[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF MICHIGAN]

Synthesis of Potential Anti-cancer Agents. II. Purine Antagonists from 2-Methylhypoxanthine-8-thiol[1]

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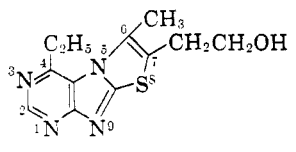
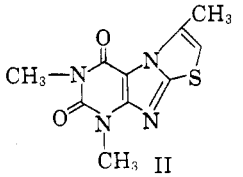
2-Methylhypoxanthine-8-thiol has been condensed with chloroacetic and β-chloropropionic acids to give the corresponding thioacetic and thiopropionic acids which on cyclization gave 4-hydroxy-2-methyl-6,7-dihydrothiazolo-[2.3-*f*]purine-6-one and 4-hydroxy-2-methyl-6,7-dihydro-1,3,6-thiazino[2.3-*f*]purine-6-one. Condensation of the former with *p*-(bis-β,β′-dichloroethyl)aminobenzaldehyde through the reactive methylene group gave the corresponding benzylidene derivative. A similar condensation with the thiazinopurine ketone failed. A series of thio ethers of 2-methylhypoxanthine-8-thiol has been prepared.

One general approach to the synthesis of compounds potentially capable of acting as purine antagonists and hence as inhibitors of tumor growth involves blocking possible sugar incorporation at the 7 or 9 positions of a purine by the introduction of suitable substituents. Alkyl groups are not particularly suited for this purpose since evidence is at hand that, at least in the case of methyl groups, considerable demethylation occurs during metabolism of such substances.[3]

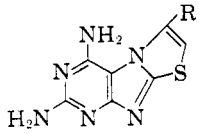
The use of fused ring systems as blocking groups has been reported by a few investigators. Todd and Bergel[4] prepared a homolog of dihydrothiazolo-[2.3-*f*]xanthine(I), Ochiai[5] prepared an analogous derivative of theophylline (II), and Gordon[6] prepared a [2.3-*f*]dihydrothiazolo derivative of 2,6-diaminopurine (III).[7]



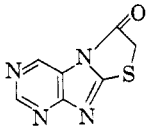
I



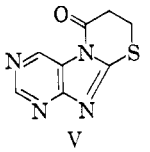
II



III. R = H or CH_3



IV



V

As far as we are aware, no purine derivatives carrying fused rings of the type of IV or V, have been described. It therefore seemed of interest to prepare representative compounds for evaluation as possible tumor inhibitors. Compounds analogous to IV have been reported by Kendall and Duffin,[8] as resulting from cyclization of 2-benzimidazolylmercaptoacetic acid. Inasmuch as a plentiful supply of 4,5-diamino-6-hydroxy-2-methylpyrimidine (VI) was available from other work, VII was selected as the starting purine.

Fusion of VI with thiourea gave 2-methylhypoxanthine-8-thiol (VII) in almost quantitative yield. When VII was refluxed with the appropriate chloro acid, the mercapto acids VIII and IX were obtained. The mercapto acids, in turn, provided 4 - hydroxy - 2 - methyl - 6,7 - dihydrothiazolo-[2.3 - *f*]purine - 6 - one (X) and 4 - hydroxy - 2-methyl - 6,7 - dihydro - 1,3,6 - thiazino - [2.3 - *f*]-purine - 6 - one (XI) respectively, when they were refluxed with acetic anhydride. Ring closure is formulated as occurring at position 7 of the purine system by analogy with the closures reported by Todd and Bergel[4] and by Ochiai.[5] It should be pointed out that no definitive evidence for excluding ring closure at the 9 position of the purine system has been offered by either of the latter workers.

Having thus obtained X and XI it seemed desirable to incorporate the *p*-[*N*,*N*-bis(2-chloroethyl)amino]benzylidene alkylating function into the molecules. Certain other purines carrying this group[9] have given evidence of tumor inhibitory activity in animals.[10] For this purpose, it was hoped to take advantage of the activity of the methylene hydrogens in the 7 positions of X and XI. X condensed readily with *p*-[*N*,*N*-bis(2-chloroethyl)amino]benzaldehyde (XII) in the presence of glacial acetic acid to give XIII. XIV, however, could not be

(1) This investigation was supported by Research Grant CY-2961 from the National Cancer Institute of the National Institutes of Health.

