analyses, and J. J. Shipman for the infrared interpretations.

BRECKSVILLE, OHIO

[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, YALE UNIVERSITY SCHOOL OF MEDICINE]

The Reaction of Chloral Hydrate with Semicarbazides and the Synthesis of Semicarbazide-C^{14} and 6-Azauracil-2-C^{14} [1]

BY PAULINE K. CHIANG AND T. L. V. ULBRICHT[2]

RECEIVED MARCH 27, 1957

Chloral hydrate reacts with semicarbazides to give chloral *hydrate* semicarbazones. The structure of these compounds is discussed in relation to the infrared spectrum of the thiosemicarbazone. Two syntheses of 6-azauracil (*asym*-triazine-3,5-dione) using chloral hydrate semicarbazones or their derivatives have been investigated. In one, heating an aqueous solution of chloral hydrate methylthiosemicarbazone gave azauracil directly. Two possible mechanisms for this unusual reaction are discussed. The other synthesis was used to prepare 6-azauracil-2-C^{14} from urea-C^{14} in an over-all yield of 30% *via* semicarbazide-C^{14}. The chlorination of 5-bromoazauracil and milliniolar syntheses of potassium thiocyanate and thiosemicarbazide also are described.

6-Azauracil (*asym*-triazine-3,5-dione, I)[3] has been shown to inhibit the growth of certain strains of microörganisms[4,5] and a number of transplantable neoplasms[5,6] in mice. The ribofuranoside of this analog of uracil has been prepared recently[7] and, unlike free azauracil, this derivative inhibits the growth of sarcoma 180 cells in tissue culture.[8] To permit metabolic studies in these systems, a synthesis of C^{14}-labeled azauracil was developed.

Two recent four-step syntheses of azauracil from thiosemicarbazide and oxomalonic acid or its methyl ester, in over-all yields of 41[9] and 22%,[10] respectively, did not appear to be satisfactory for isotopic synthesis.

(1) This paper was presented, in part, at the 131st National Meeting of the A.C.S., Miami, Fla., April, 1957.

(2) This work was supported by a grant from the National Cancer Institute, Public Health Service.

(3) This designation is adopted in view of current practice (refs 4, 6, 7 and 9) and because it probably describes the structure better than does 3,5-dihydroxy-1,2,4-triazine.[10] However, the authors suggest (in view of the confusion caused in pyrimidine literature, particularly "Chemical Abstracts" [A. Bendich, "The Nucleic Acids," ed. by E. Chargaff and J. N. Davidson, Vol. 1, p. 83, Academic Press, Inc., New York, 1955] by calling compounds pyrimidones, pyrimidols and hydroxypyrimidines) that it might be preferable to name all compounds as derivatives of the aromatic ring system, without prejudice to the preferred tautomeric form.

(4) R. E. Handschumacher and A. D. Welch, *Federation Proc.*, **15**, 267 (1956); *Cancer Research*, **16**, 1065 (1956).

(5) F. Sorm, A. Jakobovic and L. Slechta, *Experientia*, **12**, 271 (1956).

(6) M. T. Hakala, L. W. Law and A. D. Welch, *Proc. Am. Assocn. Cancer Research*, **2**, 113 (1956).

(7) R. E. Handschumacher, *Biochim. et Biophys. Acta*, **23**, 428 (1957).

(8) R. Schindler and A. D. Welch, *Science*, **125**, 548 (1957).

(9) R. B. Barlow and A. D. Welch, THIS JOURNAL, **78**, 1258 (1956).

(10) E. A. Falco, E. Pappas and G. H. Hitchings, *ibid.*, **78**, 1938 (1956).

TABLE I

INFRARED ABSORPTION SPECTRA[16]

Compound	3-μ region				6-μ region					
Chloral hydrate[17a]	2.83	3.00			5.65s				6.23	
Chloral hydrate[17b]		3.00			5.65vw					
Thiosemicarbazide[17b]	2.97		3.06	3.15		6.05	6.09	6.15		
III[17b]	2.93	3.00	3.06	3.15					6.21s	
II[17b]	2.92		3.06	3.15	5.86s	6.00			6.21	6.30

A synthesis in which glyoxylic acid semicarbazone (II)[11] was cyclized using aqueous sodium hydroxide was described by Seibert,[12] who did not record the yield (which has now been found to be about 20%) or the source of the semicarbazone. The latter is most readily prepared from chloral hydrate and semicarbazide hydrochloride, or by the hydrolysis of chloral hydrate semicarbazone.[13]

$$CCl_3CH(OH)_2 + NH_2NHCONH_2 \longrightarrow CCl_3CH(OH)NHNHCONH_2$$

$$CCl_3CH(OH)_2 + NH_2NHCONH_2HCl \xrightarrow[\Delta]{H_2O} HOOCCH{=}NNHCONH_2 \quad (II)$$

As thiourea reacts far more readily than urea to give pyrimidines, an attempt was made to prepare the thiosemicarbazone of glyoxylic acid (or a similar derivative) to see whether the yield in the cyclization step could be improved.

