Potential Antiradiation Drugs. I. Amide, Hydroxamic Acid, and Hydrazine Derivatives of Mercapto Acids. Amino Thioacids[1]

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The syntheses and radioprotective properties of 25 compounds drawn from the title classes are described. All compounds containing a thiol function show some protective activity in either mice or bacteria. N,N'-Bis-(mercaptoacetyl)hydrazine has promise as a radioprotective drug.

The purpose of the work here described was to prepare pure organic compounds which might protect against the lethal effects of ionizing radiation.[2] When this work began it was known that aminoethanethiol, 3-amino-1-propanethiol, and a number of derivatives of these substances had some radioprotective action. It was suggested that compounds related to these substances, in which the state of oxidation of the carbon and nitrogen atoms in the molecule was increased, would be of interest. Potential drugs prepared during the present study are presented in Table I; most of these substances have either not been described previously or have been inadequately characterized.

The radioprotective properties of these compounds in mice and in bacteria are presented in Table II.

Chemistry.—The syntheses of mercaptoamides were conventional except in the cases of the mercaptoacyl derivatives of alkylenediamines (see Experimental).

Hydroxamic acids containing a free thiol function should be stable, for no loss of thiol function during an attempted reaction between propionhydroxamic acid and a typical thiol such as ethyl mercaptoacetate in dilute base was observed. However, when an attempt was made to prepare mercaptohydroxamic acids by the reaction of esters with hydroxylamine, only the related disulfides (**9** and **10**) were obtained. The oxidation of the thiol function by hydroxylamine has been observed by others,[3] and this oxidation reaction with a variety of thiols and aminothiols has been observed in the present work.

We sought to protect the thiol function by means of the S-tetrahydropyranyl group.[4] The intermediate hydroxamic acid (**11**) was prepared, but we were unable to isolate the desired mercaptoacethydroxamic acid after removal of the protecting group. Similar difficulties in isolation were encountered when cleavage of the disulfides **9** and **10** was attempted.

Simple hydrazides **12–17** were prepared by the reaction of hydrazine with the appropriate ester. The isolation of intermediate hydrazinium salts and the preparation of derivatives, including the hydrazone **15**, is described under Experimental. Two of the hydrazides were acetylated to prepare **21** and **22** using a procedure described previously[5] for the preparation of **21**.

When the preparation of N,N'-bis(mercaptoacyl)-hydrazines (**18–20**) by the reaction of hydrazine with excess ester was carried out under a variety of conditions, only the simple hydrazides were obtained. This was surprising since the literature[6] cites this procedure as a standard method for the preparation of N,N'-bis-acylhydrazines. Curtius' students[7] and, more recently, Schnabel[8] have also been unable to prepare bisacylhydrazines by this method.

The compounds **18–20** were obtained successfully by a procedure discovered accidentally. When an impure hydrochloride salt of mercaptoacethydrazide (**12**) was being recrystallized from boiling 1,2-dimethoxyethane, we observed the separation of pure N,N'-bis(mercaptoacetyl)hydrazine (**18**). During the subsequent preparation of a large sample of **18** it was observed that when the hydrazide **12** was treated with 0.5 mole of HCl in boiling 1,2-dimethoxyethane the bisacylhydrazine and hydrazine monohydrochloride were formed in high yield. No reaction occurred when 1 mole of HCl was used. This same process was then successfully applied to the synthesis of **19** and **20**.

The formation of bisacylhydrazine from hydrazide in acid solution has been observed as an unexpected side reaction during an attempt to allow a hydrazide to react with phosgene in boiling chlorobenzene,[9] during an attempt to sulfonate hydrazides with sulfamic acid,[10] during the reaction of the hydrazides of acetylglycine and hippuric acid with isonicotinyl chloride,[11] and during studies of the Curtius azide method in peptide synthesis.[8,12] Of particular interest was a brief mention[13] of the formation of **18** from **12** in aqueous acid. All of these reactions seem to be related to the nitrogen-to-nitrogen rearrangements of acyl groups observed in the case of the hydrazides of acylated amino acids.[14,15]

Our procedure using the correct amount of HCl in 1,2-dimethoxyethane has particular advantages when working with substances that are highly soluble in water.

