of KEH (20 ml., 0.05 mole) in 1-butanol was added, and the MIBK was evaporated at reduced pressure (water pump) at 40° on the rotary evaporator to give 5.5 g. (38%) of white crystals after drying *in vacuo* (P_2O_5); $[\alpha]^{24}D$ +229° (*c* 1.02, water). A direct comparison of the n.m.r. spectrum of this specimen with the n.m.r. spectrum of pure potassium 6-[(+)-α-phenoxypropionamido]penicillanate[16] indicated essential optical purity.

Anal. Calcd. for $C_{18}H_{21}KN_2O_5S \cdot H_2O$: C, 49.75; H, 5.33. Found: C, 49.40; H, 5.18.

Bis(6-phenylmercaptoacetamidopenicillanyl) Disulfide (14).—To 105 mg. (0.25 mmole) of potassium 6-(phenylmercaptoacetamido)thiopenicillanate dissolved in 10 ml. of water was added concentrated HCl to pH 2.5. The solution was layered with 10 ml. of ether and treated with 2 ml. of 0.1 *N* iodine in ether. The ether was washed with 2% aqueous $NaHCO_3$ solution and finally with water and dried ($MgSO_4$). The ether was evaporated and the residue was dried *in vacuo* (0.1 mm.) for 17 hr. to yield 70 mg. (75%) of amorphous solid. See Table I for analysis and properties. The major infrared absorptions (in $cm.^{-1}$) in KBr were a broad absorption near 3370 (includes the amide NH), 1798 (β-lactam carbonyl), 1731 and 1715 (penicillanic acid disulfide carbonyl), 1685 (amide carbonyl), and 740 and 690 (monosubstituted phenyl). The n.m.r. spectrum of a solution of the disulfide in $CDCl_3$ had absorption peaks which were assigned as follows: a doublet of spacing 9 c.p.s. at δ 7.59 due to the amide proton which is coupled to the C-6 proton, a singlet at 7.36 due to the 5 aromatic protons, a quartet centered at 5.84 ascribed to the C-6 proton which is coupled to the amide proton (J = 9 c.p.s.) and to the C-5 proton (J = 4.5 c.p.s.), the C-5 proton gave rise to a doublet of spacing 4.5 c.p.s. at 5.55, a singlet at 4.37 due to the C-3 proton, a singlet at 3.68 from the protons of the methylene group, and singlets at 1.60 and 1.51 due to the *gem*-dimethyl protons.

Acknowledgments.—We wish to express our thanks to David F. Whitehead and Albert Vulcano for the interpretation of the infrared and n.m.r. spectra and to R. M. Downing and C. Kalinowski for the elemental analyses. For the microbiological data we are indebted to Dr. Joseph Lein, Dr. Alexander Gourevitch, Dr. John A. Bach, and their associates.

Studies on 2-(α-Hydroxybenzyl)benzimidazole (HBB) Analogs. I. Synthesis of 8-(α-Hydroxybenzyl)purines, the Diaza Analogs of HBB[1a,b]

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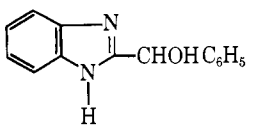
The antiviral activity displayed by a number of 2-(α-hydroxybenzyl)benzimidazoles (HBB) and several purines has initiated the synthesis of some 8-(α-hydroxybenzyl)purines. These compounds were prepared by the cyclization of 4-amino-5-(acetylmandelamino)pyrimidines which were in turn prepared by the reaction of 4,5-diaminopyrimidines with acetylmandelyl chloride. Preliminary testing results of these diaza analogs of HBB against Sarcoma 180 and KB cell culture are reported. 8-(α-Hydroxybenzyl)purine was found to be inactive against type 1 and 2 polio virus *in vitro*.