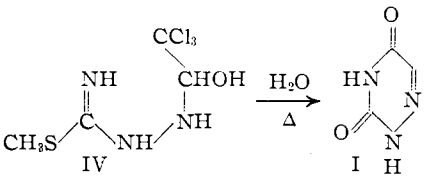
Chloral hydrate is known to condense with semicarbazide in cold aqueous solution with the elimination of only one molecule of water. It has been assumed that the remaining molecule of water is an integral part of the molecule, but this has not been established.[14,15] We found that chloral hydrate gives similar products with thiosemicarbazide and methyl thiosemicarbazide. These products might be (1) molecular hydrates of the semicarbazone IIIa; (2) molecular complexes of chloral with the semicarbazide IIIb; or (3) true chemical hydrates (such as IIIc).

$CCl_3CH{=}NNHCNH_2{\cdot}H_2O$ (IIIa) $\quad CCl_3CHO{\cdot}NH_2NHCNH_2$ (IIIb)

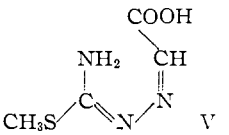
$CCl_3CH(OH)NHNHCNH_2$ (IIIc)

The structure of the product obtained from thiosemicarbazide III was investigated by comparing its infrared spectrum with those of chloral hydrate, thiosemicarbazide and glyoxylic acid semicarbazone (II) (see Table I). It will be noted that chloral hydrate has a pronounced carbonyl peak in chloroform which is barely visible in potassium bromide. The wave length (5.65 μ) is lower than in other saturated aliphatic aldehydes (5.74–5.81 μ), as in other carbonyl compounds containing strongly electron-withdrawing groups, such as acyl chlorides (5.5–5.65) and phosgene (5.47).[18] The 3-μ region is difficult to interpret with certainty, but the band at 3.0, present in chloral hydrate and III, may be OH stretching (hydrogen-bonded). In the 6-μ region, III has only one band, whereas thiosemicarbazide has three (NH_2-bending),[19] and, in particular, III does not have any bands corresponding to carbonyl or C=N absorption, the latter being visible in the spectrum of II (6.0 μ). The structure of the chloral hydrate semicarbazones is therefore best represented by a formula of the type IIIc.

Neither glyoxylic acid methylthiosemicarbazone (V) nor the thiosemicarbazone could be prepared by acid hydrolysis of the chloral hydrate thiosemicarbazones or by condensing chloral hydrate with the thiosemicarbazide hydrohalides. No crystalline product was isolated from the attempted cyclization of chloral hydrate methylthiosemicarbazone (IV) with sodium in ethanol. However, the controlled hydrolysis of IV gave 6-azauracil (I) in one step. In this unusual reaction, the hydrolysis of all the chlorines and of the methylthio group as well as cyclization have taken place.



IV $\quad$ I



V

The hydrolysis of the CCl_3 group followed by elimination of water (*cf.* the hydrolysis of chloral hydrate semicarbazone to glyoxylic acid semicarbazone (II)) would give the hypothetical glyoxylic acid methylthiosemicarbazone (V), which would not be expected to cyclize in aqueous acid solution, particularly as the corresponding semicarbazone II does not. If it is assumed that the CCl_3 group is not immediately hydrolyzed, the mechanism of the reaction can be formulated as a series of simple eliminations of hydrogen chloride and water lead-

(11) Not glyoxylic ester semicarbazone, as stated in ref. 10.

(12) W. Seibert, *Ber.*, **80**, 498 (1947).

(13) A. Darapsky and M. Prabhakar, *ibid.*, **45**, 2624 (1912). Compound II was first prepared in this way for a synthesis of azauracil by workers of the Squibb Institute for Medical Research.

(14) A. Kling, *Compt. rend.*, **148**, 569 (1909).

(15) G. Knopfer, *Monatsh.*, **32**, 768 (1911).

(16) The spectra were measured on a Perkin–Elmer double beam instrument, model 21; s = strong, vw = very weak; all other bands are of medium intensity.