(1) (a) This work was performed under contract DA-49-193-MD-2071 with the U. S. Army Medical Research and Development Command, Office of the Surgeon General. (b) Reported in part at the 141st National Meeting of the American Chemical Society, Washington, D. C., March 1962, Abstracts, p. 37 N.

(2) (a) J. F. Thomson, "Radiation Protection in Mammals," Reinhold Publishing Corp., New York, N. Y., 1962; (b) S. Fallab and H. Erlenmeyer, *Experientia*, **19**, 374 (1963).

(3) (a) M. Thiel, F. Asinger, K. Haussler, and T. Korner, *Ann.*, **622**, 107 (1959); (b) A. Chauveau and R. Mathis-Noel, *Ann. Fac. Sci. Univ. Toulouse, Sci. Math. Sci. Phys.*, **25**, 147 (1961).

(4) (a) G. F. Holland and L. A. Cohen, *J. Am. Chem. Soc.*, **80**, 3765 (1958); (b) R. G. Hiskey and W. P. Tucker, *ibid.*, **84**, 4789, 4794 (1962).

(5) R. L. Rose and B. R. Wilson, British Patent 782,420 (1957).

(6) See for example (a) T. Curtius, *J. prakt. Chem.*, [2] **50**, 275 (1894); (b) P. A. S. Smith, *Org. Reactions*, **3**, 366 (1946).

(7) T. Curtius and H. Hille, *J. prakt. Chem.*, [2] **64**, 403 (1901).

(8) E. Schnabel, *Ann.*, **659**, 168 (1962).

(9) T. S. Gardner, E. Wenis, and J. Lee, *J. Org. Chem.*, **26**, 1514 (1961).

(10) Y. Takata, T. Matsuda, and K. Yokata, *Nippon Kagaku Zasshi*, **82**, 376 (1961); *Chem. Abstr.*, **56**, 8553 (1962).

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(12) T. Curtius, *J. prakt. Chem.*, [2] **94**, 117 (1916).

(13) H. Fiesselmann, Abstract of paper in *Angew. Chem.*, **73**, 537 (1961).

(14) A. Kurtz and C. Niemann, *J. Am. Chem. Soc.*, **83**, 3309 (1961).

(15) M. Brenner and W. Hofer, *Helv. Chim. Acta*, **44**, 1794 (1961).