(2) On leave of absence from the Chemistry Department, B. N. College, Patna University, India.

(3) V. C. Meyers and R. F. Hanzal, *J. Biol. Chem.*, **162**, 309 (1946).

(4) A. R. Todd and F. Bergel, *J. Chem. Soc.*, 1559 (1936).

(5) E. Ochiai, *Ber.*, **69B**, 1650 (1936).

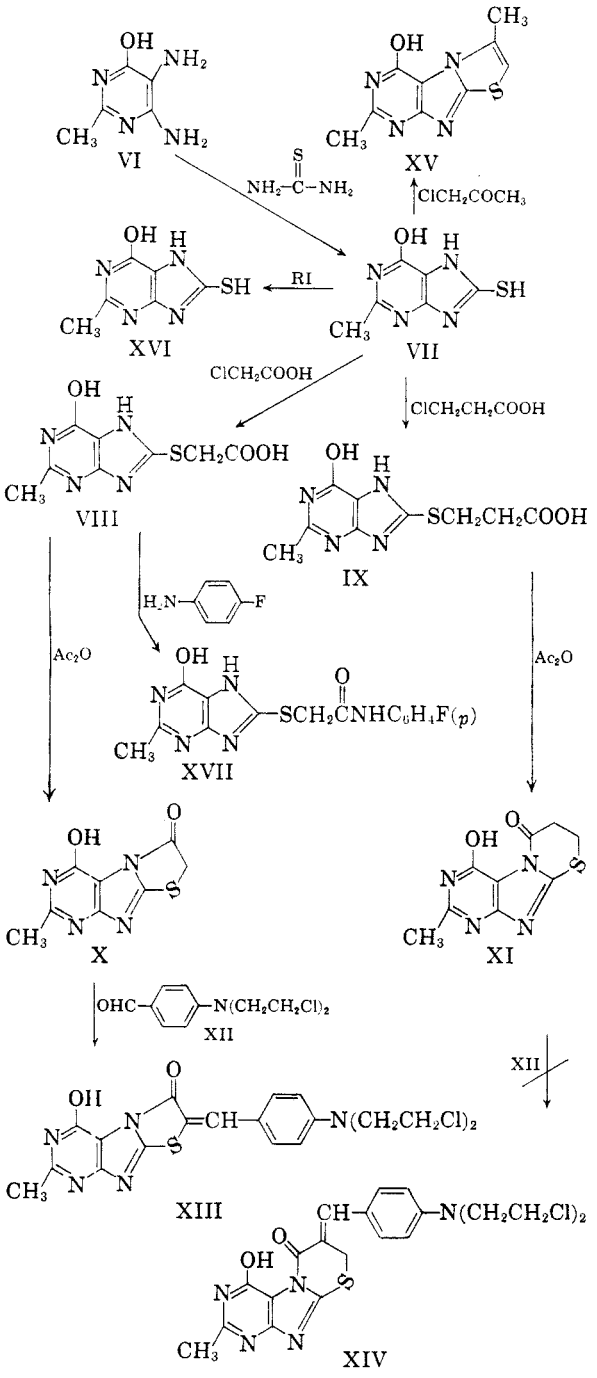
(6) M. Gordon, *J. Am. Chem. Soc.*, **73**, 984 (1951).

(7) Nomenclature and numbering of these fused ring systems is that used by Chemical Abstracts and the Ring Index and differs from that employed in refs. 4–6.

(8) J. D. Kendall and G. F. Duffin, Brit. Patent **634,951**.

(9) Unpublished work from this laboratory.

(10) Private communication from Dr. Ralph Jones, Jr.



obtained under the conditions used for the preparation of XIII, and only the unreacted materials were recovered. Even under more severe conditions (acetic anhydride and sodium acetate, triethylamine or piperidine and acetic anhydride) XI failed to condense with XII. It appears that in X, the electronegative sulfur atom exerts some activating influence on the methylene hydrogens in addition to the usual effect of the carbonyl group which is not possible in XI.

