found to be identical with that prepared by the preceding procedure.

2-Amino-6-methylthio-9-(β-chloroethyl)purine (XV, R = CH_3).—A solution of 18.1 g. (0.1 mole) of 2-amino-6-(methylthio)purine (XVI, R = CH_3) in 100 ml. of dimethyl sulfoxide was stirred at room temperature. To this solution was added anhydrous K_2CO_3 (27.6 g., 0.2 formula wt.) and 2-bromoethyl chloride (15.7 g., 0.11 mole). After stirring for 3 hr. at room temperature, the reaction mixture was evaporated to dryness under reduced pressure. The yellow residue was extracted with two 75-ml. portions of boiling benzene and the crude product was obtained by evaporation of the benzene extract. Recrystallization from a 1:10 mixture of benzene–heptane gave 11.2 g. (46%) of pure product which gave a negative ionizable halide test; m.p. 141–142°; $\lambda_{max}^{ethanol}$ 243 mμ (ε 14,800), 307 mμ (ε 11,500).

Anal. Calcd. for $C_8H_{10}ClN_5S$: C, 39.4; H, 4.1; N, 28.7. Found: C, 39.4; H, 4.4; N, 28.5.

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Nitrogen Mustards Derived from 3,4-Dihydro-2,4-dioxo-1(2H)-pyrimidinepropionic and -butyric Acids[1]

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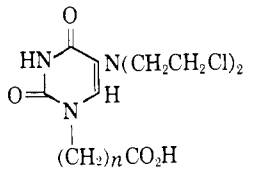
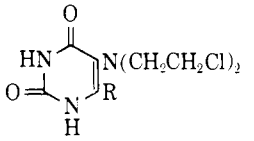
The two uracil nitrogen mustards, ω-[5-bis(2-chloroethyl)amino-3,4-dihydro-2,4-dioxo-1(2H)-pyrimidine]-propionic and -butyric acids (I and II) have been synthesized. Reaction of β-ethoxyacryloyl isocyanate with the appropriate ω-aminoalkanoic acid ester and cyclization in base afforded the uracilalkanoic acid. Nitration gave the nitrouracilalkanoic acid which was esterified, reduced to the amine, and hydroxyethylated. Chlorination with phosphoryl chloride proceeded smoothly to afford the mustards I and II. These demonstrated low toxicity and borderline activity against Walker 256 in the rat.

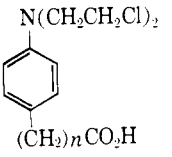
Uracil mustard (III) and its 6-methyl derivative, Dopan (IV), are useful clinically in the treatment of various malignancies in man.[2] Another nitrogen mustard that has clinical utility is chlorambucil (V, $n = 3$).[3a] Its homologs (V, $n \neq 3$) show less biological activity than V ($n = 3$) but more than the parent aromatic amine, bis(2-chloroethyl)aniline.[3b] The alkanoic acid side chain causes significant changes in chemical and biological properties. Consideration of the activity of both types of alkylating agents III and V suggested the synthesis of the uracil nitrogen mustards I and II. They contain the elements of both and may be considered as uracil analogs of chlorambucil in which alkanoic acid side chains are attached to the heterocyclic amine III. If I and II should possess antitumor activity, conceivably one may show greater activity than the other. The synthesis and antitumor evaluation of I and II are presented in this manuscript.



I, $n=2$
II, $n=3$



III, R = H
IV, R = CH_3



V

The uracilpropionic acid VIIIa was prepared by the general scheme of uracil synthesis of Shaw and his co-workers.[4] For the following steps, the procedures were simplified and the yields increased significantly. Sodium β-ethoxyacrylate was converted to the acid chloride,[4c] then to β-ethoxyacryloyl isocyanate (VI),[4a] and condensed with ethyl β-alanate, all in one step, to afford the acylurea VIIa. This was cyclized to the uracilpropionic acid VIIIa[5] in quantitative yield. The literature method[4b] was modified by heating at 60° for 2.5 hr. and using a medium of aqueous sodium hydroxide and 1,2-dimethoxyethane. Omission of the 1,2-dimethoxyethane caused incomplete solution of VIIa and gave lower yields of product. Reaction for shorter periods at higher temperatures was also less satisfactory.

