TABLE IV
EFFECT OF DOSES IN THE TREATMENT AGAINST *S. obvelata*.

No.	Treatment, mg./kg.	No. of mice	Deaths	Total elimin. *a*	Total elimin. *b*	Partial elimin. *c*
II	250, 4 days	10	2	8	100	0
	200, 4 days	30	1	21	72.4	3
	150, 4 days	20	1	10	52.6	3
	100, 4 days	20	1	7	36.8	2
	50, 4 days	10	0	2	20	2
III	250, 4 days	10	1	8	88	0
	200, 4 days	20	3	11	64.7	1
	150, 4 days	10	0	5	50	1
	100, 4 days	10	0	4	40	0
VII	250, 4 days	10	1	8	88	1
	200, 4 days	20	1	9	47.3	3
	150, 4 days	10	0	2	20	2
	120, 4 days	10	1	2	22	1
	100, 4 days	10	2	1	12.5	0
	500, once	10	0	2	20	1
	400, once	10	1	2	22	2
	200, once	10	1	2	22	0
IX	250, 4 days	10	8	1	...	0
	200, 4 days	20	7	8	61.5	1
	150, 4 days	10	0	5	50	1
	100, 4 days	10	2	1	12	1

[a] Number of mice totally free of parasite. [b] Percentage calculated on the number of living mice at the end of the test. [c] Mice with one or two pinworms in cecum.

"A." On *S. obvelata* compounds with C = 0 had no activity, while those with C = 2 and C = 1 are quite similar in potency (50 and 47%, respectively). The introduction of a double bond when C = 2 decreases this activity (XVI). Replacement of the hydrazide by an amide function (study of "B") also led to inactive compounds. The influence of anti *Hymenolepis nana* activity is not restricted to a given structure. The finding that the smallest "A" substitution was the best led us to test compounds with no "A" substitution, *i.e.*, simple hydrazides of dicarboxylic acids. Odd numbers of carbons as in II and IV seem to be the best (72.4 and 77%) and in the range of the commercial derivatives of piperazine. Introduction of an aromatic ring without changing the number of carbons between the hydrazide groups as in isophthalic hydrazide slightly increases the activity (from 72 to 83%) and considerably increases toxicity (deaths range from 1/30 for II to 4/10 for XVIII). We also tried to diminish the absorption of the active molecules by forming salts and blocking the hydrazide group with an acyl moiety which can undergo lysis in the organism. No appreciable changes were noted. In conclusion, the best structure seems to be the one of a diacid hydrazide, the most active of the compounds tested being glutaric acid dihydrazide.

Other Pharmacological Results.—A more general pharmacological study, including determination of acute toxicity in mice, measurement of variation of blood pressure and respiration in cats and dogs, and action on isolated organs: frog's heart, rat duodenum, guinea pig ileum, alone and as antagonists of acetylcholine, barium chloride, and histamine, has been performed on some of these compounds, without finding any action of interest. The toxicity is low and, in particular, they did not show any convulsive effects, contrary to what one might expect from previous studies on hydrazides.[9]

(9) H. L. Williams and J. A. Bain, *Intern. Rev. Neurobiol.*, **3**, 319 (1961).

Experimental

Synthesis.—Most of the hydrazines tested have already been described in the literature. They were easily prepared by addition of hydrazine to the corresponding ester, without solvent (sometimes followed by reflux) and careful recrystallization of the precipitate. In case of acylation the hydrazide was refluxed in the appropriate aldehyde, ketone, or anhydride.

Anthelmintic Tests. *Hymenolepis nana.*—Three weeks after infection by ingestion of 50 eggs of *H. nana*, fasted white mice received 25 mg./kg. of the test compound orally. They were then given a purgative (Na_2SO_4) 4 hr. later. The next day, the animals underwent autopsy and the percentage of parasite-free animals was determined[10] (see Tables I–IV).

Syphacia obvelata.—Mice infected with *S. obvelata* by contact with highly contaminated mice received the test compound, orally, 8–11 days after infection over a period of 4 days. On autopsy 48 hr. after completion of the treatment, the percentage of parasite-free animals was determined[11] (see Tables I–IV).

(10) R. Cavier, *Ann. pharm. franc.*, **14**, 545 (1956).

(11) R. Cavier, *Bull. Soc. Pathol. Exotique*, **54**, 850 (1961); **55**, 412 (1962).