In complete analogy with earlier reports,[4-6] when VII was boiled for several days with chloroacetone in ethanol, 2,6-dimethyl-4-hydroxythiazolo[2.3-*f*]purine (XV) resulted. It was not necessary to isolate the intermediate acetonyl derivative.

Finally, a series of 2-methyl-8-mercaptohypoxanthine derivatives of the general type of XVI were prepared for investigation for tumor-inhibiting action. These included a number of thioethers and a series of esters of 6-hydroxy-2-methyl-8-purinylmercaptoacetic acid. In the preparation of the mercaptoacetates, an observation originally made by Gordon[6] was confirmed and extended. In order to prepare such esters, it is unnecessary to start with the appropriate bromoacetic ester. Rather, merely refluxing a solution of VII with about one to two equivalents of bromoacetic acid in the appropriate alcohol until a clear solution resulted, gave excellent yields of the mercaptoacetates. In general, the time required for complete reaction decreased with the higher boiling alcohols. Obviously, the mercaptoacetic acid must be formed initially, followed by esterification catalyzed by the liberated hydrogen bromide. Also of interest is the ready formation of esters of secondary alcohols by this procedure. Pertinent data concerning the synthesis and properties of these compounds are given in Table I.

An attempt at nucleophilic displacement of the mercaptoacetic acid group of VIII by *p*-fluoroaniline did not succeed, although a similar displacement has been reported[11] with 6-purinylmercaptoacetic acid. Instead the product isolated was found to be an anilide (XVII).

Reaction of the mercaptoacetates (XVI) with hydrazine resulted in the formation of a hydrazide in agreement with Huber's[11] findings.

Results of physiological tests on the compounds described in this paper will be reported elsewhere.

EXPERIMENTAL[12,13]

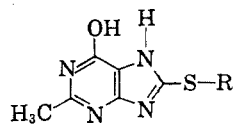
2-Methylhypoxanthine-8-thiol (VII). An intimate mixture of 9.52 g. (0.04 mole) of the sulfate of 4,5-diamino-6-hydroxy-2-methylpyrimidine (VI)[14] and 12 g. (0.157 mole) of thiourea was heated at 230–250° for 15–20 min. The mixture melted, then frothed with evolution of ammonia and finally became quite viscous. After cooling, the dark brown mass was dissolved in dilute potassium hydroxide solution, treated with decolorizing carbon and the filtered solution was acidified with acetic acid to yield 7.2 g. of light brown material. An analytical sample was obtained by repeating the above process. Ultraviolet absorption data on this and other compounds are given in Table II.

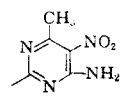
2-Methyl-8-methylmercaptohypoxanthine (XVI, R = CH_3). A solution of 4.5 g. (0.0246 mole) of VII in 100 ml. of aqueous 1.5% potassium hydroxide solution was cooled in ice. After the addition of 5 g. of iodomethane, the solution was stirred vigorously for 3 hr. and allowed to stand overnight in the refrigerator. The white precipitate was collected and thoroughly washed with water to give 4 g. of material which slowly decomposed above 290°. Recrystallization from 1.5 l. of boiling water gave 2.1 g. of cream-colored material which

(11) G. Huber, *Angew. Chem.*, **68**, 706 (1956).
(12) All melting points are uncorrected for stem exposure.
(13) Microanalysis by Spang Microanalytical Laboratory, Ann Arbor, Mich.
(14) W. Traube, *Ann.*, **432**, 287 (1923).