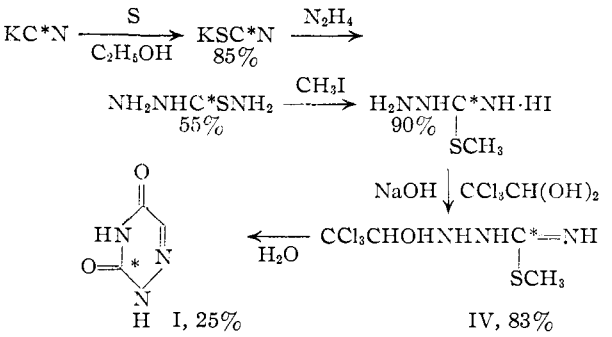
(17) (a) In chloroform; (b) in pressed potassium bromide disks.

(18) L. J. Bellamy, "The Infra-Red Spectra of Complex Molecules," Methuen & Co., Ltd., London, 1954.

(19) *Cf.*, thiourea, bands at 6.15 and 6.22 (A. Yamaguchi, R. B. Penland, S. Mizushima, T. J. Lane and J. V. Quagliano, THIS JOURNAL, **79**, 527 (1957)).

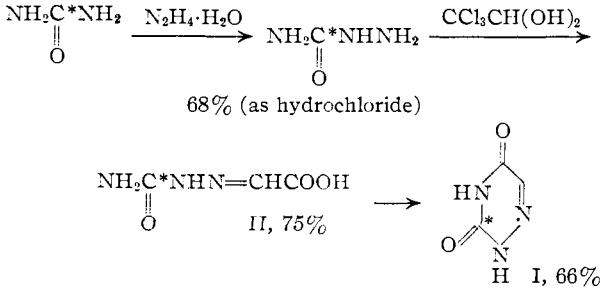
ing to a substituted *asym*-triazine which could give rise to 6-azauracil by hydrolysis.[20]

For this reaction to be used in a synthesis of 6-azauracil-2-C^{14}, a method of preparing thiosemicarbazide-C^{14} was required. Potassium cyanide was converted to the thiocyanate by refluxing with sulfur in ethanol, and the thiocyanate, by a modification of the method of Freund and Schander,[21] to thiosemicarbazide. Reasonable yields were obtained on a millimolar scale in these reactions, which would be suitable for syntheses with C^{14} or S^{35}. Thus, a possible synthesis of labeled 6-azauracil was afforded.



As the over-all yield would be less than 10%, other methods of synthesis were investigated. It was found that, using the method of Guha and Mistry,[22] urea could be converted directly to semicarbazide, which meant that glyoxylic acid semicarbazone (II) could be prepared readily from the available urea-C^{14}. Other conditions for the cyclization of II were explored. No reaction took place with sodium in absolute ethanol, apparently because the sodium salt of II is insoluble in this solvent. However, it is soluble in ethylene glycol, so that it is possible to use an ethylene glycol–ethanol mixture, varying the proportions of the solvents to give that reflux temperature which results in the best yield. In this way it is possible to carry out the cyclization in anhydrous, homogeneous solutions, raising the yield in the final step from 20 to 66%.

6-Azauracil-2-C^{14} was synthesized in three steps as



The over-all yield was 30%, based on urea-C^{14}. It may be noted that this method gives a two-step synthesis of 6-azauracil from semicarbazide hydrochloride and chloral hydrate, with a yield of 50%.

With a view to preparing an intermediate that might be used in a synthesis of the difficulty-available 6-azacytosine.[10] the chlorination of azauracil was studied. No product could be identified from reactions with phosphorus oxychloride, with or without dimethylaniline or phosphorus pentachloride. Therefore, 5-bromo-6-azauracil, which should be less reactive, was prepared and chlorinated with phosphorus oxychloride. Vacuum distillation of the product gave an oil which could not be freed completely from phosphorus oxychloride, and which did not appear to contain bromine. It is believed to be trichloro-*asym*-triazine, since treatment with methanol followed by crystallization from water gave a chloro-dihydroxy-*asym*-triazine. This compound has an infrared spectrum which is so similar to that of 5-bromo-6-azauracil in the 2–9 μ region that it may be regarded as the 5-chloro derivative.

In the course of a synthesis of thymine-$C^{14}H_3$,[23] it was found that a pyrimidinelithium derivative, prepared from the corresponding bromo compound, reacts in the usual manner with water to give the unsubstituted pyrimidine. By treating 5-bromo-6-azauracil with three equivalents of butyllithium and subsequently with water, 6-azauracil could be obtained. It was hoped to use this reaction in a synthesis of 6-azauracil-5-H^3, but the hydrogen atom in the 5-position of 6-azauracil was found to be too labile. Thus, simple recrystallization of 6-azauracil from tritiated water led to the exchange of 2.6 hydrogen atoms in the molecule, and under more vigorous conditions, all three were completely exchanged.