Deaza Analogs of 6-Mercaptopurine[1]

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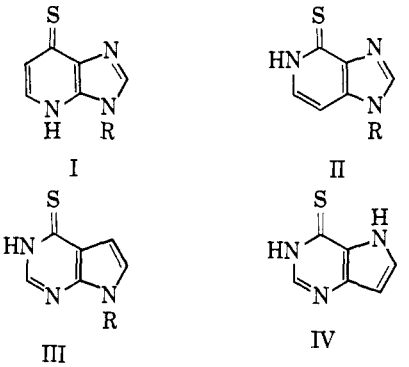
It is well known that 6-mercaptopurine is converted *in vivo* to 6-mercaptopurine ribonucleotide by cells whose growth is inhibited by 6-mercaptopurine. There is good evidence that the product of this "lethal synthesis" inhibits growth by negative pseudo-feedback, blocking the conversion of phosphoribosyl pyrophosphate to aminoribosyl phosphate, thus inhibiting *de novo* synthesis of purine nucleotides.[2]

(1) This investigation was supported by funds from the C. F. Kettering Foundation and the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. PH-43-64-51.

(2) L. L. Bennett, Jr., L. Simpson, J. Golden, and T. L. Baker, *Cancer Res.*, **23**, 1574 (1963); R. W. Brockman, *ibid.*, **23**, 1191 (1963).

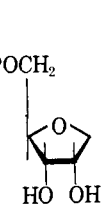
In order to learn more about the binding sites of the enzymes involved in this process of growth inhibition by 6-mercaptopurine, we have prepared the three deaza analogs (Ia–IIIa) of 6-mercaptopurine that might be converted *in vivo* to the corresponding nucleotides (Ib–IIIb) (the isomer IV, which we have also prepared,[3] cannot be).



I II III IV

a, R = H

b, R = $(HO)_2POCH_2$

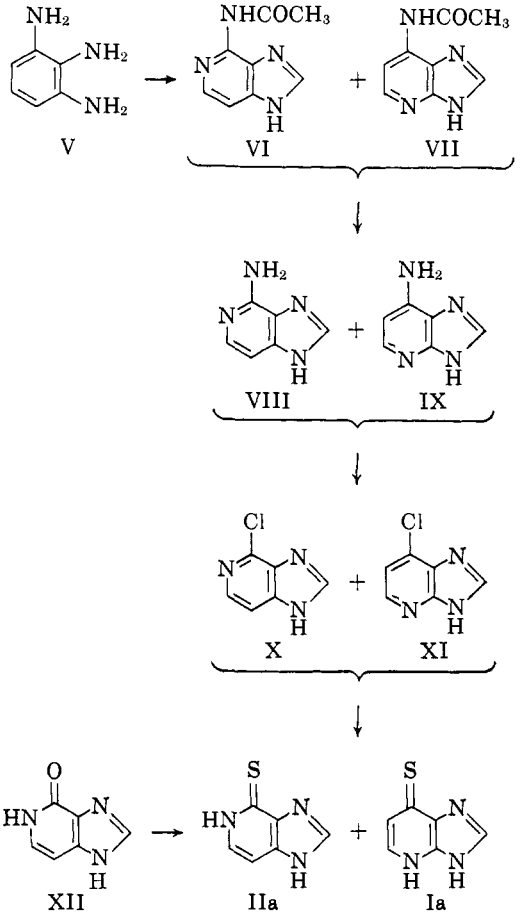


The reaction of 2,3,4-triaminopyridine (V)[5] with diethoxymethyl acetate gave a mixture of 4-acetamidoimidazo[4,5-*c*]pyridine (VI) and 7-acetamidoimidazo[4,5-*b*]pyridine (VII) from which VII was readily obtained pure in 25% yield (see Scheme I). Deacetylation of VII gave 7-aminoimidazo[4,5-*b*]pyridine (IX).[5] Deacetylation of the material obtained from the mother liquor from the isolation of VII gave a mixture of 7-aminoimidazo[4,5-*b*]pyridine (IX) and 4-aminoimidazo[4,5-*c*]pyridine (VIII)[6] from which VIII was obtained pure by several recrystallizations from water. It was more expedient, however, not to effect a separation at this point.

