TABLE III

ANALYSES OF S-BENZYLTHIOCARBONYL AMINO ACID PHENYLHYDRAZIDES

S-Benzylthiocarbonyl phenylhydrazides of	Formula	Calcd., % C	H	N	S	Found, % C	H	N	S
Glycine	$C_{16}H_{17}N_3O_2S$	61.00	5.40	13.32	10.15	60.80	5.25	13.10	9.95
L-Alanine	$C_{17}H_{19}N_3O_2S$	62.00	5.78	12.75	9.75	62.0	5.83	12.70	9.71
L-Leucine	$C_{20}H_{25}N_3O_2S$	64.70	6.75	11.3	8.63	64.5	6.62	11.5	8.53

was saturated with hydrogen sulfide. The resulting lead sulfide was filtered and filtrate was evaporated to dryness *in vacuo*. The residue was dried in a vacuum desiccator overnight. The solid residue was recrystallized three times from chloroform–petroleum ether (30–60°). The yield of colorless needles (m.p. 118–120°) was 0.2 g. (35.9%) (reported[17] 118–120°), assumed to be ethyl hydantoin-3-acetate.

Anal. Calcd. for $C_7H_{10}N_2O_4$: C, 45.16; H, 5.41; N, 15.05. Found: C, 45.30; H, 5.55; N, 15.10.

Reaction of S-Benzylthiocarbonylglycine Phenylhydrazide with Lead Acetate.—Lead acetate (10.0 g.) was dissolved in 1 l. of warm 95% ethanol. To this solution was added 15.2 g. of S-benzylthiocarbonylglycine phenylhydrazide. The temperature of the mixture was maintained at 75–80° until the suspension of lead benzyl-mercaptide settled (*ca.* 40 min.). The solution was then filtered and the filtrate was saturated with hydrogen sulfide. The resulting lead sulfide was removed by filtering through a mat of Filter-Cel. The filtrate was evaporated to dryness and the solid residue was recrystallized first from 95% ethanol then from chloroform to yield 6.05 g. (59.2%) of 2-phenyl-3,6-dioxohexahydro-1,2,4-triazine, m.p. 165.0–166.5°.

Anal. Calcd. for $C_9H_9N_3O_2$: C, 56.54; H, 4.75; N, 21.98. Found: C, 56.84; H, 4.63; N, 22.00.

The above triazine (0.5 g.) was heated with 20 g. of acetyl chloride. The mixture was evaporated on a steam-bath to form a brown oil which crystallized from benzene–petroleum ether yielding 0.22 g. of white crystals, m.p. 157–158°.

Anal. Calcd. for $C_{13}H_{13}N_3O_4$: C, 56.72; H, 4.76; N, 15.26. Found: C, 56.64; H, 4.82; N, 15.30.

Reaction of S-Benzylthiocarbonylmethionine Phenylhydrazide with Lead Acetate.—This reaction was carried out in the same manner as the above using 0.78 g. (0.002 mole) of S-benzylthiocarbonylmethionine phenylhydrazide, 0.42 g. (0.0011 mole) of lead acetate and 40 ml. of 70% ethanol. The reaction mixture was heated on a hot water-bath for 8 min. The crude product was recrystallized three times from ethanol–water. The yield of triazine (m.p. 122–123.5°) was 0.4 g. (75.5%).

Anal. Calcd. for $C_{12}H_{13}N_3O_2S$: C, 54.32; H, 5.69; N, 15.84; S, 12.08. Found: C, 54.48; H, 5.84; N, 15.61; S, 12.20.

Reaction of S-Benzylthiocarbonylglycine Phenylhydrazide with Ferric Chloride.—A solution of 16 g. of ferric chloride hexahydrate dissolved in 25 ml. of distilled water was added to a solution of 8 g. of S-benzylthiocarbonylglycine phenylhydrazide dissolved in 30 ml. of acetone. After the addition was complete, the solution was refluxed for 3 hr. The solution was distilled until 25 ml. of the acetone had been removed. The solution was then cooled overnight in a refrigerator. The resulting crystals were filtered and recrystallized from chloroform; yield 5.1 g. (94%), m.p. 151–152°, mixed melting point with an authentic sample of S-benzylthiocarbonylglycine 151–152°.

(17) R. Locquin and V. Cerchez, *Bull. soc. chim.*, **49**, 309 (1939).

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL SCIENCES, STANFORD RESEARCH INSTITUTE]

Potential Anticancer Agents.[1] XXXVI. Alkylating Agents Derived from 5-Aminouracil

BY ALLEN BENITEZ, LEONARD O. ROSS, LEON GOODMAN AND B. R. BAKER

RECEIVED MARCH 3, 1960

Two monofunctional alkylating agents, 5-(2-chloroethylamino)-uracil (V) and 5-[2-chloroethyl)-ethylamino]-uracil (VIII), were synthesized for comparison of effectiveness as antitumor compounds with difunctional alkylating agents. An unknown material, obtained *via* the hydroxyethylation of 5-(ethylamino)-uracil (III), was identified as 3-(2-chloroethyl)-5-[(2-chloroethyl)-ethylamino]-uracil (XIIa) by comparison of its ultraviolet spectra with those of the previously unknown compounds, 5-[bis-(2-chloroethyl)-amino]-3-methyluracil (XVIII) and 5-[bis-(2-chloroethyl)-amino]-1-methyluracil (XXVI).

Recently the hypothesis was put forward that alkylating agents consist of a carrier and the alkylating group and that differences in effects and side effects on tumors might be related to the differences in the carrier group.[2] More recently this hypothesis was expanded into a rationale for the design of specific irreversible enzyme inhibitors.[3] This rationale proposed that substrates, properly substituted by an alkylating group, could fit the specific enzyme site for the substrate, then replace a nearby active hydrogen by alkylation, thus resulting in specific irreversible inactivation of the enzyme.

Recent synthetic work in the nitrogen mustard field has resulted in several promising anticancer compounds, such as phenylalanine mustard (sarcolysin),[4] *m*-phenylalanine mustard,[3a,5] chlorambucil,[6] uracil mustard[7] and benzimidazole mustard.[8] All of the above compounds are so-called "two-

(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, *cf.* E. J. Reist, H. P. Hamlow, I. G. Junga, R. M. Silverstein and B. R. Baker, *J. Org. Chem.*, **25**, 1455 (1960).

(2) F. Bergel, *Ann. N. Y. Acad. Sci.*, **68**, 1238 (1958).

(3) (a) H. F. Gram, C. W. Mosher and B. R. Baker, THIS JOURNAL, **81**, 3103 (1959); (b) B. R. Baker, Cancer Chemotherapy Reports No. **4**, p. 1 (1959), a publication of the National Cancer Institute.