TABLE I

AMIDE, HYDROXAMIC ACID, AND HYDRAZINE DERIVATIVES OF MERCAPTO ACIDS. AMINO THIOACIDS

No.	Structure	M.p., °C.	Formula	% calcd. C	% calcd. H	% calcd. N	% calcd. S	% calcd. SH	% found C	% found H	% found N	% found S	% found SH[a]
						Amides							
1	$HSCH_2CONH_2$[b]	54–56	C_2H_5NOS	...	...	...	...	...	...	...	...	...	...
2	$HSCH_2CH_2CONH_2$[c]	99–100	C_3H_7NOS	34.3	6.66	13.3	...	28.6	34.3	6.62	13.75	...	28.8
3	$HSCH_2CH(NH_2)CONH_2.HCl$[d]	187–188	$C_3H_9ClN_2OS$	23.0	5.75	17.9	...	...	23.1	5.50	17.8	...	...
4	$HSCH_2CONHCH_2CH_2NH_2$[e]	120–122	$C_4H_{10}N_2OS$	35.8	7.46	20.9	23.9	24.6	35.8	7.33	20.6	24.0	24.4
5	$CH_3CH(SH)CONHCH_2CH_2CH_2NH_2$	143–144	$C_6H_{14}N_2OS$	44.4	8.64	17.3	19.7	20.4	44.3	8.54	17.2	20.0	20.4
6	$(HSCH_2CONHCH_2)_2$[f]	140–142	$C_6H_{12}N_2O_2S_2$	34.6	5.76	13.5	30.8	31.7	34.9	5.76	13.4	31.2	31.1
7	$(CH_3CH(SH)CONHCH_2)_2$	194–196	$C_8H_{16}N_2O_2S_2$	40.7	6.78	11.9	27.1	28.0	41.1	6.64	11.5	27.8	27.4[g]
8	$(HSCH_2CH_2CONHCH_2)_2$	154–156	$C_8H_{16}N_2O_2S_2$	40.7	6.78	11.9	27.1	28.0	40.9	6.67	11.8	27.4	27.5
						Hydroxamic Acids							
9	$(SCH_2CONHOH)_2$[h]	135 dec.	$C_4H_8N_2O_4S_2$	22.6	3.78	13.2	30.2	...	22.6	3.83	13.5	30.8	...
10	$(SCH_2CH(NH_2)CONHOH)_2$	144 dec.	$C_6H_{14}N_4O_4S_2$	26.6	5.18	20.8	23.7	...	26.8	5.18	19.3	23.5	...
11	$CH_2CH_2CH_2CH_2CHSCH_2CONHOH$ (cyclic, O)	100–101	$C_7H_{13}NO_3S$	44.0	6.81	7.33	16.7	...	43.9	6.73	7.42	16.5	...
						Hydrazine Derivatives							
12	$HSCH_2CONHNH_2$[i,j]	50–52	$C_2H_6N_2OS$	...	...	...	...	30.1	...	...	...	...	30.6
13	$CH_3CH(SH)CONHNH_2$[i,k]	94–95	$C_3H_8N_2OS$	...	...	...	...	27.4	...	...	...	...	27.3
14	$HSCH_2CH_2CONHNH_2$[i,k–m]	25	$C_3H_8N_2OS$	...	...	...	...	27.4	...	...	...	...	27.0
15	$HSCH_2CH_2CONHN{=}C(CH_3)_2$	89–90	$C_6H_{12}N_2OS$	45.0	7.50	17.5	...	20.6	45.1	7.17	17.2	...	20.8
16	$HSCH_2CH(NH_2)CONHNH_2$[n]	101–102	$C_3H_9N_3OS$	...	...	...	...	24.4	...	...	...	...	o
17	$HSCHCONHNH_2$ / $CH_2CONHNH_2$	141 dec.	$C_4H_{10}N_4O_2S$	27.0	5.62	31.4	18.0	18.5	27.5	5.64	31.0	17.7	18.3
18	$(HSCH_2CONH)_2$	156–158	$C_4H_8N_2O_2S_2$	26.6	4.45	15.5	35.6	36.7	27.0	4.27	15.4	36.4	36.5
19	$(CH_3CH(SH)CONH)_2$	203 dec.	$C_6H_{12}N_2O_2S_2$	34.5	5.80	13.4	30.7	31.7	34.7	5.83	13.3	30.7	31.2
20	$(HSCH_2CH_2CONH)_2$	174–175	$C_6H_{12}N_2O_2S_2$	34.5	5.80	13.4	...	31.7	34.4	5.53	13.6	...	31.4
21	$HSCH_2CONHNHCOCH_3$[m]	121–122	$C_4H_8N_2O_2S$	...	...	...	...	22.3	...	...	...	...	22.0
22	$HSCH_2CH_2CONHNHCOCH_3$	142–143	$C_5H_{10}N_2O_2S$	37.0	6.17	17.3	...	20.3	36.6	6.11	17.6	...	20.1
						Amino Thioacids							
23	NH_2CH_2COSH[p,q]	154 dec.	C_2H_5NOS	26.4	5.53	15.4	35.2	91.1[r]	27.2	5.55	15.6	35.2	90.8[r]
24	$CH_3CH(NH_2)COSH$[q,s]	245–253 dec.	C_3H_7NOS	34.3	6.60	13.3	30.4	...	34.3	6.38	13.6	31.2	...
25	$NH_2CH_2CH_2COSH$[q]	338–340 dec.	C_3H_7NOS	34.3	6.60	13.3	30.4	105.1[t]	34.4	6.78	13.5	30.3	104.7[r]