Acknowledgment.—The authors thank Dr. A. D. Welch for his interest in and encouragement of this work.

Experimental[24]

Chloral Hydrate Thiosemicarbazone (III).—To a solution of thiosemicarbazide (0.91 g., 0.01 mole) in water (10 ml.) was added chloral hydrate (1.65 g., 0.01 mole). After brief shaking, a colorless crystalline precipitate of **chloral hydrate thiosemicarbazone** separated from the clear solution; yield 2.28 g. (96%), m.p. 104–105° dec. The analytical sample was recrystallized from ethyl acetate and petroleum ether.

Anal. Calcd. for $C_3H_6ON_3Cl_3S$: C, 15.1; H, 2.5; N, 17.6. Found: C, 15.4; H, 2.5; N, 17.9.

Chloral Hydrate Methylthiosemicarbazone (IV).—A solution of S-methylthiosemicarbazide hydroiodide[25] (8.2 g., 0.035 mole) and chloral hydrate (6.3 g., 0.035 mole) in water (35 ml.) was made alkaline with sodium hydroxide solution (1 *N*) to *p*H 9 to 10. **The chloral hydrate methylthiosemicarbazone** separated as a colorless solid; yield 6.95 g. (83%). The analytical sample was recrystallized from ethyl acetate and petroleum ether; m.p. 86–87° dec.

Anal. Calcd. for $C_4H_6ON_3Cl_3S$: C, 19.0; H, 3.2; N, 16.6. Found: C, 19.2; H, 3.2; N, 16.6.

6-Azauracil (I) from IV.—A solution of IV (1.0 g.) in water (50 ml.) was refluxed gently for 1 hour. The condenser was removed and the solution concentrated by gentle boiling to a volume of 12 ml. On refrigeration overnight,

(20) P. K. Chang and T. L. V. Ulbricht, Abstracts Miami A.C.S. Meeting, April, 1957, 61-O.

(21) M. Freund and A. Schander, *Ber.*, **29**, 2500 (1896). We wish to thank our colleague, Dr. H. G. Mautner, for his advice concerning this reaction.

(22) S. M. Mistry and P. C. Guha, *J. Indian Chem. Soc.*, **7**, 795 (1930).

(23) T. L. V. Ulbricht, unpublished.

(24) Melting points are uncorrected. Analyses by Huffman Microanalytical Labs., Wheatridge, Colo. Petroleum ether refers to the fraction b.p. 65–110°.

(25) E. Cattelain, *Bull. soc. chim.*, **11**, 256 (1944).

0.2 g. (25%) of crude 6-azauracil was obtained. After recrystallization from water it had m.p. 268–270°; mixture m.p. with an authentic sample[9] 268–270°.

Potassium Thiocyanate from Potassium Cyanide.—A mixture of potassium cyanide (133.2 mg., 2.04 millimoles) and sulfur (71.8 mg., 2.24 millimoles) in absolute ethanol (2 ml.) was refluxed for three hours. The potassium thiocyanate which separated after cooling was filtered, and a second crop obtained by the addition of petroleum ether to the filtrate. The total yield of crude product, m.p. 173–176°, was 200 mg. (98%).

Thiosemicarbazide from Potassium Thiocyanate.—A mixture of hydrazine sulfate (257 mg., 1.97 millimoles) and potassium carbonate (129 mg.) was dissolved in water (1.5 ml.) with slight warming. Potassium thiocyanate (193 mg., 1.98 millimoles) was added and the solution refluxed for 10 minutes. Boiling ethanol (95%, 1.25 ml.) was added, and the hot solution filtered. The precipitate was washed with a little ethanol. The combined filtrate and washings were reduced to dryness and kept at 120° on a water aspirator for 2 hours. Water (0.3 ml.) was added to the cooled residue, which was filtered to give crude thiosemicarbazide, m.p. 175–179° (90.6 mg.). The filtrate was concentrated in the same way to give an additional 17.4 mg.; total yield 108 mg. (57%).