The mixture (VIII and IX) was diazotized in concentrated hydrochloric acid to give a mixture of the corresponding chloroimidazopyridines (X[6] and XI[5]) which, since it could not be separated, was treated with thiourea in ethanol. The resultant mixture of imidazopyridinethiones (XIII and XIV) was resolved by chromatography on a cellulose column giving pure samples of 3H-imidazo[4,5-*b*]pyridine-7(4H)-thione (1-deaza-6-mercaptopurine, Ia) and 1H-imidazo[4,5-*c*]pyridine-4(5H)-thione (3-deaza-6-mercaptopurine, IIa). The former compound was more readily prepared by the thiation of 1H-imidazo[4,5-*c*]pyridin-4(5H)-one (XII) with phosphorus pentasulfide in pyridine.

The third isomer, pyrrolo[2,3-*d*]pyrimidine-4(3H)-thione (7-deaza-6-mercaptopurine, IIIa) was prepared as described by Davoll.[7]

Scheme I



V VI VII VIII IX X XI XII IIa Ia

Table I shows the relative effectiveness of the three deaza analogs of 6-mercaptopurine in the inhibition of the growth of KB cells in culture.[8] The data show that 6-mercaptopurine is 300–500 times more effective than any of the deaza compounds. Furthermore, none of these compounds inhibited the early step of purine biosynthesis that is sensitive to inhibition by 6-mercaptopurine (in the form of its ribonucleotide) at 50 times the effective concentration of 6-mercaptopurine.[11]

Table I

Cell Culture Cytotoxicity

Compound	ED_{50}, μmole/l.[a]
6-Mercaptopurine	1.5
1-Deaza-6-mercaptopurine (Ia)	430
3-Deaza-6-mercaptopurine (IIa)	850
7-Deaza-6-mercaptopurine (IIIa)	660

[a] Lowest level inhibiting the growth of KB cells to 50% of controls.

Experimental

Melting points below 260° were determined on a Kofler Heizbank apparatus and are corrected. The ultraviolet spectra were

(3) This compound was prepared by treatment of 4-amino-5H-pyrrolo[3,2-*d*]pyrimidine[4] with nitrous acid to give 5H-pyrrolo[3,2-*d*]pyrimidin-4(3H)-one which was thiated to give IV.

(4) J. A. Montgomery and K. Hewson, *J. Org. Chem.*, **30**, 1528 (1965).

(5) F. Kögl, G. M. Van der Want, and C. A. Salemink, *Rec. trav. chim.*, **67**, 29 (1948).

(6) (a) C. A. Salemink and G. M. Van der Want, *ibid.*, **68**, 1013 (1949); (b) Y. Mizuno, T. Itoh, and K. Saito, *Chem. Pharm. Bull.* (Tokyo), **12**, 866 (1964).

(7) J. Davoll, *J. Chem. Soc.*, 131 (1960). Dr. C. C. Cheng kindly supplied our initial sample of this material.

(8) These growth inhibition studies were by the procedure of Eagle and Foley[9] and modified by the Cancer Chemotherapy National Service Center.[10]

(9) H. Eagle and G. E. Foley, *Cancer Res.*, **18**, 1017 (1958).

(10) Cancer Chemotherapy National Service Center, *Cancer Chemotherapy Rept.*, **1**, 63 (1959).

(11) L. L. Bennett, personal communication.

determined in aqueous solution with a Cary Model 14 spectrophotometer; the infrared spectra were determined in pressed potassium bromide disks with a Perkin-Elmer Model 221 spectrophotometer. The paper chromatograms were run on Whatman No. 1 paper by the descending technique.

3H-Imidazo[4,5-*b*]pyridine-7(4H)-thione (Ia) and 1H-Imidazo[4,5-*c*]pyridine-4(5H)-thione (IIa).—A suspension of 7-aminoimidazo[4,5-*b*]pyridine hydrochloride and 4-aminoimidazo[4,5-*c*]pyridine hydrochloride (3.2 g., 18.8 mmoles) in concentrated HCl (50 ml.) was cooled to 0°. With continuous stirring, solid $NaNO_2$ (2.7 g., 39.2 mmoles) was added over a 3-hr. period, and the mixture was stirred until a negative Bratton–Marshall test was obtained (18 hr.). The reaction was filtered to remove inorganic solid, and the filtrate was evaporated to dryness *in vacuo* with several additions of alcohol. Unreacted 4-aminoimidazo[4,5-*c*]pyridine hydrochloride was removed by filtration from a hot ethanol solution of the crude product. Two additional ethanol triturations followed by recrystallization of the product from ethanol removed essentially all of the starting compound; yield 2.1 g. (59%).