[a] Iodimetric, in dilute HCl. [b] P. Klason and T. Carlson, *Ber.* **39,** 736 (1906). [c] Mentioned by J. E. Jansen [U. S. Patent 2,709,706 (1955)] and by J. W. Haefele and R. W. Broge [*Am. Perfumer Aromat.*, **75,** 39 (1960)], but first described by L. Bauer and T. L. Welsh [*J. Org. Chem.*, **26,** 1443 (1961)] who used another method and reported m.p. 94–96°. [d] Free base, m.p. 98–101°, decomposes slowly at 0°. [e] H. Baron, L. Hepding, and W. Hohmann, German Patent 1,063,763 (1959). [f] N. N. Crounse, U. S. Patent 2,714,119 (1955). [g] Titration in warm methyl alcohol. [h] Ref. 3b. [i] J. W. Haefele and R. W. Broge, ref. *c*. [j] W. Schultheis, German Patent 1,108,703 (1961). [k] A. A. Chilingaryan, Y. Y. Isaevich, and A. Y. Nazarov, *Tr. Leningr. Khim.-Farmatsevt. Inst.* 5 (1962); *Chem. Abstr.*, **60,** 10541 (1964). [l] Ref. 30. [m] Ref. 5. [n] Ref. 31. [o] This hydrazide, unlike the others, gave SH values 5–10% greater than theory; oxidation of the hydrazide group may be involved [T. Curtius, *J. prakt. Chem.*, [2] **50,** 281 (1894)]. [p] T. Wieland, D. Sieber, and W. Bartmann, *Chem. Ber.*, **87,** 1093 (1954). [q] Ref. 16. [r] Neutralization equivalent. [s] T. Wieland and D. Sieber, *Naturwiss.*, **40,** 242 (1953); *ibid.*, **40,** 300 (1953).

TABLE II
PROTECTIVE ACTIVITIES

Compd.	Rodent drug dose, mg./kg.	Radiation protection: Rodent test	Radiation protection: Bacteria test (*E. coli*)
		Amides	
1	51–150	Slight	Good
2	51–150	None	Fair
3	>750	Fair	Slight
4	>750	Good	Good
	350–750	Fair	...
5	150–350	Slight	Good
6	51–150	Fair	Good
7	51–150	Not significant[a]	Slight
8	151–350	Not significant[a]	Fair
		Hydroxamic Acids	
9	51–350	None	None
10	>750	None	Slight
11	351–750	None	Slight
		Hydrazine Derivatives	
12	51–150	None	Fair
13	151–350	None	Good
14	51–150	None	Good
15	51–150	None	Fair
16	151–350	None	Good
17	351–750	Not significant[a]	Good
18	51–150	Fair	Good
	150–350	Good	...
19	150–350	Fair	Good
20	51–150	Slight	Slight
21	351–750	Fair	...
22	351–750	None	Slight
		Amino Thioacids	
23	>750	None	Fair
24	>750	None	Good
25	>750	None	Slight

[a] This compound was tested against mid-lethal radiation; the result indicates the statistical significance between survivors in treated and untreated mice.

The amino thioacids **23–25** were prepared using variations of a standard method described by Wieland,[16] who first characterized this class of compound.

Radioprotective Activities.[17]—Compounds in water solution, or suspended in a mixture of 0.2% Tween in 4% carboxymethylcellulose, were administered intraperitoneally to 15 mice. The pH of solutions and suspensions was adjusted to 7 and the drug was injected just before irradiation. The mice were tested for 30-day survival against fully lethal radiation of 1000 r. (100 r./min.) from a cobalt-60 source or 800 r. from an X-ray source. These radiations are of comparable biological effectiveness. Tests with bacteria used *Escherichia coli* b/r suspended in 15 *M* solutions of the drug in phosphate buffer and exposed to 20 kr. from a cobalt-60 source (LD_{99}). Standard technics were used to count survivors.

Protective activities are given in Table II on a scale that is correlated with similar ratings for two well-known standards. Mercaptoethylamine (cysteamine) at 150 mg./kg. is rated as "good" and N-acetylmercaptoethylamine at 300 mg./kg. is rated as "slight." More exact quantitative data on radioprotective activity have not been released by the testing agency for reasons of national security.

(16) T. Wieland and W. Bartmann, *Chem. Ber.*, **89**, 946 (1956).

(17) This work was performed by Dr. David P. Jacobus and associates, Walter Reed Army Institute of Research, Washington 12, D. C.

It is apparent that where any protection is shown the bacteria test almost always indicates a better degree of protection than does the rodent test. This higher rating of drugs in the bacteria test has been observed with all classes of radioprotective drugs so far reported and may be the result of the fact that the simpler biological system can tolerate much higher concentrations of the drug. Where no radioprotective activity is observed, as in the case of the hydroxamic acids **9–11** that contain no thiol group, both rodent and bacteria tests indicate this lack of protection.