The interesting biological activity of 2-(α-hydroxybenzyl)benzimidazole (HBB, I), which suppresses poliomyelitis virus infection in mice, was first described in 1958.[2] Later, Tamm, *et al.*,[3] reported that HBB and its 6-chloro derivative showed selective inhibition against type 2 polio virus. Recently, O'Sullivan and Wallis[4] showed that 1-alkyl-substituted HBB compounds had powerful activity in tissue culture against types 1, 2, and 3 polio virus and possessed protection to ERK cells against the cytopathogenicity of entero viruses. Compounds of this type have been shown to inhibit the synthesis of virus-directed RNA polymerase,[5] of viral RNA,[6] and viral coat protein.[6a]



I

Much light has been cast on structure–activity relationships in this series of compounds.[3,4,7,8] Hydrogen bonding and metal chelation have also been investigated. From the information available at present, it appears that the α-hydroxybenzyl moiety in I is not only of fundamental importance but rather specific for the virus inhibitory action of HBB. 2-Hydroxymethyl and 2-(α-hydroxyethyl) derivatives of benzimidazole, for instance, were inactive.[3] The corresponding 2-benzoyl and 2-benzyl derivatives were less active with little selectivity of action.[3] The fact that 2-(*o*-hydroxybenzyl)benzimidazole (II) possesses similar antiviral activities but the isomeric *p*-hydroxy derivative failed to do so[9] indicated that the existence of intramolecular hydrogen bonding and/or steric requirements[10] plays an important role in this type of

(1) (a) This investigation was supported by the Cancer Chemotherapy National Service Center, National Cancer Institute of the National Institutes of Health, Public Health Service, Contract SA-43-ph-3025. (b) Presented in part before the Division of Medicinal Chemistry, 147th National Meeting of the American Chemical Society, Philadelphia, Pa., April 1964. (c) To whom all inquiries should be directed.

(2) A. C. Hollingshad and P. K. Smith, *J. Pharmacol. Exptl. Therap.*, **123**, 54 (1958).

(3) (a) I. Tamm and M. M. Nemes, *J. Clin. Invest.*, **38**, 1047 (1959); (b) I. Tamm, R. Bablanian, M. M. Nemes, C. H. Shunk, F. M. Robinson, and K. Folkers, *J. Exptl. Med.*, **113**, 625 (1961); (c) H. J. Eggers and I. Tamm, *ibid.*, **113**, 697 (1961).

(4) (a) D. G. O'Sullivan and A. K. Wallis, *Nature*, **198**, 1270 (1963); **200**, 1101 (1963); (b) D. G. O'Sullivan, P. W. Sadler, and D. J. Baner, *Antibiot. Chemotherapia*, **2**, 403 (1963); (c) D. G. O'Sullivan, *New Scientists*, **19**, 539 (1963); (d) D. G. O'Sullivan, D. Pantic, and A. K. Wallis, *Nature*, **201**, 378 (1964); (e) D. G. O'Sullivan, A. K. Wallis, and D. Pantic, Annual Report of British Empire Cancer Campaign for Research, Part II, 1963, p. 23.

(5) D. Baltimore, H. J. Eggers, R. M. Franklin, and I. Tamm, *Proc. Natl. Acad. Sci. U. S.*, **49**, 843 (1963).

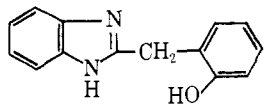
(6) (a) H. J. Eggers and I. Tamm, *Virology*, **18**, 426 (1962); (b) H. J. Eggers and I. Tamm, *Nature*, **197**, 1327 (1963); **199**, 513 (1963); (c) H. J. Eggers, E. Reich, and I. Tamm, *Proc. Natl. Acad. Sci. U. S.*, **50**, 183 (1963).

(7) A. F. Wagner, P. E. Wittreich, A. Lusi, and K. Folkers, *J. Org. Chem.*, **27**, 3236 (1962).

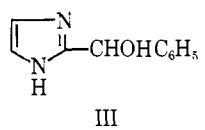
(8) I. Tamm and H. J. Eggers, *Science*, **142**, 24 (1963).

(9) D. G. O'Sullivan and P. W. Sadler, *Nature*, **192**, 341 (1961).

(10) Examination of LaPine atomic models of I and II confirmed that the hydroxy group in II can take relatively the same position that is occupied by the hydroxyl group in I.



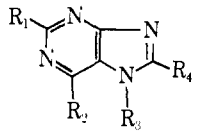
II



III

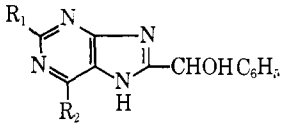
molecule. In addition, the inactivity of a very close analog of HBB, 2-(α-hydroxybenzyl)imidazole (III), reported by Tamm and Eggers,[8] suggested that a fused, bicyclic ring system is also definitely required.