Nitration of VIIIa proceeded in either sulfuric acid or acetic acid–sulfuric acid to give the nitrouracil IXa. Esterification to Xa, followed by catalytic hydrogenation over palladium on charcoal afforded the aminouracil XIa.

Hydroxyethylation of XIa with 2 moles of ethylene oxide in glacial acetic acid gave a chromatographically

(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. PH-43-64-500. The opinions expressed in this paper are those of the authors and not necessarily those of the Cancer Chemotherapy National Service Center.

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(5) After the completion of this work, the preparation of VIIIa by the reaction of uracil with acrylic acid derivatives in liquid ammonia[6] and with acrylonitrile in aqueous alkali[7] was reported.

(6) Y. P. Shvachkin, M. T. Azarova, and I. L. Rapanovich, *Vestn. Mosk. Univ. Ser. II, Khim.*, **18**, 68 (1963); *Chem. Abstr.*, **59**, 15283 (1963).

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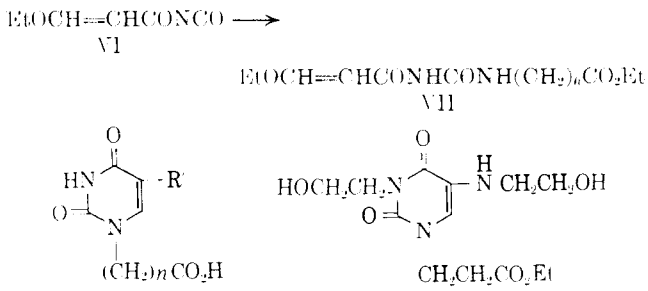
TABLE I

URACILALKANOIC ACID MUSTARDS AND INTERMEDIATES

Compd.	Yield,[a] %	M.p.,[a,b] °C.	R_f[c]	Formula	Calcd., %				Found, %			
					C	H	Cl	N	C	H	Cl	N
VIIa	45	114.0–114.5, A	0.40 (A) 0.84 (B)	$C_{11}H_{14}N_2O_4$	51.2	7.03		10.9	51.4	6.93		10.7
VIIIa	(100)	188.0–188.5, B (184–186)	0.05 (A) 0.91 (C)	$C_7H_{10}N_2O_4$	45.7	4.38		15.2	45.7	4.29		15.2
IXa[d]	94	268–270	0.04 (B) 0.67 (C)	$C_7H_7N_3O_6$	36.7	3.08		18.3	36.9	3.30		18.2
Xa	64 (100)	175.0–175.5, A (170–171)	0.66 (B) 0.81 (D)	$C_9H_{11}N_3O_6$	42.0	4.31		16.4	41.9	4.43		16.3
XIa	63 (92)	140–141, A	0.49 (B) 0.73 (D)	$C_9H_{13}N_3O_4$	47.6	5.77		18.5	47.6	5.70		18.7
XIIa	100	Gum	0.61 (B) 0.87 (D)	$C_{13}H_{21}N_3O_6$				13.3				13.1
I	71	206–300,[e] C	0.24 (A) 0.82 (D)	$C_{11}H_{12}Cl_2N_2O_3$	40.7	4.67	21.7		40.7	4.97	21.6	
VIIIb	34	70.0–70.5, A	0.50 (A) 0.85 (B)	$C_{12}H_{20}N_2O_4$	52.9	7.40		10.3	53.0	7.36		10.3
IXb	64	189–190, D	0.76 (A) 0.23 (B)	$C_8H_{10}N_2O_4$	48.5	5.09		14.1	48.3	5.05		14.0
Xb	(89)	178–180, D (172–173)	0.33 (A) 0.20 (B)	$C_8H_{11}N_3O_6$	39.5	3.73		17.3	39.2	3.46		17.7
XIb	67 (100)	133–134, A (130–132)	0.18 A 0.67 (B)	$C_{10}H_{15}N_3O_6$	44.3	4.83		15.5	44.5	4.63		15.6
XIb	65 (86)	126.5–127.5, B (126–127)	0.25 (A)	$C_{10}H_{15}N_3O_4$	49.8	6.27		17.4	49.6	6.45		17.3
XIIb	(100)	(Gum)	0.82 (A)	$C_{14}H_{23}N_3O_6$								
II	43	130.0–130.5, A	0.13,[f] 0.28 (A)	$C_{12}H_{17}Cl_2N_3O_3$	42.6	5.07	21.0	12.4	42.7	5.03	20.9	12.4