In the compounds described here, an aliphatic thiol group appears to be a necessary structural feature for radioprotective action in rodents; in this respect the compounds are similar to the simple aminothiols.[2] The several states of oxidation in the molecule have no significant effect on drug activity except in the case of the amino thioacids where the thiol group is well known to differ chemically from that in the other drugs, and where no radioprotective activity was observed. In the case of the hydrazides (**12–17**) the protective quality of the thiol group is apparently interfered with by the hydrazide function. This interference is noted in mice, but not in bacteria, suggesting that a transport mechanism is involved.

The only amide of interest was N-(2-aminoethyl)-mercaptoacetamide (**4**) and then only at high doses. Among the hydrazine derivatives, N,N′-bis(mercaptoacetyl)hydrazine (**18**) has aroused considerable interest as a potential radioprotective drug. This good protective action may be because it has the highest per cent thiol function of all the drugs reported here. The ability of thiol groups to trap active free radicals or to bring about hypoxia in cells is an important feature of popular mechanisms to account for radioprotective action.[2]

Experimental[18]

Amides 1–8.—The simple amides **1** and **2** were prepared from methyl or ethyl esters by reaction with concentrated aqueous ammonia. Boiling with *n*-butyl alcohol during recrystallization from the solvent dried the materials effectively. Cysteineamide (**3**) was prepared by dissolving freshly prepared ethyl etser[19] in excess concentrated aqueous ammonia at 0° and allowing the solution to stand at 0° for 3 days before evaporating to dryness under vacuum. The hydrochloride was prepared by the action of methanolic HCl in methyl alcohol–ether.

N,N′-Ethylenebis(2-mercaptopropionamide) (7).—Methyl 2-mercaptopropionate, b.p. 58–60° (39 mm.), was prepared in 80% yield by Baker's procedure[20] for the analogous 3-mercapto ester; 100 g. (0.83 mole) was stirred while 15 g. (0.25 mole) of ethylenediamine was added slowly. The mixture was heated on the steam bath for 2 hr. while methyl alcohol escaped. Excess ester was then removed under vacuum at 90° and the crude residue (obtained in quantitative yield) was recrystallized from ethyl alcohol. The bisamides **6** and **8** were prepared similarly, but in methyl alcohol solution held at 45–55° for 2–4 days and then cooled to 0°. When higher temperatures were used (as in the preparation of **7**) high-melting materials containing no free thiol groups were obtained, and similar difficulties were encountered during attempts to prepare bismercaptoacyl derivatives of 1,3-diaminopropane. Erratic behavior during the synthesis of bisamides by the reaction of alkylenediamines with simple aliphatic esters has been reported by others.[21] The

(18) All melting points and boiling points are uncorrected; analyses were by S. M. Nagy (M.I.T.) or by C. K. Fitz (Needham, Mass.). With few exceptions all substances prepared in this work were very soluble in water. Most manipulations were carried out under dry nitrogen.

(19) M. Frankel and E. Katchalski, *J. Am. Chem. Soc.*, **64**, 2264 (1942).

(20) B. R. Baker, M. V. Querry, S. Bernstein, S. R. Saffir, and Y. Subbarow, *J. Org. Chem.*, **12**, 167 (1947).

(21) S. L. Vail, C. M. Moran, and H. B. Moore, *ibid.*, **27**, 2067 (1962).

presence of free thiol groups in our reactants precluded the use of special techniques recommended recently for difficult amide synthesis.[22,23]

The monoamide **4** precipitated when the related bisamide synthesis described above was carried out at 25°. After being washed with cold methyl alcohol and ether, it required no additional purification. The monoamide **5** was obtained during an unsuccessful attempt to prepare the bisamide by a reaction of 1,3-diaminopropane with excess methyl 2-mercaptopropionate in refluxing methyl alcohol.

Dithiodiglycolicdihydroxamic Acid (9).—In a typical run 187 g. (2.7 moles) of hydroxylamine hydrochloride was stirred into a solution of 144 g. (3.6 moles) of NaOH in 450 ml. of water. To this solution there was added dropwise during 20 min. 108 g. (0.9 mole) of freshly distilled ethyl mercaptoacetate. The homogeneous reaction mixture was stirred at room temperature for 15 hr. and deposited 76.6 g. (73%) of **monosodium dithiodiglycolicdihydroxamate,** m.p. 137° dec.