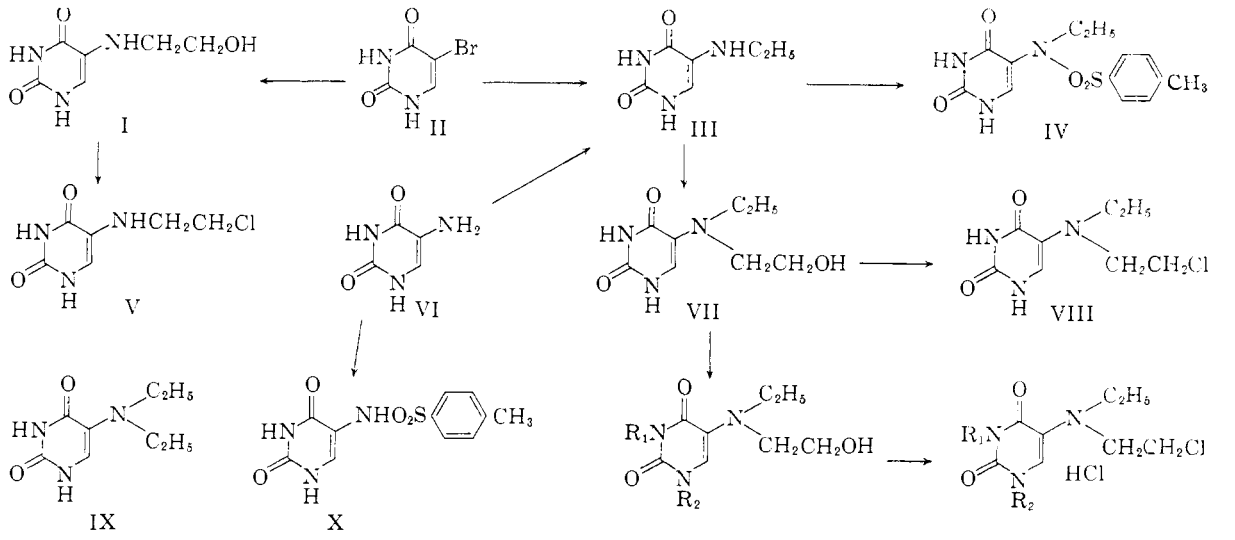
(4) F. Bergel, V. C. E. Burnop and J. A. Stock, *J. Chem. Soc.*, 1223 (1955); L. F. Larionov, A. S. Khokhlov, E. N. Shkodinskaia, O. S. Vasina, V. I. Trusheikina and A. M. Novikova, *Lancet*, **269**, 169 (1955).

(5) T. S. Osdene, D. N. Ward, W. H. Chapman and H. Rakoff, THIS JOURNAL, **81**, 3100 (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, THIS JOURNAL, **80**, 6459 (1958); D. A. Lyttle and H. G. Petering, *J. Natl. Cancer Inst.*, **23**, 153 (1959).

(8) E. Hirschberg, A. Gellhorn and W. S. Gump, *Cancer Research*, **17**, 904 (1957).



XIa, $R_1 = CH_2CH_2OH$, $R_2 = H$
b, $R_1 = H$, $R_2 = CH_2CH_2OH$

XIIa, $R_1 = CH_2CH_2Cl$, $R_2 = H$
b, $R_1 = H$, $R_2 = CH_2CH_2Cl$

armed" mustards in which a bis-(chloroethyl)-amino group is attached to a carrier which is a metabolite or resembles a metabolite. Keeping in mind the rationale for the design of specific irreversible enzyme inhibitors,[3] it can be seen that the attachment of a monochloroethylamino group to the proper substrate should give such an enzyme inhibitor and that such a "one-armed" mustard might possess a much more specific action as an anticancer agent than the corresponding "two-armed" mustard. This manuscript reports the preparation of some compounds designed to test the rationale.

Uracil was chosen as the carrier (substrate) for the synthesis of a number of monofunctional alkylating agents. The biological importance of uracil and of compounds containing the uracil moiety[9] leads logically to its choice as a carrier; the recent announcement of the broad spectrum of anticancer activity of the difunctional uracil mustard (XXV)[7] in animals lends added interest to the synthesis of the monofunctional compounds and gives a standard of comparison in testing. In the course of the work two bis-(2-chloroethyl)-amino compounds were also prepared and their syntheses are described.

The first efforts toward the synthesis of 5-(ethylamino)-uracil (III), a precursor of the desired "one-armed" mustard (VIII), involved the catalytic reductive alkylation of 5-aminouracil (VI) with acetaldehyde using Raney nickel.[10] Mixtures of III and 5-(diethylamino)-uracil (IX) resulted regardless of the relative amounts of VI and acetaldehyde that were used. Macro amounts of the mixture of III and IX were readily separable by chromatography on seed test paper,[11] but this method was not practical for the large amounts of III required. The reaction of 5-bromouracil (II) with aqueous ethylamine at 160° gave a 66% yield of III as a crystalline, chromatographically homogeneous solid. Previously, Johnson and Matsuo[12] had reported the similar reaction of II with aqueous methylamine to give 5-(methylamino)-uracil. Under similar conditions the reaction of 5-bromouracil (II) with aqueous 2-aminoethanol gave 68–72% of 5-(2-hydroxyethylamino)-uracil (I). In the course of the work directed toward the synthesis of III, both 5-aminouracil (VI) and 5-(ethylamino)-uracil (III) were converted to their N-*p*-toluenesulfonyl derivatives X and IV, respectively.

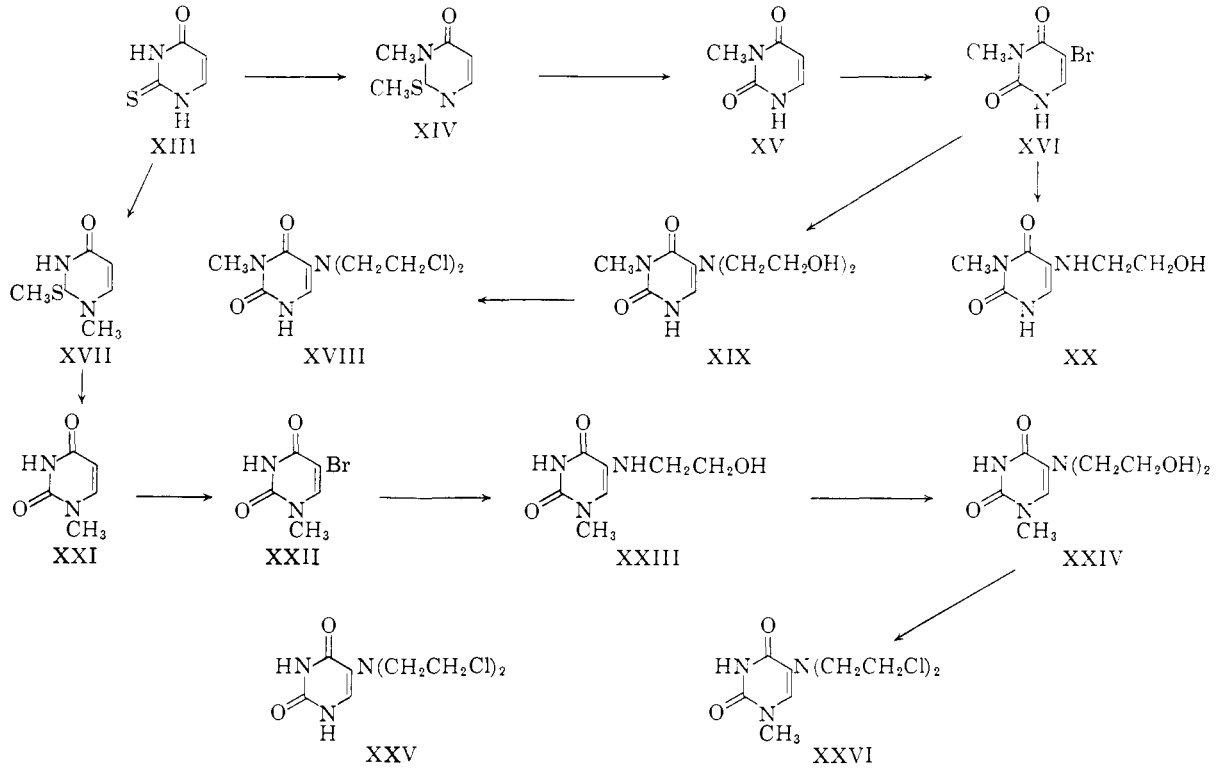
When the reaction of III with ethylene oxide was carried out using the conditions described for the bis-hydroxyethylation of 5-aminouracil (VI),[7] the product, isolated in small yield as the picrate, was shown by analysis to have incorporated two hydroxyethyl groups and proved to be XIa (*cf.* below). The hydroxyethylation of III, therefore, required attention as to the reaction time and relative quantities of III and ethylene oxide used. The disappearance of III from the reaction mixture was followed by paper chromatography using solvent system A (*cf.* Experimental). When chromatography showed that no more III was present, the excess ethylene oxide was evaporated and the product was adsorbed on an acid ion exchange resin[7] to separate it from polymers derived from ethylene oxide. Elution with ammonium hydroxide gave, after evaporation of the eluate, the water-soluble solid which could be recrystallized from acetonitrile to give 44% of VII. When the reaction of III with a larger excess of ethylene oxide was allowed to run for much longer times, the major product, isolated as in the case of VII, was a crystalline solid whose melting point and paper chromatographic behavior were very similar to those of VII, but whose analysis showed the presence of an