[a] Melting points and yields are for the analytical samples. Corresponding values in parentheses are for less highly purified products of suitable purity for use in the next step. [b] Recrystallization solvents are indicated by letter after the melting point: A, ethanol–water; B, ethanol; C, ethyl acetate; D, water. [c] The R_f values are given for the appropriate solvent systems listed in ref. 15. [d] Nitration done by two different procedures. See Experimental. [e] Softens at 206° and gradually decomposes without completely melting when heated to 300°. [f] Trace spot.

homogeneous product, believed to be the desired bis(2-hydroxyethyl)aminouracil XIIa. Hydroxyethylation in the ring at the 3-position to give a 1,3-disubstituted uracil (such as XIII) is believed to be unlikely under the conditions employed, but not impossible. It is known[8] that reaction of excess ethylene oxide with 5-(ethylamino)uracil in aqueous acetic acid causes some hydroxyethylation at the 3-position, as established by comparison of wave-length shifts in the ultraviolet spectra. Similar comparisons cannot be used to distinguish between 1-monosubstituted and 1,3-disubstituted uracils[9,10] such as XIIa and XIII. However, XIIa has an acidic proton at N-3 while XIII does not. By titration in a nonaqueous solvent, the product was shown to be XIIa.



VIII, R = H; R' = H
IX, R = H; R' = NO_2
X, R = Et; R' = NO_2
XI, R = Et; R' = NH_2
XII, R = Et; R' = $N(CH_2CH_2OH)_2$

XIII
a series, n = 2
b series, n = 3

The chlorination of XIIa was expected to be the most troublesome step as in the case of the preparation of the uracil mustard III[11] and related compounds. Indeed, treatment of XIIa with thionyl chloride gave dark, intractable materials. Phosphoryl chloride, on the other hand, proved to be an excellent chlorinating agent for XIIa, and other similar uracils investigated here when used at 80°. At this temperature, no conversion of ring hydroxyl to chlorine[12] was observed. Better yields were obtained in the chlorination with the hydrochloride of the bishydroxyethylamine than with the free base. The mustard ester formed in the chlorination was hydrolyzed to yield the mustard I.

The butyric acid analog II was prepared in the same way. The physical properties and yields of the intermediates are summarized in Table I. Some representative ultraviolet spectra are given in Table II. Since both the propionic and the butyric series show essentially the same spectra, only one example of the latter series is included.

The mustards I and II were screened against Walker carcinosarcoma 256[13] in rats, a tumor system against which uracil mustard (III) was effective.[14] Both I and II

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(12) D. J. Brown, "The Pyrimidines," John Wiley and Sons, Inc., New York, N. Y., 1962, p. 16.

(13) Screening was performed under the auspices of the Cancer Chemotherapy National Service Center according to its protocol outlined in "Protocols for Screening Chemical Agents and Natural Products against Animal Tumors and Other Biological Systems," *Cancer Chemotherapy Rept.*, **25**, 1 (1962).

(14) D. A. Lytle and H. G. Petering, *J. Am. Chem. Soc.*, **80**, 6459 (1958).

TABLE II

ULTRAVIOLET SPECTRA FOR SOME REPRESENTATIVE URACILS IN AQUEOUS SOLUTION

Compd.	pH 1[a] λ, mμ	pH 1[a] ε × 10⁻³	pH 7[b] λ, mμ	pH 7[b] ε × 10⁻³	pH 13[c] λ, mμ	pH 13[c] ε × 10⁻³
VIIIa	265	9.21	267	9.99	265	7.00
IXa	307	10.02	313	9.85	324	9.80
	240	7.20	242	7.25	241[d]	6.55
Xa	308	10.28	313	8.85	324	9.85
	241	7.10	239[d]	7.05	244[d]	6.55
XIa	267	8.27	297	6.64	292	5.60
			224[d]	6.60	255[d]	8.68
XIIa	268	7.89	302[d]	2.97	279[d]	3.70
			255[d]	5.07		
I	268	6.31	305[d,e]	3.00	257[d,e]	5.98
			260[d]	5.02		
II	304[d]	3.10	304[d,e]	3.25	253[d,e]	6.25
	266	6.50	262[d]	5.46		