Anal. Calcd. for $C_4H_7N_2NaO_4S_2$: C, 20.5; H, 3.0; N, 11.9; Na, 9.8. Found: C, 19.0; H, 3.6; N, 11.1; Na, 9.4.

The dihydroxamic acid was displaced from its salt by addition of acetic acid to a warm aqueous solution of the salt. On prolonged standing at 0° the solution deposited the dihydroxamic acid, which was then recrystallized from methyl alcohol.

The monosodium salt was obtained in the same yield when the reaction was carried out in ethyl alcohol solution; in this case the sodium chloride which precipitated during the preparation of the hydroxylamine solution was removed by filtration. A similar procedure, involving an uncharacterized potassium salt, has been described[30]; the infrared spectrum of dithiodiglycolicdihydroxamic acid obtained was identical with that observed in our work. Formation of dithiodiglycolic acid[24] upon hydrolysis of our dihydroxamic acid in boiling 6 *N* HCl was additional evidence for our assigned structure.

An alternative procedure, based on that described for the synthesis of aminohydroxamic acids[25] gave pure dithiodiglycolicdihydroxamic acid directly, but in reduced yield.

Cystinedihydroxamic Acid (10).—The synthesis of hydroxamic acid derivatives of a number of amino acids has been described.[26] A general procedure described for this class[25] of compounds was used. Cysteine ethyl ester hydrochloride (30 g., 0.162 mole) and 33.6 g. (0.49 mole) of hydroxylamine hydrochloride were dissolved in 600 ml. of anhydrous methyl alcohol and the solution was cooled to 5°. A cold solution of 66 g. (0.65 mole) of triethylamine in 200 ml. of anhydrous methyl alcohol was added during 4 min.; no heat was evolved. The mixture was stored in the dark at room temperature for 24 hr. and then at 0° for 3 days. It was evaporated to dryness in a rotary evaporator at about 20 mm. The solid residue was leached with 100 ml. of water at room temperature leaving a 7.7-g. residue of crude cystinedihydroxamic acid, m.p. 130° dec. An additional 2.6 g. was recovered from the filtrate by partial evaporation and storage at 0° (47% yield). When shorter reaction times were used, the odor of unreacted ester was noted during work-up of the reaction mixture. When the reaction mixture was allowed to stand at room temperature during the entire reaction period, it became yellow and deposited elemental sulfur along with the principal product.

Cystinedihydroxamic acid was purified by a process designed to eliminate small amounts of thiol by-products[27]; 17 g. was stirred for 60 min. with 200 ml. of 2% aqueous $NaHCO_3$ while air was passed through the suspension. The material was then washed successively with water and methyl alcohol and dried. In addition to the analytical data presented in Table I a neutralization equivalent (as a base) of 136 (calcd., 135) was obtained by dissolving the substance in excess 0.1 *N* HCl and back-titrating with standard alkali. The substance formed a red color with ferric ion in dilute HCl, resembling other aminohydroxamic acids in this respect.[26]

Cystinedihydroxamic acid was refluxed for 5 hr. in 3 *N* HCl and gave L-cystine, m.p. 228°, $[\alpha]D$ −215.7° (*c* 1, *N* HCl); lit.[28] $[\alpha]D$ −215.7° (*c* 1, *N* HCl). The infrared spectra of the hydrolysis product and authentic L-cystine were identical.

(2-Tetrahydropyranylmercapto)acethydroxamic Acid (11). A mixture of 84 g. (1 mole) of redistilled dihydropyran, 60 g. (0.5 mole) of ethyl mercaptoacetate, and 2 ml. of 1,2-dimethoxyethane containing 7 mequiv. of HCl was refluxed for 4 hr. and was then allowed to stand for 8 days at room temperature. The reaction mixture was washed with 5% aqueous bicarbonate solution and then was dried over $MgSO_4$. The mixture was distilled to give 98 g. (96%) of ethyl (2-tetrahydropyranylmercapto)-acetate, b.p. 96–105° (5 mm.). A portion was redistilled for analysis, b.p. 91–92° (2 mm.).

Anal. Calcd. for $C_9H_{16}O_3S$: C, 52.9; H, 7.84; S, 15.7. Found: C, 53.4; H, 7.89; S, 14.8.