It has also been reported that 2,6-diaminopurine (IVa) inhibits the multiplication of vaccinia virus *in*



IVa, R_1, R_2 = NH_2; R_3, R_4 = H
b, R_1, R_2 = Cl; R_3 = H; R_4 = OH
c, R_1, R_2, R_4 = Cl; R_3 = H
d, R_1, R_2 = Cl; R_3 = CH_3; R_4 = H
e, R_1 = NH_2; R_2, R_3, R_4 = H

vitro,[11] prevents multiplication of Russian spring-summer encephalitis virus *in vivo* and *in vitro*,[12,13] inhibits the growth of Lansing-type polio virus in monkey testicular cells,[14] has mutagenic effects on T4 bacteriophages,[15] and possesses activity against the 6-BC strain of psittacosis agent (PA) virus.[16] 2,6-Dichloro-8-hydroxypurine (IVb), 2,6,8-trichloropurine (IVc), and 2,6-dichloro-7-methylpurine (IVd) have shown virustatic but irreversible activity against vaccinia *in vitro*, but were inactive *in vivo*,[11] and 2-aminopurine (IVe) has a mutagenic effect on T4 bacteriophage.[15] These activities in conjunction with the known anticancer activities of benzimidazole derivatives[17] and substituted purines[18] suggest that the synthesis of 8-(α-hydroxybenzyl)purines (V) (see Table I), the diaza analogs of HBB, might lead to some interesting anticancer and antiviral compounds.



Va-k

The purine analog (Va) of HBB was prepared as follows. Reaction of 4,5-diaminopyrimidine (VIa)[19] with acetylmandelyl chloride (VII)[20] in hot pyridine readily gave a good yield of 4-amino-5-(acetylmandelamido)pyrimidine (VIIIa) (see Table II). Cyclization of VIIIa to 8-(α-hydroxybenzyl)purine (Va) was achieved by means of potassium hydroxide or potassium ethoxide. The latter was the preferred cyclization

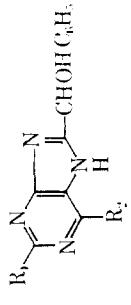


TABLE I: 8-(α-HYDROXYBENZYL)PURINES

R_1	R_2	Recrystn. solvents	Yield, %	M.p., °C. dec.	Formula	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N	pH 1 λmax, mμ	pH 1 ε × 10⁻³	pH 11 λmax, mμ	pH 11 ε × 10⁻³
H	H	Acetone	60	217–219	$C_{12}H_{10}N_4O$	63.7	4.45	24.8	64.0	4.77	24.6	268	10.9	223	8.6
														275	12.9
H	CH_3	Water	74	260–262	$C_{13}H_{12}N_4O$	65.0	5.04	23.3	65.3	5.15	23.0	270	11.4	275	9.7
H	NH_2	Methanol	72	274–275	$C_{12}H_{11}N_5O$	59.8	4.60	29.1	59.5	4.76	28.9	266	16.6	271	15.4
H	CH_3S	Water–acetone	74	222–225	$C_{13}H_{12}N_4OS$	57.3	4.45	20.6	57.2	4.52	20.5	227	12.5	230	14.2
												299	15.8	293	16.9
NH_2	H	Methanol	88	283–284	$C_{12}H_{11}N_5O \cdot H_2O$	55.6	5.05	27.0	55.8	5.40	27.1	236 (s)	11.7		6.0
												315	5.7	303	9.3
CH_3S	H	Water–acetone	81	193–195	$C_{13}H_{12}N_4OS$	57.3	4.45	20.6	57.0	4.51	20.4	240	23.4	240	21.2
												312	6.0	305	7.9
NH_2	CH_3S	Ethanol	60	272–273	$C_{13}H_{12}N_4OS$	54.4	4.56	24.4	54.3	4.71	23.9	274	7.8		
												324	12.3	316	12.9
CH_3	NH_2	Water–ethanol	70	280–281	$C_{13}H_{12}N_5O$	61.2	5.13	27.4	61.0	5.53	27.2	269	17.8	273	15.8
H	$C_6H_5CH_2S$	Ethyl acetate–heptane	80	190–191	$C_{19}H_{16}N_4OS$	65.5	4.63	16.1	65.3	4.96	16.0	298	16.7	297	18.5
H	OH	Water	50	>230	$C_{12}H_{10}N_4O_2$	59.5	4.17	23.1	59.5	4.38	22.6	253	16.1	262	14.7
H	SH	Water–ethanol	62	>200	$C_{12}H_{10}N_4OS$	55.8	3.91	21.7	56.2	4.28	21.6	228	10.6	234	18.3
												326	17.3	312	23.5