A solution of the mixture of 7-chloroimidazo[4,5-*b*]pyridine and 4-chloroimidazo[4,5-*c*]pyridine (540 mg., 2.9 mmoles) and thiourea (218 mg., 2.9 mmoles) in ethanol (20 ml.) was allowed to stand at room temperature, under anhydrous conditions, overnight. The reaction mixture was evaporated to dryness and the residue was chromatographed on a cellulose column using butanol–water (86:14) as the eluent. The green fluorescent product that was eluted from the column first was identified as essentially pure 1H-imidazo[4,5-*c*]pyridine-4(5H)-thione (77 mg.). The yellow fluorescent material that was eluted second was the desired 3H-imidazo[4,5-*b*]pyridine-7(5H)-thione (152 mg.). This almost pure product was dissolved in 1 *N* NaOH (3 ml.), the solution was filtered through dry Celite, and the filtrate was acidified with acetic acid. The crystals that precipitated on standing were collected by filtration and recrystallized from water to give the pure product: yield 62 mg.; m.p. dec. above 200°; R_f (water-saturated butanol) 0.44; neut. equiv., 167 (calcd., 165); λ_{max} in mμ ($\epsilon \times 10^{-3}$), pH 1—244 (7.9), 285 (9.7), 339 (17.9); pH 7—285 (10.9), 336 (12.3); pH 13—228 (14.3), 302 (17.0); $\tilde{\nu}_{max}$ in cm.$^{-1}$, 3000–2500 (acidic H), 1600, 1510 (C=C, C=N).

Anal. Calcd. for $C_6H_5N_3S \cdot 0.75H_2O$: C, 43.67; H, 3.94; N, 25.47. Found: C, 43.99; H, 4.18; N, 25.82.

1H-Imidazo[4,5-*c*]pyridine-4(5H)-thione (IIa). B.—A mixture of 1H-imidazo[4,5-*c*]pyridin-4-ol (0.5 g., 2.7 mmoles) and phosphorus pentasulfide (3 g., 13.7 mmoles) in pyridine (50 ml.) was refluxed for 4 hr. under anhydrous conditions. The cooled reaction mixture was poured into ice water (300 ml.) and the resulting solution was acidified with glacial acetic acid and concentrated to one-third volume *in vacuo*. The sulfur that precipitated was removed by filtration, and the filtrate was concentrated to one-third volume and extracted with butanol. Evaporation of the butanol extract gave 320 mg. of crude product, which was dissolved in 1 *N* NaOH (5 ml.), and the solution was filtered through dry Celite. The purified product that precipitated on acidification of the filtrate with glacial acetic acid was collected by filtration, washed with water, and dried *in vacuo*; yield 149 mg. (26%). A second precipitation from base gave the pure product: 97 mg. (17%); m.p. dec. above 350°; R_f (water-saturated butanol) 0.51; λ_{max} in mμ ($\epsilon \times 10^{-3}$), pH 1—222 (12.7), 285 (sh), 338 (14.0); pH 7—224 (12.5), 283 broad (3.5), 325 (15.3); pH 13—225 (15.2), 246 (11.5), 316.5 (14.5), 325 (sh); $\tilde{\nu}_{max}$ in cm.$^{-1}$, 3170, 3000, 2910 (NH, CH), 2800–2500 (acidic H), 1605, 1600, 1580, 1545 (C=C, C=N).

Anal. Calcd. for $C_6H_5N_3S$: C, 47.68; H, 3.34; N, 27.81; S, 21.21. Found: C, 47.64; H, 3.34; N, 27.40; S, 21.13.

7-Aminoimidazo[4,5-*b*]pyridine (IX) Hydrochloride and 4-Aminoimidazo[4,5-*c*]pyridine (VIII) Hydrochloride.[3,4]—A mixture of 2,4-diamino-3-nitropyridine (1 g., 6.5 mmoles) in ethanol (125 ml.) was hydrogenated at atmospheric pressure in the presence of platinum dioxide catalyst (100 mg.). After reduction was complete, the catalyst was removed by filtration in a nitrogen atmosphere, and the filtrate was evaporated to dryness *in vacuo*. The oily residue was dissolved in diethoxymethyl acetate (10 ml.), and the resulting solution was allowed to stand at room temperature under anhydrous conditions for 1 hr. before it was evaporated to dryness *in vacuo* with several additions of ethanol. The residue was dissolved in ethanol and diluted to cloudiness with water. The product that precipitated from the solution was collected by filtration and identified by its spectra as the 7-acetamidoimidazopyridine: yield 278 mg. (24%). A sample did not melt but sublimed above 260°; R_f (water-saturated butanol) 0.63; λ_{max} in mμ ($\epsilon \times 10^{-3}$), pH 1—286 (21.7), pH 7—273 (17.3), pH 13—282 (16.2); $\tilde{\nu}_{max}$ in cm.$^{-1}$, 3200–2600 (CH, acidic H), 1710 (C=O), 1630, 1585 (C=C, C=N), 1450 (CH).