(9) For a review of uracil and compounds containing uracil, *cf.* chapters by A. Bendich, p. 81, and J. Baddiley, p. 137, in "The Nucleic Acids," Vol. I, E. Chargoff and J. N. Davidson, ed., Academic Press, Inc., New York, N. Y., 1955.

(10) W. S. Emerson in "Organic Reactions," Vol. IV, Roger Adams, ed., John Wiley and Sons, Inc., New York, N. Y., 1948, p. 174.

(11) H. H. Brownell, J. G. Hamilton and A. A. Casselman, *Anal. Chem.*, **29**, 550 (1957).

(12) T. B. Johnson and I. Matsuo, THIS JOURNAL, **41**, 782 (1919).



additional hydroxyethyl group and whose mixed melting point with VII was depressed. The new compound had the general ultraviolet and infrared spectral behavior expected for a uracil derivative still in the lactam form and was assigned the (di-2-hydroxyethyl)-aminouracil structure XI; it could be isolated in 44% yield as a crystalline compound. The picrates of VII and XI melted similarly but gave a strongly depressed mixed melting point.

The reactions of I, VII and XI with thionyl chloride to give the desired chloroethyl compounds required individual study. 5-(2-Hydroxyethylamino)-uracil (I) was heated at reflux for several hours with excess thionyl chloride to give, after evaporation, a very dark residue of the hydrochloride of V. The aqueous solution of this salt readily gave the free base V with aqueous sodium bicarbonate and this could be recrystallized with good recovery.

Compound VII, on prolonged heating with thionyl chloride, gave much black, tarry material; when the time of heating was carefully controlled, a fair yield of the hydrochloride of VIII was obtained. The use of the conditions of Lyttle and Petering,[7] thionyl chloride in 1,2-dimethoxyethane with small amounts of water and ethanol, gave good yields of the hydrochloride of VIII, which was easily converted to the free base VIII by precipitation from aqueous solution with sodium bicarbonate.

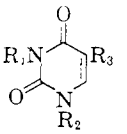
The *di*-hydroxyethyl compound XI seemed to be the most stable of the three compounds, I, VII and XI, in hot thionyl chloride. It darkened only slightly after being heated with a large excess of the halogenating agent at 80° for six hours and, on evaporation, gave an excellent yield of the dichloroethyl hydrochloride XII as an analytically pure compound without further purification. Efforts to precipitate the free base of XII from its aqueous solution were unsuccessful.

In an effort to assign structure XIa or XIb to the di-hydroxyethylated compound from the reaction of III and ethylene oxide, two series of compounds based on 5-amino-3-methyluracil and 5-amino-1-methyluracil were prepared as ultraviolet spectral standards. The conversion of 2-thiouracil (XIII) to 3-methyluracil(XV), *via* 3-methyl-2-(methylthio)-4(3H)-pyrimidinone (XIV), and to 1-methyluracil (XXI), *via* 1-methyl-2-(methylthio)-4(1H)-pyrimidinone (XVII), was carried out by the excellent procedure of Brown, Hoerger and Mason.[13] Both XV and XXI reacted with bromine in glacial acetic acid[12] to give good yields of the corresponding 5-bromouracils XVI and XXII. Both the bromo compounds XVI and XXII reacted readily with amines at high temperatures, as previously noted by Johnson and Matsuo,[12] and behaved like II in this respect. The 3-methyluracil XVI with diethanolamine gave a fair yield of the crystalline bis-(2-hydroxyethyl)-amine XIX and with 2-aminoethanol gave a good yield of the mono-(2-hydroxyethyl)-amine XX. The 1-methyluracil XXII with diethanolamine gave a sirup from which the bis-(2-hydroxyethyl)-amine XXIV could not be isolated in any state of purity. The reaction of XXII with 2-aminoethanol, however, gave a fair yield of the mono-(2-hydroxyethyl)-amine XXIII as a crystalline compound which could be converted in good yield to crystalline XXIV by a further reaction with ethylene oxide in aqueous acetic acid. The ultraviolet spectral data for these substituted uracils are collected in Table I.

(13) D. J. Brown, E. Hoerger and S. F. Mason, *J. Chem. Soc.*, 211 (1955).

TABLE I

ULTRAVIOLET SPECTRA OF SUBSTITUTED URACILS[c]



Compound	R_1	R_2	R_3	pH 1 λ	pH 1 ε	pH 7[a] λ	pH 7[a] ε	pH 13 λ	pH 13 ε
Uracil	H	H	H	259	8200			283	6150
XXI	H	CH_3	H	267	9360	268	9400	265	6840
XV	CH_3	H	H	258	7180	259	7180	283	10600
II	H	H	Br	276	7770			291	7070
XXII	H	CH_3	Br	283	8840	284	8640	279	6190
XVI	CH_3	H	Br	276	7380	276	6640	298	10400
I	H	H	$NHCH_2CH_2OH$	261	7150	234	7490	293	4150
						296	5190		
XXIII	H	CH_3	$NHCH_2CH_2OH$	270	9020	303	6040	294	5180
XX	CH_3	H	$NHCH_2CH_2OH$	261	6640	296	4750	309	7250
III	H	H	NHC_2H_5	262	6390	234	7940	228[b]	8660
						296	5190	291	3830
VII	H	H	$N(Et)CH_2CH_2OH$	262	7440	298[b]	2800	292	5460
XXIV	H	CH_3	$N(CH_2CH_2OH)_2$	272	8680	264[b]	4650	263[b]	4010
						305[b]	2800		
XIX	CH_3	H	$N(CH_2CH_2OH)_2$	262	6440	253[b]	4550	291	7580
						295[b]	2080		
XI	?	?	$N(Et)CH_2CH_2OH$	260	5660	264	4430	277	5560
						290[b]	2710		
VIII	H	H	$N(Et)CH_2CH_2Cl$	263	6790	262[b]	4100	292	5220
						295[b]	2500		
XXVI	H	CH_3	$N(CH_2CH_2Cl)_2$	271	7890	263[b]	4900	260[b]	4400
						310[b]	2700	305[b]	2100
XVIII	CH_3	H	$N(CH_2CH_2Cl)_2$	263	6140	255	4110	291	7380
						303[b]	1700		
XII	?	?	$N(Et)CH_2CH_2Cl$	262	6720	284	6500	227	9010
								282	5000

[a] *p*H 7 buffer. [b] Shoulder. [c] An excellent study of the ultraviolet spectra of substituted pyrimidines with emphasis on the uracils is found in a paper by D. Shugar and J. J. Fox, *Biochim. et Biophys. Acta*, **9**, 199 (1952).