[a] 0.1 *N* HCl. [b] Buffer at pH 7. [c] 0.1 *N* NaOH. [d] Shoulder. [e] Intense end absorption which decreases with increasing wave length, but no well-defined maximum.

showed borderline activity with therapeutic indices of *ca.* 2 and 3, respectively. (Compounds having therapeutic indices of 4 or more are considered active.[13]) Perhaps the most outstanding characteristic of I and II was the low toxicity. Their LD_{10}[13] values were over 200 and over 100 mg./kg./day, respectively, while uracil mustard (III) and Dopan (IV) both had LD_{50} values of less than 3 mg./kg.[2] Compound I was also tested and found inactive by cell culture methods against KB cells.[13]

Experimental[15]

Ethyl β-[3-(β-Ethoxyacryloyl)ureido]propionate (VIIa).—A well-stirred mixture of 27.6 g. (0.200 mole) of sodium β-ethoxyacrylate, 200 ml. of dry benzene, and 15.0 ml. (0.207 mole) of thionyl chloride was heated at reflux for 60 min. The mixture containing the acid chloride was cooled to about 50°, and 35.0 g. (0.233 mole) of dry silver cyanate was added. The mixture was agan stirred and heated at reflux for 60 min., then cooled in ice. To this stirred mixture of VI was added a slurry of 33.5 g. (0.218 mole) of ethyl β-alanate hydrochloride, 21.4 g. (0.211 mole) of triethylamine, and 200 ml. of dioxane (the compounds of this slurry had previously been combined and stirred at room temperature for 60 min.). The cold mixture was stirred for 45 min., filtered, allowed to stand overnight, then filtered again. The filtrate was evaporated to dryness *in vacuo* to leave 40 g. (78%) of a yellow solid that was crystallized from ethanol–water to give 23.1 g. (45%) of VIIa with the properties described in Table I.

3,4-Dihydro-2,4-dioxo-1(2H)-pyrimidinepropionic Acid (VIIIa).—The literature method[4a,b] for cyclizing acylureas was followed except that 19.0 g. (73.7 mmoles) of the acylurea VIIa was heated at 58–60° for 2.5 hr. in 70 ml. of 1,2-dimethoxyethane and 147 ml. of 1 *N* NaOH solution to give 14.0 g. (a quantitative yield) of VIIIa, m.p. 184–186°, sufficiently pure for use in the next step. See Table I for properties of VIIIa.

3,4-Dihydro-2,4-dioxo-5-nitro-1(2H)-pyrimidinepropionic Acid (IXa). A.—To a solution of 0.25 g. (1.36 mmoles) of the uracil VIIIa in 10 ml. of concentrated H_2SO_4 (sp. gr. 1.84) was added 0.30 ml. of red fuming nitric acid. After standing for 30 min., the solution was poured into 25 g. of crushed ice. The white crystalline precipitate was collected, washed with 25 ml. of water, and dried *in vacuo* to afford 0.29 g. (94%) of IXa, m.p. 268–270°, as white, shiny plates.

B.—To the slightly turbid solution of 0.64 g. of the uracil VIIIa in 10 ml. of glacial acetic acid was added 0.20 ml. of concentrated H_2SO_4 (sp. gr. 1.84), followed by 0.80 ml. of red fuming nitric acid. Stirring was continued for 18 hr. During this time a white crystalline precipitate gradually formed. The crystals were collected, washed with 25 ml. of cold water, and dried to give 0.55 g. (69%) of IXa, m.p. 261–262°, with the same infrared spectrum as above product.

No nitration occurred when H_2SO_4 was omitted.

Ethyl 3-[3,4-Dihydro-2,4-dioxo-5-nitro-1(2H)-pyrimidine]-propionate (Xa).—The acid IXa (0.31 g., 1.35 mmoles) was esterified by refluxing for 1.5 hr. in 15 ml. of absolute ethanol, previously saturated with HCl at 0°, to afford 0.34 g. (100%) of the ester Xa, m.p. 170–171°.