TABLE I

DERIVATIVES OF 2-METHYLHYPOXANTHINE-8-THIOL



R	Reaction Time[a]	Recrystallization solvent[b]	Analysis Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	M.P., °C.
H		A	39.56	3.30	30.77	39.60	3.21	30.85	>300
CH_3		A	42.85	4.08	28.57	42.81	4.07	28.58	>300
$CH_2(C{=}O)OH$[c]		A	37.21	3.87	21.70	37.62	4.01	21.48	>300
$CH_2CH_2(C{=}O)OH$		B	42.52	3.93	22.04	42.37	4.08	22.11	>300
$CH_2(C{=}O)OCH_3$	18	C	42.54	3.93	22.28	42.74	4.09	21.63	250–251.5 dec.
$CH_2(C{=}O)OCH_2CH_3$	16	D	44.77	4.47	20.88	44.72	4.48	21.05	232.6–233.6 dec.
$CH_2(C{=}O)OCH_2CH_2CH_3$	8	D	46.80	4.96	19.85	46.76	4.96	19.95	230–231 dec.
$CH_2(C{=}O)OCH(CH_3)_2$	48[d]	D	46.80	4.96	19.85	46.88	5.17	20.14	223–226 dec.
$CH_2(C{=}O)O(CH_2)_3CH_3$	2	D	48.64	5.38	18.91	48.69	5.52	19.13	204–205 dec.
$CH_2(C{=}O)OCH_2CH(CH_3)_2$	18	D	48.64	5.38	18.91	48.82	5.62	19.11	221–222 dec.
$CH_2(C{=}O)OCH(CH_3)CH_2CH_3$	20	D	48.64	5.38	18.91	48.72	5.61	18.89	234–235 dec.
$CH_2(C{=}O)O(CH_2)_4CH_3$	1	D	50.32	5.80	18.06	50.25	5.84	18.09	201–203 dec.
$CH_2(C{=}O)OCH_2CH_2Cl$	20 min.	E	39.66	3.63	18.51	39.72	3.61	18.55	180–184 dec.
$CH_2(C{=}O)NHNH_2$[e]		A	37.13	4.06	32.49	37.46	3.94	32.44	300
CH_2CH_2OH		A or B	42.47	4.42	24.77	42.37	4.37	24.78	>300
$CH_2(C{=}O)NH(C_6H_4)F$[f]		A or B	49.77	3.70	20.74	50.04	3.92	20.94	>300
		A	39.52	2.99	33.53	39.24	3.24	33.26	>300

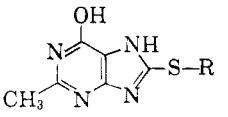
[a] Reflux time (in hours unless otherwise specified) required to obtain a clear solution. [b] A solution in dilute potassium hydroxide and reprecipitation by acetic acid; B, water; C, methanol and benzene; D, dilute methanol; E, acetone and ether. [c] Analysis calculated for $C_8H_8N_4O_3S \cdot H_2O$. [d] The solution was not quite clear after 48 hr of refluxing. [e] Analysis calculated for $C_8H_{10}N_6O_2S \cdot 0.25\ H_2O$. [f] Analysis calculated for $C_{14}H_{12}FN_5O_2S \cdot 0.25\ H_2O$.

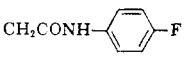
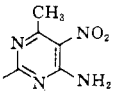
was further purified by solution in cold dilute potassium hydroxide and reprecipitation by acetic acid. The decomposition point was substantially unchanged.

2-Methyl[8-(β-hydroxyethyl)-mercaptohypoxanthine (XVI, R = CH_2CH_2OH). A solution of 1.82 g. (0.01 mole) of VII in a solution of 1.2 g. (0.022 mole) of potassium hydroxide in 30 ml. of water was stirred and cooled in ice. After the addition of 1 g. (0.0125 mole) of 2-chloroethanol, the mixture was vigorously stirred for 24 hr. at room temperature. The yellow solution was then treated with decolorizing carbon, filtered, and acidified with acetic acid. On standing in the refrigerator for 24 hr., 1.9 g. of yellow crystalline solid