A comparison of the ultraviolet spectrum of XI with the spectra of the 1-methyl- and 3-methyl-5-(N,N-disubstituted)-aminouracils XXIV and XIX indicates that XI is most probably the 3-(2-hydroxyethyl)-substituted compound XIa. The spectra of XI and XIX agree well in acid solution; it is noteworthy in Table I that, at *p*H 1, 3-methyl substitution does not change significantly the position of the wave length maximum of the parent uracil (compare uracil and XV, II and XVI, I and XX, and VII and XIX). At *p*H 13, this regularity does not exist and the large difference in the wave length maximum between XI and the model compound XIX in basic solution does not seem to be an argument against the assumption of structure XIa.

The reaction of the bis-(2-hydroxyethyl)-amines XXIV and XIX with hot thionyl chloride required careful control to avoid extensive decomposition. The mild procedure of Lyttle and Petering[7] was preferable and gave a good yield of the (bis-2-chloroethyl)-uracil XVIII as the hydrochloride, which was converted to the free base XVIII by neutralization with aqueous sodium bicarbonate. The same procedure with XXIV gave a product as a hydrochloride which seemed to lose easily part of the hydrogen chloride on standing; it was best isolated as the free base XXV by neutralization of the aqueous solution of the salt with sodium bicarbonate and extraction of the soluble XXV with chloroform.

Comparison of the ultraviolet spectral data (Table I) for the di-2-chloroethylamine XII with XVIII and XXVI also suggests that XII is the 3,5-disubstituted compound XIIa, in agreement with the conclusions reached regarding its precursor, di-2-hydroxyethylamine XI.

Biological evaluation of the chloroethylamino compounds against animal tumor systems is in progress.

Experimental[14]

5-(2-Hydroxyethylamino)-uracil (I).—A mixture of 1.00 g. (5.23 mmoles) of 5-bromouracil (II) and 1.27 g. (20.9

(14) Melting points are uncorrected and 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,[15] isopropyl alcohol–2 *N* aqueous hydrochloric acid (65:35); B,[16] 1-butanol saturated with water; C,[17] 5% disodium hydrogen phosphate (no organic phase); D,[18] 1-butanol–acetic acid–water (5:2:3), and E,[19] 2-methoxyethanol–water (9:1).

(15) G. R. Wyatt, *Biochem. J.*, **48**, 584 (1951).

(16) J. G. Buchanan, C. A. Dekker and A. G. Long, *J. Chem. Soc.*, 3162 (1950).

(17) C. E. Carter, THIS JOURNAL, **72**, 1835 (1950).

(18) D. M. Brown, A. Todd and S. Varadarajan, *J. Chem. Soc.*, 2388 (1956).

mmoles) of 70% aqueous 2-aminoethanol was heated at 160° for 5 hours in a stainless steel bomb. The bomb was cooled to room temperature and 20 ml. of methanol was added. Filtration gave 0.70 g. (80%) of product, m.p. 258–261° dec. It was recrystallized from 10 ml. of hot water with the aid of Norit to give 0.50 g. (56%) of I, m.p. 275–277° dec.; $\lambda_{max\mu}^{Nujol}$ 2.95 and 3.05 (OH), 3.14 and 3.26 (NH), 5.71, 5.90 and 5.99 (uracil C=O), 9.46 (C—OH). On paper chromatography in solvents A and B, it moved as a single spot with R_{ad} 1.10 and 0.60, respectively.

Anal. Calcd. for $C_6H_9N_3O_3$: C, 42.1; H, 5.39; N, 24.5. Found: C, 41.8; H, 5.32; N, 24.3.

The use of 15 g. of 5-bromouracil in the above procedure gave 9.2–9.8 g. (67–72%) of I, which was identical with the analytical sample according to infrared spectrum and paper chromatography.

The picrate of I was formed by mixing hot aqueous solutions of I and picric acid. It was recrystallized from water (40 ml./g.) and had m.p. 207–208° dec.

Anal. Calcd. for $C_{12}H_{12}N_6O_{10}\cdot 1/2H_2O$: C, 35.2; H, 3.20; N, 20.5. Found: C, 35.0; H, 3.44; N, 20.2.

Reductive Ethylation of 5-Aminouracil (VI).—A mixture of 2.0 g. (15.7 mmoles) of 5-aminouracil (VI) and approximately 12 g. of Raney nickel[20] (washed with three 50-ml. portions of 95% ethanol and two 50-ml. portions of absolute ethanol and finally with 100 ml. of absolute ethanol) was placed in a pressure bottle and the bottle flushed with nitrogen. Acetaldehyde (2.5 ml., 44 mmoles) was added while the nitrogen stream was maintained. The resulting mixture was shaken with hydrogen at 23–29° and 30 p.s.i. for 8 hours. The pressure bottle was heated on the steam-bath and the contents filtered by gravity. The Raney nickel residue was washed with two 30-ml. portions of hot absolute ethanol and the filtrate plus washings were evaporated *in vacuo*, leaving 1.79 g. of a white solid which showed two spots on paper chromatography in solvent A with R_{ad} 1.23 (III) and R_{ad} 1.69 (IX). Efforts to separate the two materials by crystallization were unsuccessful.

In another reductive alkylation where a much larger amount of acetaldehyde was employed, a non-volatile oil was isolated from the hydrogenation mixture. On standing at room temperature for several weeks, the oil deposited a small amount of a crystalline solid consisting of two distinct types of crystals which could be manually separated. One of the crystal forms was chromatographically homogeneous III (R_{ad} 1.23 in solvent A) and had m.p. 265–285°.

Anal. Calcd. for $C_6H_9N_3O_2$: C, 46.4; H, 5.85. Found: C, 45.7; H, 5.78.

The other crystal form was chromatographically homogeneous IX (R_{ad} 1.72 in solvent A) and had m.p. 258–262°.

Anal. Calcd. for $C_8H_{13}N_3O_2$: C, 52.4; H, 7.15. Found: C, 52.0; H, 7.49.