Semicarbazide-C^{14}-Hydrochloride from Urea-C^{14}.—Mistry and Guha[22] gave no experimental details of the conversion of urea to semicarbazide. The following conditions gave the best yield: a mixture of urea-C^{14} (240 mg., 4 millimoles) and hydrazine hydrate (99–100%) (210 mg., 4.2 millimoles) in isoamyl alcohol (0.64 ml.) and absolute ethanol (0.60 ml.) was refluxed with a double-surface condenser for 12 hours. Some solid separated on cooling, and the solution was reduced to dryness on a water aspirator at room temperature. The residue of crude semicarbazide was dried in a desiccator *in vacuo* and dissolved in absolute ethanol (3 ml.). Dry hydrogen chloride was passed into the solution to give semicarbazide-C^{14} hydrochloride (354 mg., 79%) (m.p. of product obtained in cold run, 163–167°).

Glyoxylic Acid Semicarbazone-C^{14} (II) from Semicarbazide-C^{14} Hydrochloride.—A solution of semicarbazide-C^{14} hydrochloride (354 mg., 3.17 millimoles) and chloral hydrate (542 mg., 3.28 millimoles) in water (7 ml.) was refluxed gently for 25 minutes. The solution was chilled in ice, filtered and the product washed with ethanol and ether. After drying in air the yield was 318 mg. (77%) (m.p. of product obtained in cold run, 200–202°).

6-Azauracil-2-C^{14} (I) from II.—A solution of glyoxylic acid semicarbazone-C^{14} (318 mg., 2.42 millimoles) in ethylene glycol (10 ml.) was added rapidly to sodium (180 mg., 7.8 millimoles) dissolved in absolute ethanol (5 ml.) and the clear solution refluxed gently for 24 hours. After reducing the solution to dryness on a water aspirator at 120°, the residue was dissolved in hot water (5 ml.) and the hot solution adjusted to *p*H 2 with concentrated hydrochloric acid. 6-Azauracil-2-C^{14} crystallized on cooling; yield 160 mg. (66%). After recrystallization from water it had m.p. 268–270°, mixture m.p. with an authentic sample 268–270°. (6-Azauracil-2-C^{14} was recovered from the mother liquors by ion-exchange chromatography by our colleague, Dr. R. E. Handschumacher.)

5-Bromoazauracil.[26]—A mixture of 6-azauracil (5 g.), bromine (5 ml.) and water (75 ml.) was stirred with a magnetic stirrer for 27 hours. The colorless crystalline product was filtered and dried (4.7 g.). Concentration of the filtrate gave an additional 2.94 g. (total yield of **5-bromo-6-azauracil** 90%). After recrystallization from water it had m.p. 232–234°.

Anal. Calcd. for $C_3H_2BrN_3O_2$: C, 18.8; H, 1.0; N, 21.9; Br, 41.6. Found: C, 18.8; H, 1.0; N, 22.0; Br, 41.6.

Chlorination of 5-Bromo-6-azauracil and Hydrolysis to Chlorodihydroxy-*asym*-triazine.—A mixture of 5-bromo-6-azauracil (7.8 g.) and phosphorus oxychloride (40 ml.) was refluxed (bath temp. 125°) until the mixture became homogeneous (48 hours). Most of the excess phosphorus oxychloride was removed on a water aspirator, and the residue distilled *in vacuo*, to give a colorless oil, b.p. 72° (3 mm.) (yield 2.2 g.). The oil (1.93 g.) was added dropwise to methanol (6 ml.). Hydrogen chloride was evolved and the solution became hot. The solution was concentrated to give a colorless substance (1.2 g.) which was recrystallized three times from water. The **chlorodihydroxy-*asym*-triazine** had m.p. 225–227° (begins to soften at 200°).

Anal. Calcd. for $C_3H_2ClN_3O_2$: C, 24.4; H, 1.4; N, 28.5; Cl, 24.0. Found: C, 24.5; H, 1.3; N, 28.3; Cl, 23.8.

6-Azauracil from 5-Bromo-6-azauracil.—A solution of 5-bromo-6-azauracil (200 mg.) in absolute tetrahydrofuran (25 ml.) was cooled to −70°, and ethereal *n*-butyllithium[27] (0.82 *N*, 5 ml.) added slowly. To the stirred orange-colored solution was added water (1 ml.), the cooling bath removed, and the solution allowed to warm up to room temperature. The solvents were removed under reduced pressure, and the residue dissolved in hot water (3 ml.) and immediately acidified to *p*H 2 with 6 *N* hydrochloric acid. 6-Azauracil (15 mg., 17%) crystallized from the cooled solution; m.p. and mixture m.p. 268–270°.

(26) This compound was first prepared by Dr. R. E. Handschumacher.

(27) H. Gilman, E. A. Zoellner and W. M. Selby, THIS JOURNAL, **55**, 1252 (1933).

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