(11) (a) R. L. Thompson, M. L. Price, S. A. Minton, Jr., G. B. Elion, and G. H. Hitchings, *J. Immunol.*, **65**, 529 (1950); (b) W. Munyon, *Virology*, **22**, 15 (1964).

(12) C. Friend, *Proc. Soc. Exptl. Biol. Med.*, **78**, 150 (1951).

(13) A. E. Moore and C. Friend, *ibid.*, **78**, 153 (1951).

(14) G. C. Brown, *J. Immunol.*, **69**, 441 (1952).

(15) E. Freese, *J. Mol. Biol.*, **1**, 87 (1959).

(16) B. Dalen and H. R. Morgan, *Proc. Soc. Exptl. Biol. Med.*, **110**, 251 (1962).

(17) For a comparison of various activities, see E. Hirschberg, *Cancer Res.*, **23**, 522 (1963).

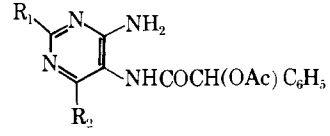
(18) For a recent review of antitumor activity among purine derivatives, see R. K. Robins, *J. Med. Chem.*, **7**, 186 (1964).

(19) D. J. Brown, *J. Appl. Chem.*, **2**, 239 (1952).

(20) F. K. Thayer, "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 12.

TABLE II

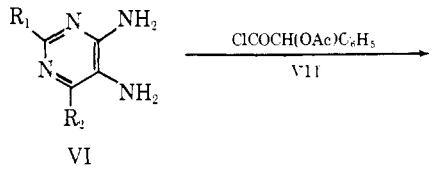
4-AMINO-5-(ACETYLMANDELAMIDO)PYRIMIDINES[a]



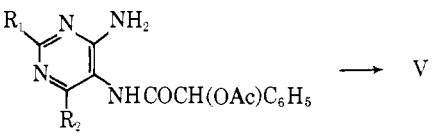
R1	R2	Recrystn. solvents	Yield, %	M.p., °C. dec.	Formula	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N	pH 1 λmax. mμ	pH 1 ε × 10⁻³	pH 11 λmax. mμ	pH 11 ε × 10⁻³
H	H	Ethyl acetate	53	198–200	$C_{14}H_{14}N_4O_3$	58.7	4.93	19.6	58.6	5.08	19.1	251	12.0	236	8.5
														281	7.4
H	CH_3	Ethanol–ethyl acetate	83	240–242	$C_{15}H_{16}N_4O_3 \cdot HCl$	53.5	5.06	16.6	53.6	5.21	16.5	249	11.1	234	7.7
														274	4.0
H	NH_2	Water–methanol	56	268	$C_{14}H_{15}N_5O_3$	55.8	5.01	23.2	55.3	5.33	23.2	263	12.0	257	6.0
H	CH_3S	Acetone	71	230–232	$C_{15}H_{16}N_4O_3S$	54.2	4.85	16.9	54.3	5.14	16.6	242	17.3	230	18.6
												294	12.6	286	8.0
NH_2	H	Water–ethanol	81	252	$C_{14}H_{15}N_5O_3 \cdot HCl$	49.9	4.77	20.8	49.8	4.97	20.9	265 (s)	5.7	227	13.4
														287	7.4
CH_3S	H	Water–acetone	80	180–181	$C_{15}H_{16}N_4O_3S$	54.2	4.85	16.9	54.5	5.23	16.8	243	25.2	226	15.3
														253	11.6
														291	8.0
NH_2	CH_3S				See Ref. 27										
CH_3	NH_2	Ethanol–ether	64	250–252	$C_5H_{17}N_5O_3 \cdot HCl$	51.3	5.15	19.9	51.6	5.48	20.2	264	10.6	258	9.9
H	$C_6H_5CH_2S$	Hexane–acetone	66	176–178	$C_{21}H_{20}N_4O_3S$	61.8	4.95	13.7	61.8	5.21	13.7	240	16.3	233	20.4
												297	11.4	280	11.0
H	OH	Water–dimethyl formamide	59	295–300	$C_{14}H_{14}N_4O_4 \cdot 0.5H_2O$	54.0	4.83	18.0	54.3	4.86	18.2	258	8.7	255	6.3