5-(Ethylamino)-uracil (III).—A mixture of 3.0 g. (15.7 mmoles) of 5-bromouracil (II) and 4.1 g. (*ca.* 20.8 mmoles) of 70% aqueous ethylamine was heated in a stainless steel bomb at 160° for 6 hours. The bomb was chilled in ice and opened. The crystalline solid was collected by filtration and washed with cold water; yield 1.41 g. (58%) of product, m.p. 273–276°, which was suitable for conversion to the hydroxyethyl compound VII. A portion of the material (0.37 g.) was dissolved in 75 ml. of absolute ethanol, the solution decolorized with Norit, filtered, and the filtrate chilled to yield 0.061 g. of crystalline solid, m.p. 268.0–274.5°; $\lambda_{max(\mu)}^{KBr}$ 3.15, 3.26 and 6.56 (NH), 5.8–6.0 (uracil C=O). On paper chromatography in solvent A, the material showed a single spot with R_{ad} 1.26.

Anal. Calcd. for $C_6H_9N_3O_2$: C, 46.5; H, 5.85; N, 27.1. Found: C, 46.6; H, 6.13; N, 27.7.

When the reaction was carried out using 60 g. of 5-bromouracil in a stirred autoclave, the yield of III was 66% of material that compared well with the analytical sample in infrared spectrum and paper chromatographic behavior.

The picrate of III was prepared by mixing warm aqueous solutions of III and picric acid and chilling the resulting solution. The precipitate, after recrystallization from water (60 ml./g.), had m.p. 192–193°.

(19) A. E. Bender, *Biochem. J.*, **48**, xv (1951) (Proc. Biochemical Society).

(20) Sponge nickel catalyst, Davison Chemical Co., Cincinnati 29, Ohio.

Anal. Calcd. for $C_{12}H_{12}N_6O_9$: C, 37.3; H, 3.15; N, 21.9. Found: C, 37.4; H, 3.37; N, 21.9.

N-Ethyl-N-(1,2,3,4-tetrahydro-2,4-dioxo-5-pyrimidinyl)-*p*-toluenesulfonamide (IV).—To a warm (70°) solution of 2.60 g. (65.0 mmoles) of sodium hydroxide in 500 ml. of water was added 10.0 g. (64.4 mmoles) of 5-(ethylamino)-uracil (III). After the solid had dissolved, the solution was cooled to room temperature and, while stirring vigorously, 12.3 g. (64.5 mmoles) of *p*-toluenesulfonyl chloride was added in small portions. The mixture was stirred for 22 hours at room temperature at which time the *p*H was *ca.* 7. Pyridine (3 ml.) was added and the mixture was stirred for 10 minutes, then 1.5 ml. of concentrated hydrochloric acid was added to bring the *p*H to 5.5–6.0. The white precipitate was collected and washed to yield 16.7 g. (84.0%) of crystalline solid, m.p. 248–251°, whose infrared spectrum was in excellent agreement with that of the analytical sample below.

In an earlier, small-scale run, the product (0.83 g.), m.p. 243–248°, was dissolved in 14 ml. of hot 2-methoxyethanol and the solution diluted with 47 ml. of hot water. On chilling, 0.68 g. of crystalline solid precipitated, m.p. 249–254°; $\lambda_{max(\mu)}^{KBr}$ 3.05–3.25 (uracil NH), 5.70–5.95 (uracil C=O), 7.47 and 8.66 (SO_2N), 12.32 (*p*-disubstituted phenyl).

Anal. Calcd. for $C_{13}H_{15}N_3O_4S$: C, 50.5; H, 4.89; N, 13.6; S, 10.4. Found: C, 50.0; H, 4.86; N, 13.3; S, 10.1.

5-(2-Chloroethylamino)-uracil (V).—A stirred suspension of 5.00 g. (29.0 mmoles) of 5-(2-hydroxyethylamino)-uracil (I) in 50 ml. of thionyl chloride was heated under reflux for 3 hours using an oil-bath at 90°. The reaction mixture was evaporated *in vacuo* using a bath temperature of 45–50° and to the residue was added 250 ml. of cold water. The aqueous mixture was filtered and the filtrate was adjusted to *p*H 7.5 by the addition of solid sodium bicarbonate to the stirred, chilled filtrate. The resulting dried precipitate (4.1 g., 75%) was dissolved in 150 ml. of hot 2-methoxy-ethanol, the solution treated with *ca.* 0.50 g. of Norit, and the filtrate diluted with 150 ml. of hot acetonitrile. The resultant crystalline product weighed 2.8 g. (51%), m.p. >300°; $\lambda_{max(\mu)}^{Nujol}$ 2.96, 3.15 and 3.25 (NH), 5.69 and 5.97 (uracil C=O), no C—OH at 9.5. On paper chromatography in solvents A and B, the compound moved as a single spot with R_{ad} 1.00 and 1.30, respectively.

Anal. Calcd. for $C_6H_8ClN_3O_2$: C, 38.5; H, 4.25; N, 22.2. Found: C, 38.7; H, 4.67; N, 22.1.

5[(2-Hydroxyethyl)-ethylamino]-uracil (VII).—To a cold solution of 2.0 g. (12.9 mmoles) of 5-(ethylamino)-uracil (III) in 56 ml. of 50% aqueous acetic acid was added 3.0 ml. (*ca.* 60 mmoles) of cold ethylene oxide. The reaction mixture was allowed to stand at room temperature in a stoppered flask for 15 hours. The reaction mixture was evaporated *in vacuo* at 25° to a volume of about 6 ml. Water (35 ml.) and 9 g. of Dowex 50(H) were added to the residue and the resulting mixture was stirred at room temperature for 2 hours and filtered. The resin was washed with three 10-ml. portions of water and the filtrate and washings were discarded. The resin was stirred with 20 ml. of 10% ammonium hydroxide solution for 1.5 hours, the ammoniacal mixture was filtered and the resin was washed with 70 ml. of 5% ammonium hydroxide solution and 50 ml. of water. The combined filtrate and washings were evaporated to dryness *in vacuo*, yielding 2.92 g. of solid. A 1.0-g. portion of the residue was dissolved in 90 ml. of hot acetonitrile, the solution was decolorized with Norit and filtered through Celite. On chilling, 0.42 g. (44%) of crystalline product precipitated, m.p. 207.0–208.5°; $\lambda_{max(\mu)}^{KBr}$ 2.90–2.95 (OH), 3.10 and 3.25 (NH), 5.78 and 6.00 (uracil C=O), 9.55 (C—OH). On paper chromatography in solvent A, the compound moved as a single spot with R_{ad} 1.28.

Anal. Calcd. for $C_8H_{13}N_3O_3$: C, 48.2; H, 6.58; N, 21.1. Found: C, 48.1; H, 6.45; N, 20.7.

The picrate of VII was prepared by mixing aqueous solutions of VII and picric acid and chilling. The compound was recrystallized from water (35 ml./g.) and had m.p. 198.5–199.5°.

Anal. Calcd. for $C_{14}H_{16}N_6O_{10}$: C, 39.3; H, 3.77; N, 19.6. Found: C, 39.2; H, 3.86; N, 19.5, 19.6.