TABLE II

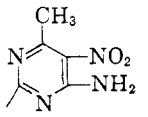
ULTRAVIOLET ABSORPTION SPECTRA OF SOME 8-SUBSTITUTED 2-METHYLPOXANTHINES



R	*p*H 1		*p*H 11	
	λmax., mμ	ε max.	λ max., mμ	ε max.
H	232	10,400	234	21,100
	289	25,200	291	21,500
CH_3	277	19,300	280	18,500
CH_2COOH	276	17,200	280	17,500
CH_2CH_2COOH	277	18,300	280	19,100
CH_2CH_2OH	277	18,700	280	18,400
CH_2COOCH_3	276	17,800	280	18,600
$CH_2COOC_2H_5$	276	17,500	280	18,500
$CH_2COOC_3H_7$-(*n*)	276	17,300	280	18,900
$CH_2COOC_3H_7$-(*i*)	276	17,500	280	18,100
$CH_2COOC_4H_9$-(*n*)	276	17,000	280	18,000
$CH_2COOC_4H_9$-(*i*)	277	18,000	280	18,800
$CH_2COOC_4H_9$-(*sec.*)	276	17,600	281	17,900
$CH_2COOC_5H_{11}$-(*n*)	277	17,700	280	18,300
$CH_2COOCH_2CH_2Cl$	271	19,400 (in methanol)		
$CH_2CONHNH_2$	276	17,500	279	17,100
	275	18,200	279	16,500
	230	17,600	232	31,300
	289	37,400	290	30,400
	297 (shoulder)	34,000	297 (shoulder)	28,300
			345	5,000

was collected. Solution in 150 ml. of boiling water and subsequent concentration to about 100 ml. gave, on cooling, 1.1 g. of yellow solid, which did not melt at 300°.

2-Methyl-8-[2'-(4'-amino-6'-methyl-5'-nitro)-pyrimidyl]-mercaptohypoxanthine (XVI R =



A solution of 0.96 g. (0.0051 mole) of 4-amino-2-chloro-6-methyl-5-nitropyrimidine in 30 ml. of absolute ethanol was added to an ice cold solution of 0.91 g. (0.005 mole) of VII in 50 ml. of aqueous 0.6% potassium hydroxide solution, and the mixture was vigorously stirred for 8 hr., the ice bath being removed after 1 hr. of stirring. The deep brown solution was left overnight at room temperature, then mixed with decolorizing carbon, filtered, and acidified with acetic acid, to give 0.9 g. of brownish yellow solid, which decomposed slowly above 300°. The material was purified by repeating the above process, to give pale yellow crystals (0.5 g.).

6-Hydroxy-2-methyl-8-purinylmercaptoacetic acid. (VIII). A mixture of 5.5 g. (0.03 mole) of VII, 3.0 g. (0.0317 mole) of chloroacetic acid and 5.1 g. (0.091 mole) of potassium hydroxide in 50 ml. water was heated under reflux for 2 hr. The solution was treated with decolorizing carbon and filtered. After acidification with acetic acid, 5.0 g. of light yellow crystalline granules separated on cooling. Reprecipitation from its solution in potassium hydroxide by acetic acid gave analytically pure material as a monohydrate. The acid showed infrared absorption bands at 970, 1210, 1590, and 1700 cm.$^{-1}$.

β-(6-Hydroxy-2-methyl-8-purinylmercapto)propionic acid. (IX). This was prepared by the same procedure as that used for VIII, except that β-chloropropionic acid was substituted for chloroacetic acid. The product (56%) was recrystallized first from dilute methanol and then from water to give cream-colored material. This substance showed infrared absorption bands at 970, 1200, 1600, and 1680 cm.$^{-1}$.

4-Hydroxy-2-methyl-6,7-dihydrothiazolo[2.3-f]purine-6-one. (X). A solution of 1.2 g. (0.005 mole) of the thioacid (VIII) in 30 ml. of acetic anhydride was heated under reflux for 30 min. The solvent was removed from the brown solid under reduced pressure. The residue was purified by reprecipitation from cold dilute aqueous potassium hydroxide by acetic acid. The yield of material, m.p. above 300°, was 1.0 g. The substance showed infrared absorption bands at 940, 1250, 1285, 1600, 1700, and 1870 cm.$^{-1}$ It gave analytical data corresponding to the retention of 1.75 moles of water of crystallization. The ultraviolet spectrum showed λ_{max} 276 mμ (ε 15,400) at pH 1 and λ_{max} 280 mμ (ε 17,100) at *p*H 11.