[a] Pyrimidine precursors (see text): VIa, D. J. Brown, *J. Appl. Chem.*, **2,** 239 (1952); VIb and VId, A. Albert, D. J. Brown, and H. C. S. Wood, *J. Chem. Soc.*, 3832 (1954); VIc, prepared by the reduction of 4,6-diamino-5-nitropyrimidine, R. K. Robins, K. J. Dille, C. H. Willits, and B. E. Christensen, *J. Am. Chem. Soc.*, **75,** 263 (1953); VIe and VIj, A. Albert, D. J. Brown, and G. Cheeseman, *J. Chem. Soc.*, 474 (1951); VIf, A. Albert and D. J. Brown, *ibid.*, 2060 (1954); VIg, G. D. Daves, Jr., C. W. Noell, R. K. Robins, H. C. Koppel, and A. G. Beaman, *J. Am. Chem. Soc.*, **82,** 2633 (1960); VIh, prepared by the reduction of 2-methyl-4,6-diamino-5-nitrosopyrimidine, E. C. Taylor, O. Vogl, and C. C. Cheng, *ibid.*, **81,** 2442 (1959); VIi, G. B. Elion, W. H. Lange, and G. H. Hitchings, *ibid.*, **78,** 2858 (1956).

agent since the former caused some hydrolysis of the amide. In a similar manner, several substituted purine analogs (Vb-i) were also prepared. These purine analogs were either patterned after the known HBB derivatives, naturally occurring purines, pseudo-vitamin B_{12} components,[21] or purines possessing antitumor activity. The ultraviolet absorption maxima were in accord with those of the corresponding 8-unsubstituted purines.



VI

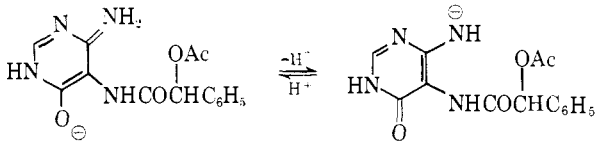


V

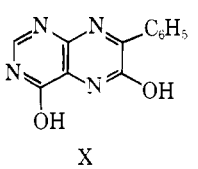
VIII

a, R_1, R_2 = H
b, R_1 = H; R_2 = CH_3
c, R_1 = H; R_2 = NH_2
d, R_1 = H; R_2 = CH_3S
e, R_1 = NH_2; R_2 = H
f, R_1 = CH_3S; R_2 = H
g, R_1 = NH_2; R_2 = CH_3S
h, R_1 = CH_3; R_2 = NH_2
i, R_1 = H; R_2 = $C_6H_5CH_2S$
j, R_1 = H; R_2 = OH
k, R_1 = H; R_2 = SH

The pure hypoxanthine analog (Vj) of HBB could not be synthesized by this general procedure. Cyclization of the intermediate amide VIIIj led to the isolation of a bright yellow product which showed an ultraviolet absorption maximum in the 380–390-mμ region in addition to the characteristic ultraviolet absorption spectrum for hypoxanthine. Paper chromatographic and other studies revealed the presence of a small amount of a side product, which is believed to be 4,6-dihydroxy-7-phenylpteridine (X). Compound X is presumably formed through a competitive cyclization *via* the intermediate IXb (formed in the presence of $^-OC_2H_5$). Studies dealing with the formation of the by-product pteridine were not carried further in the present work.