5-[(2-Chloroethyl)-ethylamino]-uracil (VIII).—A mixture of 0.25 g. (1.26 mmoles) of 5-[(2-hydroxyethyl)-ethylamino]-uracil (VII) and 3.0 ml. (41.3 mmoles) of thionyl chloride

was stirred under reflux for 15 minutes using an oil-bath at 80°, and then was allowed to stand at room temperature for 15 minutes. The reaction mixture was evaporated to dryness *in vacuo* at 40°, leaving 0.33 g. of a dark residue. The residue was extracted with 15 ml. of boiling acetonitrile and the brown extract was filtered. The filtrate was reduced to 5 ml. *in vacuo* and the solution chilled at −20°. The crystalline hydrochloride, 0.17 g. (53%), m.p. >300°, was collected; $\lambda_{max(\mu)}^{Nujol}$ 3.23 and 3.30 (NH), 3.80, 3.90 and 4.00 (NH^+), 5.66 and 5.79–5.95 (uracil C=O). On paper chromatography in solvent A, the product moved as a single spot with R_{ad} 1.43.

Anal. Calcd. for $C_8H_{12}ClN_3O_2 \cdot HCl$: C, 37.8; H, 5.16; Cl, 27.9; N, 16.5. Found: C, 38.2; H, 5.23; Cl, 27.9; N, 16.6.

When 1.40 g. (7.03 mmoles) of VII was treated with 2.10 ml. (29.0 mmoles) of thionyl chloride in 1,2-dimethoxyethane in the presence of small amounts of water and ethanol as described by Lyttle and Petering,[7] 1.62 g. (91%) of crude hydrochloride of VIII was obtained. A portion of this material, 0.50 g., was dissolved in 15 ml. of water and the solution filtered. To the cold (0°) filtrate was added dropwise a saturated aqueous sodium bicarbonate solution to bring the *p*H to 8. The precipitate was collected and washed with 25 ml. of cold water to yield 0.32 g. (67%) of VIII, m.p. 166.0–166.5°; $\lambda_{max(\mu)}^{Nujol}$ 3.17 and 3.25 (NH), 5.81 and 5.95 (uracil C=O), 6.11 (C=C), no C—OH absorption near 9.5.

Anal. Calcd. for $C_8H_{12}ClN_3O_2$: C, 44.1; H, 5.56; Cl, 16.3. Found: C, 44.4; H, 5.70; Cl, 16.0.

The free base VIII could be recrystallized from ethyl acetate (100 ml./g.) to give a 90% recovery of product with essentially unchanged melting point and equally good analyses.

N-(1,2,3,4-Tetrahydro-2,4-dioxo-5-pyrimidinyl)-*p*-toluenesulfonamide (X).—To a solution of 0.30 g. (2.36 mmoles) of 5-aminouracil (VI), 0.10 g. (2.50 mmoles) of sodium hydroxide and 20 ml. of water was added 0.45 g. (2.36 mmoles) of *p*-toluenesulfonyl chloride. The aqueous mixture was vigorously stirred for 4 hours at room temperature. Filtration of the mixture yielded 0.61 g. (91%) of product, m.p. >300°. A portion of the material (0.59 g.) was recrystallized from 180 ml. of methanol and yielded 0.32 g. (49%) of purified product, m.p. >300°; $\lambda_{max(\mu)}^{KBr}$ 3.15–3.32 (NH), 5.87–6.02 (uracil C=O), 7.40 and 8.62 (SO_2N), 12.33 (*p*-disubstituted phenyl).

Anal. Calcd. for $C_{11}H_{11}N_3O_4S$: C, 47.0; H, 3.94; S, 11.40. Found: C, 46.9; H, 4.49; S, 11.1.

3-(2-Hydroxyethyl)-5-[(2-hydroxyethyl)-ethylamino]-uracil (XIa).—To a cold (0°) solution of 6.75 g. (43.5 mmoles) of 5-(ethylamino)-uracil (VII) in 150 ml. of 50% aqueous acetic acid was added 60 ml. (*ca.* 1.21 moles) of cold ethylene oxide. The reaction mixture was stored at room temperature in a stoppered flask for 142 hours and was evaporated *in vacuo* to about 110 ml. Water (90 ml.) and 30 g. of Dowex 50 (H) resin were added to the residue and the basic reaction-product was isolated as described for the preparation of VII. After evaporation of the ammoniacal solutions, 9.64 g. of solid residue was obtained. This was dissolved in 400 ml. of hot methanol, the solution filtered, and 400 ml. of hot acetonitrile added to the filtrate. On chilling, 4.60 g. (44%) of product precipitated, m.p. 197.5–199.5° and 177–188° when mixed with VII. Evaporation of the mother liquor gave 4.4 g. of solid whose infrared spectrum indicated it to be mainly VII. From a previous, smaller run, analytical data were obtained from a sample, m.p. 196°; $\lambda_{max(\mu)}^{KBr}$ 2.96 (OH), 6.02–6.10 (uracil C=O), 9.18 and 9.52 (C—OH). On paper chromatography in solvent A the compound moved as a single spot with R_{ad} 1.37.

Anal. Calcd. for $C_{10}H_{17}N_3O_4 \cdot 1/4H_2O$: C, 48.5; H, 7.12; N, 17.0. Found: C, 48.6; H, 7.22; N, 16.9.

The picrate of XI was prepared by mixing hot, aqueous solutions of XI and picric acid. The solid was recrystallized from water (30 ml./g.), m.p. 203.0–204.5°. The mixed melting point with the picrate of VII was 184–189°.

Anal. Calcd. for $C_{16}H_{20}N_6O_{11}$: C, 40.7; H, 4.27; N, 17.8. Found: C, 40.9; H, 4.44; N, 17.5, 17.7.

3-(2-Chloroethyl)-5-[(2-chloroethyl)-ethylamino]-uracil Hydrochloride (XIIa).—A mixture of 1.00 g. (4.11 mmoles) of 3-(2-hydroxyethyl)-5-[(2-hydroxyethyl)-ethylamino]-uracil (XIa) and 12 ml. (0.17 mole) of thionyl chloride was stirred at 80° for 6 hours and the reaction mixture was evaporated to dryness *in vacuo*. The residue was stirred with 20 ml. of hot benzene and the mixture filtered. The filter cake was washed with 20 ml. of dry ether to yield 1.20 g. (92%) of product, m.p. 167–169° dec.; $\lambda_{max(\mu)}^{Nujol}$ 3.24 and 3.30 (NH), 5.80 and 5.94 (uracil C=O), 6.05 (C=C), no C—OH absorption near 9.5. On paper chromatography in solvent A, the compound moved as a single spot with R_{ad} 1.38.

Anal. Calcd. for $C_{10}H_{15}Cl_2N_3O_2 \cdot HCl$: C, 37.9; H, 5.09; N, 13.3. Found: C, 37.7; H, 5.39; N, 13.2.