In a typical run 10.4 g. (0.05 mole) of the ester was added to a mixture prepared by addition of 6 g. (0.15 mole) of NaOH in 30 ml. of anhydrous methyl alcohol to a solution of 6.9 g. (0.1 mole) of hydroxylamine hydrochloride in 45 ml. of anhydrous methyl alcohol. The reaction mixture was stirred for 2 min. and 84% of the theoretical yield of NaCl was filtered off. After standing overnight at room temperature the reaction mixture was neutralized by stirring with the acid form of IRC-50[29] and then was evaporated to dryness in a rotary evaporator. The crude hydroxamic acid (10 g.) was recrystallized from ethyl acetate to remove salt. In large-scale runs the yield was 30%.

Mercaptoacethydrazide (12).—Ethyl mercaptoacetate (40 g., 0.334 mole) was added during 1 hr. to a stirred mixture of 42 g. (1.2 moles) of 95% hydrazine and 5 ml. of water at 5–10°. The viscous solution was diluted with 20 ml. of methyl alcohol and stored at 0° to yield 74–94% of the crystalline hygroscopic **mercaptoacethydrazide hydrazinium salt,** m.p. 76–78°.

Anal. Calcd. for $C_2H_{10}N_4OS$: C, 17.4; H, 7.25; N, 40.6. Found: C, 17.8; H, 6.85; N, 39.1.

This salt (40 g.) was dissolved in 200 ml. of dry methyl alcohol (strongly endothermic process) and carbon dioxide was passed through the solution until precipitation of gummy hydrazine carbonate was complete. The clear supernatant solution was evaporated to dryness and the crude hydrazide was recrystallized from methyl alcohol. The related disulfide, m.p. 106–107°,[5] was obtained when the hydrazide was exposed to air for several days.

2- and 3-Mercaptopropionhydrazides (13 and 14) were prepared by procedures similar to that described for **12**. The intermediate hydrazinium salts were not purified. The **maleate salt of 3-mercaptopropionhydrazide,** m.p. 88–89°, was prepared by neutralization in dry methyl alcohol and precipitation by addition of ether.

Anal. Calcd. for $C_7H_{12}N_2O_5S$: C, 35.6; H, 5.08; N, 11.9; SH, 14.0. Found: C, 35.0; H, 5.11; N, 12.3; SH, 13.9.

Air oxidation of **14** gave the related disulfide, m.p. 125–127°.[26] When **14** was refluxed in acetone, acetone 3-mercaptopropionhydrazone (**15**) separated on cooling.

Cysteinehydrazide (16).—Crude cysteine ethyl ester,[31] prepared from 32 g. (0.172 mole) of its hydrochloride, was added during 25 min. to a stirred mixture of 17.5 g. (0.515 mole) of 95% hydrazine, 2.5 ml. of water, and 15 ml. of methyl alcohol at 5–10°. The mixture was stored at −15° for 4 days and deposited 18.2 g. (79%) of crude product, which was then recrystallized from ethyl alcohol. A general procedure for the synthesis of hydrazides[32] was not successful because extensive desulfurization by hydrazine[32,33] was observed.

Mercaptosuccindihydrazide (17).—Diethyl mercaptosuccinate[34] (20 g., 0.1 mole) was heated at 80° with 7 g. (0.2 mole) of 95% hydrazine until the mixture became solid. After recrystallization from methyl alcohol, **17** was obtained in 63% yield.

N,N′-Bis(mercaptoacetyl)hydrazine (18).—Crude mercaptoacethydrazide (**12**) was prepared from 3 moles of ethyl mercapto-

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acetate as described above and then was refluxed with a mixture of 1500 ml. of peroxide-free 1,2-dimethoxyethane and 425 ml. of 3.56 *N* hydrogen chloride (1.51 moles) in the same solvent. After 2 hr. an insoluble oily phase became solid. The suspension was filtered hot and insoluble hydrazine hydrochloride was extracted with three 1-l. portions of fresh solvent. The filtrate and extracts deposited a total of 204 g. of fairly pure **18** on cooling and an additional 26 g. was obtained by partial concentration of the filtrate (85% yield). The product was recrystallized from peroxide-free 1,2-dimethoxyethane (33 ml./g.).

The bisacylhydrazines **19** and **20** were prepared from the corresponding mercaptopropionhydrazides in the same way.

When the aqueous or alcoholic solutions of these substances were warmed with a little acid, rapid conversion to the corresponding mercapto acid or its ester occurred.