IXa IXb



X

Since much difficulty has been encountered in the separation of the purine (Vj) from the contaminated pteridine (X), pure Vj was obtained by the oxidation of 4-(methylthio)-8-(α-hydroxybenzyl)purine (Vd) with hydrogen peroxide followed by acid hydrolysis.[22]

The corresponding 8-(α-hydroxybenzyl)-4-thiopyrimidine (Vk) was prepared by the debenzylation of Vi by means of sodium and liquid ammonia.[23]

Biological Results and Discussion

Preliminary anticancer testing results of some of the 8-(α-hydroxybenzyl)purines are given in Table III. 8-(α-Hydroxybenzyl)purine (Va), one of the diazo analogs of HBB, has been studied for possible antiviral properties by techniques similar to those used by Tamm and associates[3] for HBB.[24] These tests were made in roller-tube tissue cultures of the LLC–MK_2 continuous cell line derived from rhesus monkey kidney.[25] The cultures had been grown in medium 199 supplemented with 1% normal horse serum. When confluent cell sheets had developed, the culture medium was removed, the cultures were rinsed with serum-free medium, fresh medium 199 (without serum) containing the compound in the desired concentration was added, and the tubes were inoculated with polio virus. Incubation was at 36–37° and cytopathogenic changes were observed daily. Control cultures were prepared and observed in the same manner except that the compound was not incorporated into the medium. Likewise, cultures were prepared and observed utilizing medium 199 containing HBB. Thus compound Va was directly compared to a known antiviral compound. (Compound Va and HBB were dissolved in absolute ethyl alcohol as concentrates and then diluted in medium 199 for addition to the cultures.)

When compound Va was tested against type 1 polio virus (Brunhilde strain), no antiviral activity was observed with concentrations of 400 μM of the compound in the medium. This was true for the two different levels of virus used: 10^4 plaque-forming units (PFU)/tube and 10^2 PFU/tube. However, HBB demonstrated significant antiviral activity in the same test. Both the 10^4 and 10^2 PFU concentrations of virus were suppressed for 7 days by 400 μM HBB. With 100 μM HBB in the medium the type 1 polio virus cytopathogenic effects were delayed approximately 2 days longer than observed with the untreated controls.

Data obtained with compound Va against polio virus type 2 (Brooks strain) were similar to those obtained for type 1 polio virus. Compound Va at 200 and 50 μM levels failed to protect cultures against 1000 and 10 PFU of polio virus type 2. In the same test 200 μM HBB delayed the cytopathologic changes due to the virus for approximately 3 days. No antiviral activity was observed against polio virus 2 when 50 μM HBB was tested.

HBB and compound Va demonstrated very similar toxicity in the LLC–MK_2 cells. Both compounds were toxic to about 50% of the cells when used in 400 μM concentrations. At a 100-μM concentration HBB exhibited no toxicity and Va only caused a very few cells to become granular, somewhat elongated, and slough from the glass. These toxic effects were more pronounced from the third through the seventh days of

(21) 2-Methyladenine was found to be a component in pseudo-vitamin B_{12}; *cf.*, E. L. Smith in "The Chemistry and Biology of Purines," Little, Brown and Co., Boston, Mass., 1957, pp. 160–168.

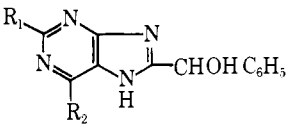
(22) Similar conversion of a methylthio group to a hydroxyl group by oxidation has been successfully applied in the pyrazolo[3,4-*d*]pyrimidine system. See R. K. Robins, *J. Am. Chem. Soc.*, **79**, 6407 (1957).

(23) The detailed debenzylation conditions were described by G. B. Elion, W. H. Lange, and G. H. Hitchings, *ibid.*, **78**, 2858 (1956).

(24) J. O. MacFarlane and R. D. Lamb, to be published.

(25) R. N. Holl, W. R. Cherry, and O. J. Tritch, *J. Exptl. Med.*, **115**, 903 (1962).