3-Methyl-2-(methylthio)-4(3H)-pyrimidinone (XIV) and 1-methyl-2-(methylthio)-4(1H)-pyrimidinone (XVII) were prepared from 2-thiouracil (XIII) by the method of Brown, Hoerger and Mason.[13] The use of 50 g. (0.39 mole) of XIII gave 13.8 g. (23%) of 3-methyl-2-(methylthio)-4(3H)-pyrimidinone (XIV), after recrystallization, and 14.4 g. (23%) of 1-methyl-2-(methylthio)-4(1H)-pyrimidinone (XVII), after recrystallization. An intermediate fraction of the mixed isomers (11.0 g., 18%), m.p. 68–138°, was also isolated. Compound XIV had m.p. 126.5–127.9° (lit.[13] m.p. 122–123°); $\lambda_{max(\mu)}^{Nujol}$ 5.99 (C=O), 6.61, 7.08, 7.48, 9.18, 12.11 and 12.71 (unassigned), no OH or NH absorption near 3.0; $\lambda_{max(m\mu)}^{pH\ 1}$ 284 (ε 7810), $\lambda_{max(m\mu)}^{pH\ 7}$ 290 (ε 9720), $\lambda_{max(m\mu)}^{pH\ 13}$ 284 (ε 9960); and on paper chromatography in solvents C, D and E it showed single spots with R_{ad} 1.98, 1.57 and 1.38, respectively. Compound XVII had m.p. 170–171° (lit.[13] m.p. 168–169°); $\lambda_{max(\mu)}^{Nujol}$ 2.88–2.92 (unassigned), 6.10 (C=O), 6.23 (C=C), 9.00 (unassigned); $\lambda_{max(m\mu)}^{pH\ 1}$ 231 (ε 15,500), $\lambda_{max(m\mu)}^{pH\ 7}$ 232 (ε 23,300), $\lambda_{max(m\mu)}^{pH\ 13}$ 263 (ε 6540); and on paper chromatography in solvents C and D, it showed main spots with R_{ad} 2.22 and 1.31, respectively, accompanied by a trace contaminant which was not compound XIV; in solvent E it showed a single spot with R_{ad} 1.22.

3-Methyluracil (XV) was prepared by acid hydrolysis of XIV[13] in 58% yield (after recrystallization from absolute ethanol). It had m.p. 184.0–184.5° (lit. m.p. 179°,[13] 174–175°,[21] 174°,[22] 174–175°[23]); $\lambda_{max(\mu)}^{Nujol}$ 3.15 (NH) 5.81 (C=O), 6.02–6.12 (C=O and C=C), 8.16 and 8.88 (unassigned), and on paper chromatography in solvents C, D and E, it showed single spots with R_{ad} 2.37, 1.21 and 1.33, respectively.

1-Methyluracil (XXI) was prepared by acid hydrolysis of XVII[13] in 68% yield (after recrystallization from water). It has m.p. 238–239° (lit. m.p. 232–233°,[13] 232°,[21] 229°[12]); $\lambda_{max(\mu)}^{Nujol}$ 3.2–3.35 and 3.55–3.78 (broad absorptions, unassigned), 5.90–6.00 (C=O), 7.01, 7.52, 8.74, 12.43 and 13.16 (unassigned); and on paper chromatography in solvents C, D and E, it showed single spots with R_{ad} 2.44, 1.05 and 1.27, respectively.

5-Bromo-3-methyluracil (XVI).—To a stirred solution of 2.0 g. (15.9 mmoles) of XV in 16 ml. of glacial acetic acid at room temperature was added dropwise 2.60 g. (16.3 mmoles) of bromine. After the addition, the mixture was stirred for 2 hours, cooled to 15°, and the solid material collected by filtration and washed with 7 ml. of cold acetic acid. The product weighed 2.55 g. (79%), m.p. 238–241° (lit. m.p. 228–229°,[13] 228–229°[21]); $\lambda_{max(\mu)}^{Nujol}$ 3.12 and 3.30 (NH), 5.82 and 5.99 (C=O), 6.17 (C=C), 10.01 and 13.18 (unassigned); on paper chromatography in solvents A and B, it showed single spots with R_{ad} 1.59 and 2.30, respectively.

5-Bromo-1-methyluracil (XXII).—Using the procedure for XVI (*cf.* above), 2.0 g. of XXI gave 2.43 g. (75%) of XXII, m.p. 267–272° dec. (lit. m.p. 266° dec.,[13] 260°[21]); $\lambda_{max(\mu)}^{Nujol}$ 5.78–6.07 (C=O), 6.18 (C=C), 7.00, 7.51, 8.49, 10.37 and 13.33 (unassigned); on paper chromatography in solvents A and B, it showed single spots with R_{ad} 1.49 and 1.81, respectively.

5-(2-Hydroxyethylamino)-3-methyluracil (XX).—A mixture of 0.75 g. (3.66 mmoles) of the bromouracil XVI, 0.88 g. (14.4 mmoles) of 2-aminoethanol and 0.37 g. of water was heated in a stainless steel bomb at 160° for 6 hours. After standing at room temperature for 15 hours, the bomb was cooled to 0°, opened and the contents filtered using 5 ml. of chloroform to rinse the bomb. The crystalline solid, 0.62 g. (91%), was recrystallized from 15 ml. of aboslute methanol, yielding 0.39 g. (57%) of product, m.p. 198–200°. A second recrystallization from methanol yielded the analytical sample, m.p. 198–200°; $\lambda_{max(\mu)}^{Nujol}$ 2.93, 3.01, 3.15, 3.22 (OH, NH), 5.85 and 6.18 (C=O and C=C), 9.40 and 9.54 (C—OH).

(21) T. B. Johnson and F. W. Heyl, *Am. Chem. J.*, **37**, 628 (1907).
(22) H. L. Wheeler and T. B. Johnson, *ibid.*, **42**, 30 (1909).
(23) C. W. Whitehead, THIS JOURNAL, **74**, 4267 (1952).

On paper chromatography in solvents A and B, the product moved as a single spot with R_{ad} 1.37 and 1.54, respectively.

Anal. Calcd. for $C_7H_{11}N_3O_3$: C, 45.4; H, 5.99; N, 22.7. Found: C, 45.4; H, 5.96; N, 22.4.

5-[Bis-(2-hydroxyethyl)-amino]-3-methyluracil (XIX).—A mixture of 3.75 g. (18.3 mmoles) of XVI and 11.0 g. (73.3 mmoles) of 70% aqueous 2,2′-iminodiethanol was heated in a stainless steel bomb at 160° for 6 hours. After being allowed to cool to room temperature overnight, the bomb was opened and the reaction mixture was transferred with the aid of 10 ml. of methanol. The mixture and washings were evaporated *in vacuo* (60°, 2 mm.) and 30 ml. of reagent methanol was added to the residue. The methanolic solution was evaporated on the steam-bath to a volume of 9 ml. and was chilled at 0°. The solid was collected and washed with 3 ml. of cold absolute methanol to yield 2.47 g. (59%) of product, m.p. 161–162°. From an earlier run, an analytical sample was obtained by several recrystallizations from methanol, m.p. 165–166°; $\lambda_{max(\mu)}^{Nujol}$ 3.11, 3.15 and 3.24 (OH, NH), 5.81 (C=O), 6.00–6.10 (C=O and C=C), 9.25, 9.48 and 9.64 (C—OH). On paper chromatography in solvents A and B, the compound moved as a single spot with R_{ad} 1.45 and 1.52, respectively.

Anal. Calcd. for $C_9H_{15}N_3O_4$: C, 47.2; H, 6.60; N, 18.3. Found: C, 47.1; H, 6.59; N, 18.3.

5-(2-Hydroxyethylamino)-1-methyluracil (XXIII).—A mixture of 0.75 g. (3.66 mmoles) of the bromouracil XXII, 0.88 g. (14.4 mmoles) of 2-aminoethanol and 0.37 g. of water was heated in a stainless steel bomb at 160° for 6 hours and the bomb was allowed to cool to room temperature overnight, then chilled to 0° before opening. The reaction mixture was transferred to a filter with the aid of 5 ml. of chloroform, yielding 0.46 g. (68%) of product, m.p. 180–195°. The product was recrystallized from 10 ml. of 95% ethanol to yield 0.29 g. (43%) of XXIII, m.p. 195–198°; $\lambda_{max(\mu)}^{Nujol}$ 2.85 (OH), 3.00 (NH), 5.90–6.10 (C=O and C=C), 9.27–9.46 (C—OH). On paper chromatography in solvents A and B, the compound moved as a single spot with R_{ad} 1.24 and 0.84, respectively.