Ethyl 3-[5-Amino-3,4-dihydro-2,4-dioxo-1(2H)-pyrimidine]-propionate (XIa).—A mixture of 0.80 g. (3.1 mmoles) of the nitrouracil Xa, 0.20 g. of 5% palladium on charcoal, and 35 ml. of 2-methoxyethanol was hydrogenated at room temperature and 1 atm. for 6 hr. The catalyst was filtered and washed with 2-methoxyethanol. The filtrate and washings were evaporated to dryness *in vacuo* to leave 0.65 g. (92%) of a light amber oil which crystallized on standing.

A chemical reduction with zinc dust, ammonium chloride, and aqueous ethanol was tried but gave very poor recovery of product.

Ethyl 3-[5-Bis(2-hydroxyethyl)amino-3,4-dihydro-2,4-dioxo-1(2H)-pyrimidine]propionate (XIIa).—A mixture of 1.14 g. (5.0 mmoles) of the amine XIa and 15 ml. of glacial acetic acid solution containing 0.453 g. (10.3 mmoles) of ethylene oxide was placed in a stoppered flask and stirred at room temperature for 60 hr. (24 hr. was found sufficient in later runs), during which time complete solution was attained. The solution was evaporated to dryness *in vacuo*, then two 25-ml. portions of toluene were successively added and evaporated to leave 1.60 g. (100%) of XIIa as an amber gum which could not be induced to crystallize, but was homogeneous on paper chromatography (see R_f and analysis, Table I).

Compound XIIa was dissolved in distilled *N*,*N*-dimethylformamide and titrated with tetramethylammonium hydroxide in 1-butanol–methanol, using an automatic Sargent recording titrator Model D and maintaining a nitrogen atmosphere. Similar titration curves were obtained for 1-methyluracil and 1,3-dimethyluracil. The curves for XIIa and 1-methyluracil were alike and totally different from that of 1,3-dimethyluracil which had no acidic proton and essentially consumed no base. Equivalent weights obtained for XIIa were low: 278, 267 (calcd., 315). The presence of about 3–4% acetic acid, not readily removed by the evaporation from the gum, would lower the equivalent weight to these values without grossly affecting the nitrogen analysis or the shape of the nonaqueous titration curve.

3-[5-Bis(2-chloroethyl)amino-3,4-dihydro-2,4-dioxo-1(2H)-pyrimidine]propionic Acid (I).—A solution of 8.00 g. (25.7 mmoles) of the bishydroxyethylamine (XIIa) in 75 ml. of methylene chloride and 20 ml. of ether was stirred and gradually saturated with HCl until there was no further precipitation. The mixture was evaporated to dryness to leave the hydrochloride of XIIa as a tan solid. This residue was protected from moisture while 70 ml. of phosphoryl chloride was added, and the mixture was heated at 80° for 3 hr. with stirring. The resultant solution was evaporated to dryness *in vacuo*. The residue was combined with 125 ml. of concentrated HCl and heated on a steam bath for 3 hr. Evaporation *in vacuo* left an amber gum which was triturated with 200 g. of ice and water until all the gum had crystallized. The product was collected, washed with 25 ml. of ice water, and triturated with 25 ml. of hot ethyl acetate to afford, after drying, 5.71 g. (71%) of I, m.p. 206 (softening) to over 300° with gradual decomposition.

Acknowledgments.—The authors thank Dr. Peter Lim for interpretation of the infrared spectra and his staff for the ultraviolet spectra and paper chromatography. The authors are also indebted to Mr. O. P. Crews, Jr., and his staff for large-scale preparation of some intermediates.

(15) Melting points were determined with the Fisher-Johns apparatus and are corrected. Paper chromatography was done by the descending technique on Whatman No. 1 paper except for solvent A where Schleicher and Schuell No. 2496 acetylated paper was used. The spots were detected by visual examination under ultraviolet light. The solvent systems were: A, benzene–methanol–water (2:6:1); B, 1-butanol–water (saturated); C, 2-propanol–2 *N* HCl (65:35); D, 5% aqueous Na_2HPO_4, pH 8.9. In all experiments, anhydrous $MgSO_4$ was used as the drying agent. Since the same experimental procedures were used for both the propionic and butyric acid series, only those for the propionic acid series are described.