Table III
Preliminary Anticancer Testing Results of Some 8-(α-Hydroxybenzyl)purines[a]



R_1	R_2	NSC No.	Dose, mg./kg.	Test/Control[b] S180	L1210	Ca755	KB cell culture Slope[c]	KB cell culture ED_{50}, γ/ml.[d]
H	H	80599	500.0	(Toxic)				
			125.0	1.29				
			100.0		0.94	0.82		
							...	$>1.0 \times 10^2$
H	CH_3	82268	500.0	0.62				
			400.0		1.07	0.99		
							...	$>1.0 \times 10^2$
H	NH_2	83675	500.0	(Toxic)				
			250.0	1.20				
			200.0		1.05	1.06		
							...	$>1.0 \times 10^2$
H	CH_3S	82449	500.0	(Toxic)				
			250.0	0.95				
			200.0		0.95	1.04		
							...	$>1.0 \times 10^2$
H	OH	85786	500.0	0.61				
			400.0		0.97			
							...	$>1.0 \times 10^2$
H	SH	85787	250.0	0.80				
			200.0		0.97			
							−0.43	1.1×10^2
NH_2	H	84584	500.0	(Toxic)				
			125.0	(Toxic)				
			63.0	1.14				
			50.0		1.09			
							−1.10	2.6×10^1
CH_3S	H	82269	500.0	(Toxic)				
			100.0	(Toxic)				
			50.0	0.57				
			40.0		1.12	1.05		
NH_2	CH_3S	83676	500.0	0.40				
			500.0	0.97				
			400.0		1.09	(Toxic)		
							−1.10	2.5×10^1
CH_3	NH_2	87095	500.0	(Toxic)				
			125.0	0.72				
			100.0		0.96			
							−0.56	6.6×10^1

[a] The biological testing was performed by the screening contractors of the Cancer Chemotherapy National Service Center. [b] S180 = Sarcoma 180, L1210 = lymphoid leukemia L1210, Ca755 = Adenocarcinoma 755. [c] Slope: change of response for each one-log change of dose. [d] ED_{50} is the dose that inhibits growth to 50% of control growth.

culture observation. It was also observed that refeeding the cultures on the third day with fresh medium containing each of these two purine analogs caused the toxicity to be greatly enhanced. This was true for all levels and even at the 50 μ*M* concentrations.

It appears that the purine analog **Va** has no antiviral activity against the polio viruses. This has been determined in simultaneous tests in which HBB has again been demonstrated to exert a pronounced effect on this RNA virus. Whether the absence of antiviral activity of Va will carry over to other RNA viruses is yet to be determined. Also, the antiviral activity of compound Va against DNA viruses remains to be examined. It is recognized that the failure of compound Va in tissue culture tests for antiviral activity does not prevent it or its metabolic breakdown products from being active *in vivo*.

Experimental Section[26]

General Preparation of 4-Amino-5-(acetylmandelamido)pyrimidines (VIII, Table II). A. VIIIa, b, d, and f–i.—A mixture of 5 g. of the corresponding 4,5-diaminopyrimidine in 150 ml. of dry pyridine was warmed to 60–70° while solution resulted. The source of heat was removed and an equimolar amount of acetylmandelyl chloride[20] was added dropwise over a period of 20–30 min. (The heat generated from the reaction kept the temperature of the mixture just below boiling.) After the addition, the reaction mixture was allowed to stir for 4 hr. The solvent was removed *in vacuo* and the resulting syrup was triturated with cold water until a solid formed.

The crude product was then separated by filtration and recrystallized. In some cases, when the hydrochloride salt of the

(26) All melting points (corrected) were taken on a Thomas–Hoover melting point apparatus. The ultraviolet absorption spectra were determined with a Beckman DK-2.

amide (VIIb and h) was formed, solid product could be obtained by triturating the resulting syrup with acetone rather than with water.[27]

B. VIIIc and e.—An equimolar mixture of finely ground triaminopyrimidine (free base, *freshly* prepared from the corresponding 5-nitropyrimidine by Raney nickel reduction) and acetylmandelyl chloride was cautiously warmed on the steam bath. At *ca.* 90° a vigorous reaction occurred. After the reaction subdued the heterogeneous mixture was warmed for another 15 min. with constant stirring. The reaction mixture was then diluted with a large amount of acetone. The crude solid product was then filtered and purified.

8-(α-Hydroxybenzyl)purines (Va-i, Table I).—To a potassium ethoxide solution (prepared by dissolving 2.1 g. of K in 20 ml. of absolute ethanol) was added 5 g. of the amide. The mixture was warmed on a steam bath for 4 hr., poured into 250 ml. of water, and acidified with glacial acetic acid. The resulting precipitate was filtered and recrystallized.