Anal. Calcd. for $C_7H_{11}N_3O_3$: C, 45.4; H, 5.99; N, 22.7. Found: C, 45.6; H, 6.21; N, 22.5.

5-[Bis-(2-hydroxyethyl)-amino]-1-methyluracil (XXIV).—To a cold (0°) solution of 1.63 g. (8.80 mmoles) of the monohydroxyethyl compound XXIII in 39 ml. of 50% aqueous acetic acid was added 2.1 ml. (42 mmoles) of ethylene oxide. The reaction mixture was allowed to stand at room temperature in a stoppered flash for 16 hours and was then evaporated *in vacuo*. The viscous residue was dissolved in 30 ml. of water and the aqueous solution was stirred with 8 g. of Dowex 50(H) at room temperature for 5 hours and the product isolated by the method used to prepare VII. The residue from the ammonia elution (2.14 g., 106%) was dissolved in 15 ml. of reagent methanol, 25 ml. of benzene was added to the solution, which was then evaporated on the steam-bath to a volume of 12 ml. On chilling and scratching, the product precipitated and was collected and washed with 5 ml. of cold benzene to yield 1.36 g. (67%) of material, m.p. 126–127°. A second recrystallization using the methanol–benzene mixture gave the analytical sample, m.p. 126–127°; $\lambda_{max(\mu)}^{Nujol}$ 3.1–3.2 (OH, NH), 5.77 (C=O), 6.02–6.12 (C=O and C=C), 9.31 and 9.50 (C—OH). On paper chromatography in solvents A and B, the product moved as a single spot with R_{ad} 1.42 and 0.97, respectively.

Anal. Calcd. for $C_9H_{15}N_3O_4$: C, 47.2; H, 6.60; N, 18.3. Found: C, 47.3; H, 6.87; N, 18.4.

5-[Bis-(2-chloroethyl)-amino]-3-methyluracil (XVIII) and Hydrochloride.—To a stirred solution of 14.5 ml. of 1,2-dimethoxyethane, 0.37 ml. of absolute ethanol and 0.01 ml. of water was slowly added 0.51 ml. (7.0 mmoles) of thionyl chloride. After about 5 minutes, the mixture was cooled to 10° and 1.40 g. (6.11 mmoles) of the bis-hydroxyethyl compound XIX was added, followed by 1.6 ml. (22 mmoles) of thionyl chloride. The reaction mixture was allowed to come to room temperature, was stirred for 20 hours and was then diluted with 46 ml. of benzene. After being stirred for an additional hour, the slurry was filtered using a sintered glass filter, and the solid was washed with 40 ml. of benzene; yield 1.44 g. (78%) of crude XVIII hydrochloride, m.p. 145–153°.

A portion of the crude hydrochloride (0.10 g.) was dissolved in 1.5 ml. of reagent methanol and the solution was filtered. To the filtrate was added 2.0 ml. of dry ether to yield, after chilling, 0.034 g. of crystalline solid m.p. 145–155°; $\lambda_{max(\mu)}^{Nujol}$ 3.19 (NH), 4.05–4.16 (R_3NH^+), 5.75 and 5.94 (C=O), 6.07 (shoulder, C=C); the C—OH bands present in the starting material were absent. On paper chromatography in solvents A and B, the compound moved as a single spot with R_{ad} 1.58 and 1.33, respectively.

Anal. Calcd. for $C_9H_{13}Cl_2N_3O_2 \cdot HCl$: C, 35.7; H, 4.66; Cl, 35.2. Found: C, 36.2; H, 4.99; Cl, 35.0.

A second portion of the crude product (0.50 g.) was suspended in 5 ml. of water and to the stirred solution was added dropwise a saturated aqueous solution of sodium bicarbonate until the reaction mixture was slightly basic. The mixture was evaporated to dryness *in vacuo* (2 mm. and 25°) and the residue was broken up and stirred with 5 ml. of water, then filtered. The filter cake was washed with two 5-ml. portions of water to leave 0.37 g. (84%) of solid, m.p. 154–156°. A portion of the material (0.20 g.) was dissolved in 6 ml. of ethyl acetate, the solution filtered and the filtrate evaporated to 2 ml. and chilled, giving 0.12 g. of product, m.p. 154–156°. A second such recrystallization from ethyl acetate with the use of Norit gave 0.077 g. of XVIII, m.p. 154–157°; $\lambda_{max(\mu)}^{Nujol}$ 3.15 (NH), 3.29 (NH and ethylenic CH), 5.82 (C=O), 6.10 (C=O and C=C), 13.61 (C—Cl); there was essentially no C—OH absorption at 9.25 μ, where the starting material XIX showed a strong band. On paper chromatography in solvent B the product moved as a single spot with R_{ad} 3.25.

Anal. Calcd. for $C_9H_{13}Cl_2N_3O_2$: C, 40.6; H, 4.92; Cl, 26.7; N, 15.8. Found: C, 40.8; H, 4.96; Cl, 26.6; N, 16.0.

5-[Bis-(2-chloroethyl)-amino]-1-methyluracil (XXVI).—Using the same procedure and the same proportions of reagents as in the preparation of XVIII, 1.10 g. (4.80 mmoles) of the bis-hydroxyethylamine XXIV was converted to the hydrochloride of XXVI; yield 1.31 g. (90%) of crude product. Attempted purification of the salt by the methanol–ether reprecipitation scheme described for XVIII gave a solid whose analysis indicated the loss of part of the hydrogen chloride. In order to convert the crude product to its free base, 0.50 g. (1.65 mmoles) of the salt was dissolved in 5 ml. of water and the solution treated with aqueous sodium bicarbonate solution and worked up by the procedure described for the isolation of the free base XVIII. The isolated solid (0.30 g., 68%) had m.p. 144–150°. Three recrystallizations from ethyl acetate by the technique used for XVIII gave 0.16 g. of pure XXVI, m.p. 147–151°; $\lambda_{max(\mu)}^{Nujol}$ 3.11 (NH), 3.35 (CH of C=C), 5.90 (C=O), 6.13 (C=C), 13.70 (C—Cl); there was essentially no C—OH absorption at 9.50 μ.

Paper chromatography on this analytically pure material was not satisfactory, possibly because of instability during chromatography. In solvent B, there were two fluorescent spots with R_{ad} 1.60 and 2.86, and in solvent A, there were fluorescent spots with R_{ad} 0.96 and 1.74.

Anal. Calcd. for $C_9H_{13}Cl_2N_3O_2$: C, 40.6; H, 4.92; Cl, 26.7; N, 15.8. Found: C, 40.9; H, 5.06; Cl, 26.3; N, 16.2.

Acknowledgment.—The authors are indebted to Dr. Peter Lim and staff for the interpretation of infrared spectra and for the paper chromatography; and to Mr. O. P. Crews, Jr., and staff for large-scale preparation of intermediates.

MENLO PARK, CALIF.