Anal. Calcd. for $C_8H_6N_4O_2S \cdot 1.75\ H_2O$: C, 37.86; H, 3.75; N, 22.08. Found: C, 37.53, 37.65; H, 3.83, 3.97; N, 22.35, 22.41.

4-Hydroxy-2-methyl-6,7-dihydro-1,3,6-thiazino[2.3-f]-purine-6-one (XI). This was prepared by the procedure used for X, except that refluxing was continued for 1 hr. The yield of white crystalline material, m.p. above 300°, was 78%. The substance showed infrared absorption bands at 960, 1250, 1280, 1580, 1650, 1700, and 1730 cm.$^{-1}$ The ultraviolet spectrum showed λ_{max} 276 mμ (ε 18,800) at *p*H 1 and λ_{max} 281 mμ (ε 19,000) at *p*H 11.

Anal. Calcd. for $C_9H_8N_4O_2S \cdot H_2O$: C, 42.51; H, 3.93; N, 22.04. Found: C, 42.35; H, 3.91; N, 22.34.

7-[p-Bis(β-chloroethyl)amino]benzylidene-4-hydroxy-2-methyl-6,7-dihydrothiazolo[2.3-f]purine-6-one (XIII). A mixture of crude X prepared from 2.4 g. (0.01 mole) of VIII, 2.5 g. (0.0102 mole) of XII and 15 ml. of glacial acetic acid was heated under reflux. The color changed from yellow to

brown within 5 min. After addition of 0.1 g. of fused potassium acetate, heating was continued with good stirring for 2 hr. The mixture was stirred at room temperature for an additional 12 hr. and poured into 250 ml. of anhydrous ether. The orange-yellow material (3.0 g.) which separated, was purified by solution in hot dimethylformamide and filtration of the solution into hot benzene. After cooling, addition of ether gave 1.3 g. of product, m.p. above 300°. The ultraviolet spectrum showed λ_{max} 270 mμ (ϵ 11,300) in dimethylformamide.

Anal. Calcd. for $C_{19}H_{17}Cl_2N_5O_2S$: C, 50.66; H, 3.77; N, 15.55. Found: C, 50.87; H, 3.97; N, 15.42.

2,6-Dimethyl-4-hydroxythiazolo[2.3-f]purine (XV). A mixture of 1.8 g. (0.01 mole) of VII, 1.2 g. (0.013 mole) of chloroacetone and 200 ml. of absolute ethanol was refluxed for 5 days. After 24 hr. an additional 1.2 g. of chloroacetone was added. The precipitate was filtered from the hot mixture and dissolved in cold dilute potassium hydroxide solution. After filtering from a small amount of insoluble material, acidification of the filtrate with acetic acid gave 1.75 g. of white crystalline material. After recrystallization by concentration of its solution in 500 ml. of ethanol to about half its volume, the substance formed fibrous crystals, m.p. above 300°. The ultraviolet spectrum showed λ_{max} 241 mμ (ϵ 20,300) and a shoulder at 272 mμ (ϵ 12,100) at *p*H 1; λ_{max} 242 mμ (ϵ 27,100) and λ_{max} 271 mμ (ϵ 12,600) at *p*H 11.

Ethyl 6-hydroxy-2-methyl-8-purinylmercaptoacetate (XVI), R = $CH_2COOC_2H_5$). A. A mixture of 1.8 g. (0.01 mole) of VII, 5.0 g. of ethyl chloroacetate and 200 ml. of 95% ethanol was heated under reflux with stirring for 65 hr. The resulting clear solution was filtered from a small amount of impurities and concentrated to about 50 ml. on the steam bath. Dilution of the hot concentrate with hot water and cooling gave crystalline material. After purification by solution in methanol, dilution and distillation of the methanol, 0.5 g. of fine needles, m.p. 233–235° (dec.), was obtained.

When ethyl bromoacetate was substituted for ethyl chloroacetate, a clear solution (indicating complete reaction) was obtained after refluxing for 3 hr.

B. A mixture of 1.82 g. (0.01 mole) of VII, 2.0 g. (0.014 mole) of bromoacetic acid and 110 ml. of 95% ethanol was heated under reflux with stirring. After 16 hr., a clear brown solution resulted, from which 1.5 g. of XVI (R = $CH_2COOC_2H_5$) was isolated.