A cyclic (or polymeric) disulfide derivative of **18** was prepared by dissolving 15 g. (0.075 mole) in 300 ml. of hot water and stirring in aqueous iodine–potassium iodide until a persistent yellow color formed. The product, which is quite insoluble in water, precipitated as it formed and was washed with dilute sodium thiosulfate to remove entrapped iodine. There was obtained 9.6 g. (63%), m.p. 236–237° dec.

Anal. Calcd. for $C_4H_6N_2O_2S_2$: C, 27.0; H, 3.37; N, 15.7; S, 35.9. Found: C, 26.9; H, 3.36; N, 15.3; S, 35.9.

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Enzyme Inhibitors. VI. Studies on the Bulk Tolerance of Adenosine Deaminase for 6-Substituted Amino-9-(3-hydroxypropyl)purines[1]

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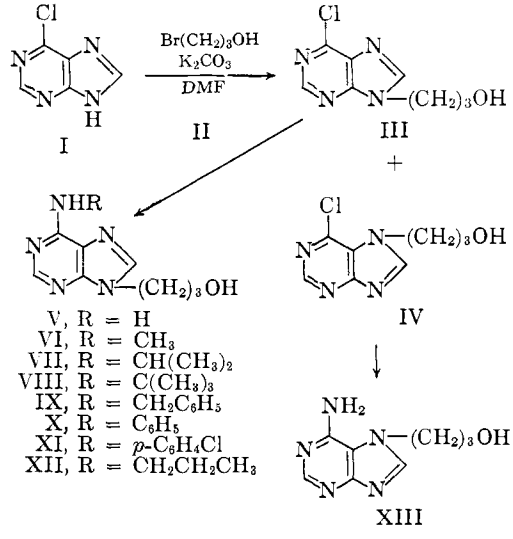
In order to study the ability of the enzyme adenosine deaminase to tolerate bulk at the 6-position of certain purine nucleoside analogs, several 6-substituted amino-9-(3-hydroxypropyl)purines were prepared. These compounds were synthesized by allowing 6-chloro-9-(3-hydroxypropyl)purine to react with the appropriate amines. Enzymatic evaluation of these compounds revealed that increasing the size of the substituent on the 6-amino group decreased the inhibitory property of the compound. These results establish that adenosine deaminase has little bulk tolerance for substituents on the 6-amino group of the purine nucleus.

In several previous studies on the enzyme adenosine deaminase, it has been found that the formation of a complex with the enzymes by a purine is dependent on several factors. For example, the purine must be substituted at the 9-position since it was observed that adenine itself was not an inhibitor.[2] It has also been determined that the substituent at the 6-position of a 9-substituted purine is critical for binding, and for compounds that are exclusively inhibitors, it has been found that a basic or neutral group at the 6-position of the purine nucleus is essential for inhibition.[3] In general, a 9-substituted purine with an amino group at the 6-position is a more effective inhibitor than the corresponding compound with a 6-methylamino group. Furthermore, the corresponding purine with a 6-dimethylamino group is either only weakly inhibitory or noninhibitory when evaluated at concentrations 2–3 times that of substrate.[2,3] Thus, it appeared that steric factors play an important role in the formation of a complex between a 9-substituted 6-aminopurine and adenosine deaminase, although it is possible to suggest that other factors such as electronic effects of the 6-substituent are the determinate factors in binding to the enzyme. The present paper describes the synthesis and enzymatic evaluation of some 6-substituted 9-(3-hydroxypropyl)purines so that the tolerance of adenosine deaminase for bulky substituents at the 6-position of the purine nucleus could be measured.

Chemistry.—Because 6-amino-9-(3-hydroxypropyl)-purine (V) has been found to be a good inhibitor of adenosine deaminase,[4] we decided to prepare some derivatives of V which were substituted at the 6-amino group by a variety of alkyl and aryl groups in order to study the ability of the enzyme to tolerate bulk at that position. The compounds which we selected for synthesis were the methyl, isopropyl, *t*-butyl, benzyl, phenyl, *p*-chlorophenyl, and *n*-propyl analogs of V. For the preparation of the 6-substituted analogs of V, it appeared that 6-chloro-9-(3-hydroxypropyl)purine (III) would be an ideal intermediate. We have previously employed this intermediate (III) which was syn-

CHART I



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