Deacetylation was effected by dissolving the acetamidoimidazopyridine in hot 6 *N* HCl. The product crystallized on cooling the acid solution and the crystals were collected by filtration and recrystallized from ethanol–water to give the 7-aminoimidazo[4,5-*b*]pyridine as the hydrochloride salt; yield 120 mg. (11%); m.p. 330–335°; R_f (water-saturated butanol) 0.48; λ_{max} in mμ ($\epsilon \times 10^{-3}$),[12] pH 1—263 (12.0), 280 (18.4), 287 (sh); pH 7—263 (12.0), 277 (13.2), 286 (sh); pH 13—275 (13.9); $\tilde{\nu}_{max}$ in cm.$^{-1}$, 3300, 3100 (NH, CH), 2860–2400 (acidic H), 1660–1610 (NH, C=C, C=N), 1540, 1510 (C=C, C=N).

Anal. Calcd. for $C_6H_6N_4 \cdot HCl$: C, 42.27; H, 4.14; N, 32.88. Found: C, 42.07; H, 4.16; N, 32.51.

Deacetylation of the filtrate from the isolation of the 7-acetamidoimidazopyridine gave a mixture of both the 7- and 4-aminoimidazopyridine hydrochloride. A pure sample of the 4-aminoimidazo[4,5-*c*]pyridine hydrochloride was isolated as the hemihydrate after several fractional recrystallizations from water: m.p. >300°; R_f (water-saturated butanol) 0.35; λ_{max} in mμ ($\epsilon \times 10^{-3}$),[12] pH 1—260 (8.5), 275 (8.9); pH 7—258 (8.9), 267 (8.9); pH 13—274 (7.5); $\tilde{\nu}_{max}$ in cm.$^{-1}$, 3350–2600 (NH, CH, acidic H), 1685, 1630 (NH, C=C, C=N) (absence of strong 1510 band found in 7-aminoimidazo[4,5-*b*]pyridine·HCl).

Anal. Calcd. for $C_6H_6N_4 \cdot HCl \cdot 0.5H_2O$: C, 40.26; H, 4.51; N, 31.30. Found: C, 40.52; H, 4.40; N, 31.32.

Acknowledgment.—The authors are indebted to Dr. W. J. Barrett and members of the Analytical Section of Southern Research Institute who performed the spectral and microanalytical determinations reported and to Dr. G. J. Dixon under whose direction the cell culture cytotoxicity tests were done.

(12) Although numerical values for λ and ε for the ultraviolet spectra of VIII and IX are not given,[5,6] our curves agree reasonably well with the published curves.[5,6]

Enzyme Inhibitors. X. A Reinvestigation of the Alkylation of 6-Chloropurine by 3-Bromo-1-propanol[1]

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Recently, we described the alkylation of 6-chloropurine by 3-bromo-1-propanol and reported that the major product of reaction was the corresponding 9-isomer (61% yield).[2] In addition, a second alkylated 6-chloropurine was obtained[2] as a minor product (14% yield), and it was considered to be the corresponding 7-isomer by analogy with the results of Montgomery and Temple[3] who originally developed this method of synthesis. Because of some recent work on the position

(1) This investigation was supported by Public Health Service Research Grant CA-06388-03 from the National Cancer Institute, by a Public Health Service research career program award 5-K3-CA-18718 from the National Cancer Institute, by Training Grant 5-T1-JM-555 from the Division of General Medical Sciences, and by Grant T-337 from the American Cancer Society.

(2) H. J. Schaeffer and R. Vince, *J. Med. Chem.*, **8**, 33 (1965).

(3) J. A. Montgomery and C. Temple, Jr., *J. Am. Chem. Soc.*, **83**, 630 (1961).