The other esters of 6-hydroxy-2-methylpurine-8-thioacetic acid were prepared by method B, using the appropriate alcohol as solvent. Melting points and analytical data are given in Table I. All the acetates showed characteristic infrared absorption peaks at 1160–1190 and 1720–1750 cm.$^{-1}$

6-Hydroxy-2-methyl-8-purinylmercaptoacet-p-fluoroanilide. [XVI, R = $CH_2CONHC_6H_4F$-(*p*)]. A mixture of 2.4 g. (0.01 mole) of VIII, 10 ml. of benzene, and 10 ml. of *p*-fluoroaniline was heated under reflux with stirring, using a water separator, for 24 hr. The deep brown mixture was cooled, diluted with methanol and filtered to give 2.1 g. of nearly white solid, which did not melt at 300°. The solid was dissolved in one l. of boiling water, filtered and concentrated to about 250 ml. On cooling 1.1 g. of white solid, m.p. above 300°, separated.

6-Hydroxy-2-methyl-8-purinylmercaptoacethydrazide. (XVI, R = $CH_2CONHNH_2$). A solution of 1.0 g. of 95% hydrazine hydrate in 10 ml. of ethanol was added to a solution of 0.74 g. (0.0025 mole) of XVI (R = $CH_2COOCH_2CH_2CH_2CH_3$) in 20 ml. ethanol. The mixture was heated under reflux for about 1.5 hr. The mixture was refrigerated for 12 hr. and the white precipitate (0.6 g.) filtered. It was purified by solution in cold dilute potassium hydroxide and reprecipitation by acetic acid. The cream-colored solid did not melt until 300°.

Acknowledgment. We acknowledge the valuable assistance of James Hudson in the preparation of certain of the intermediates used in this work.

ANN ARBOR, MICH.

[CONTRIBUTION FROM THE RESEARCH LABORATORY, PUREX CORPORATION, LTD.]

N-Halogen Compounds. I. Decomposition of 1,3-Dichloro-5,5-dimethylhydantoin in Water at *p*H 9[1]

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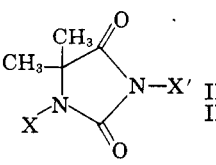
When 1,3-dichloro-5,5-dimethylhydantoin(I) dissolved in water at *p*H 9, it decomposed rapidly and completely; 1-chloro-5,5-dimethylhydantoin(IIIa), *N*-chloroisopropylamine(IV), chloride ion, nitrogen, and carbon dioxide were the major products. Nitrogen chloride was a transient intermediate. *N*-chloro-α-aminoisobutyric acid (VIII) was shown to give mainly acetone, and not more than 12% of IV, on decomposition at *p*H 9, which excludes it as an intermediate. All known monochloro-5,5-dimethylhydantoins proved to be identical and are assigned the 1-chloro structure.

1,3-dichloro-5,5-dimethylhydantoin(I) is one of a number of *N*-halogen compounds under study in this laboratory with regard to their utility in powdered bleaching and disinfecting compositions. While several studies[2,3] of the hydrolysis of hydantoins in alkaline solutions have been made, none has dealt with *N*-chloro derivatives except that of Biltz and Behrens[3] who made a few observations on 1,3-dichloro-5,5-diphenylhydantoin(V).



I. X = X′ = Cl
II. X = X′ = H
IIIa. X = Cl, X′ = H
IIIb. X = H, X′ = Cl

It has commonly been assumed that weakly alkaline solutions of I, which are of interest for bleaching fabrics, contain only I and products resulting from hydrolysis of the N—Cl bonds,

(1) Presented in part before the Organic Division at the New York Meeting of the American Chemical Society, September 1957.

(2) C. K. Ingold, S. Sako, and J. F. Thorpe, *J. Chem. Soc.*, **121**, 1177 (1922); L. A. Cohen and E. M. Fry, *J. Am. Chem. Soc.*, **78**, 5863 (1956).

(3) H. Biltz and O. Behrens, *Ber.*, **43**, 1984 (1910).