6-Hydroxy-8-(α-hydroxybenzyl)purine (Vj).—To a solution of 200 ml. of water, 10 ml. of concentrated HCl and 10 ml. of 30% of H_2O_2 was added 4.5 g. of 6-methylthio-8-(α-hydroxybenzyl)purine (Vd). The mixture was refluxed for 20 min. and, without cooling, excess acid was carefully neutralized with dilute NaOH. After refrigeration overnight, 2.0 g. of white, analytically pure product was isolated from the reaction mixture (see Table I for additional data).

Preparation of Vj by the cyclization of 4-amino-5-acetylmandelamino-6-hydroxypyrimidine (VIIIj) in potassium ethoxide yielded a yellow solid which gave an additional ultraviolet absorption maximum at 380–390 mμ. Proper analysis for Vj was obtained (*Anal.* Calcd. for $C_{12}H_{10}N_4O_3$: C, 59.5; H, 4.17; N, 23.1. Found: C, 59.3; H, 3.78; N, 23.0.) after the crude product was repeatedly recrystallized from water and ethanol. However, the 380–390-mμ absorption was still present in the final product and paper chromatographic measurements of the recrystallized product still indicated the presence of a trace amount of a fluorescent substance. Work on the formation of this by-product, which is believed to be a pteridine, is not included in the present work.

6-Thio-8-(α-hydroxybenzyl)purine (Vk).—To a three-necked 500-ml. flask equipped with stirrer, Dry Ice cold finger, and drying tube was introduced 200–250 ml. of liquid ammonia followed by the addition of 5.0 g. of 6-benzylthio-8-(α-hydroxybenzyl)purine (Vi). To the light yellow solution was added portionwise, with stirring, 0.9 g. of sodium. After the addition was complete, the mixture was stirred for 30 min. Excess NH_3 was allowed to evaporate and the residue was dissolved in 60 ml. of water. This was then acidified with glacial acetic acid and the resulting yellow gum was recrystallized from a mixture of water and ethanol to yield 2.3 g. of a bright yellow solid (see Table I for additional data).

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(27) In the case of the preparation of VIIIg, a mixture of mono- and bis(acetylmandelamino)pyrimidine[28] was formed. The monoacetyl derivative was difficult to purify. The bisacetyl derivative, after recrystallization from ethanol, gave the following information: m.p. 208–209°; λ_{max}^{pH1} 231 mμ (ε 23,000), 303 (14,700); λ_{max}^{pH11} 290 mμ (ε 10,400). *Anal.* Calcd. for $C_{35}H_{28}N_8O_8S \cdot 0.5H_2O$: C, 56.4; H, 4.88; N, 12.9. Found: C, 56.5; H, 5.11; N, 12.8. The crude intermediate, however, was readily cyclized to the desired compound Vg by normal procedure in a 60% over-all yield.

(28) Formation of bis(acylamido)pyrimidines has also been reported by other investigators; *cf.* A. Albert and D. J. Brown, *J. Chem. Soc.*, 2060 (1954).

Substituted 5,6-Dihydro-2-(2-, 3-, and 4-pyridyl)-4H-1,3,4-oxadiazines[1]

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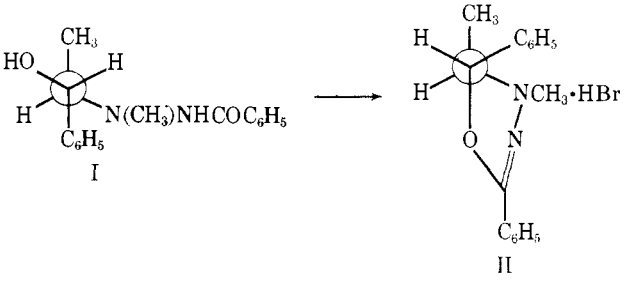
Various 2-(β-hydroxyalkyl)nicotinic, -isonicotinic, and -picolinic acid hydrazides were prepared by treating appropriate hydrazino alcohols with either the chloride, mixed anhydride, or anhydride of nicotinic, isonicotinic, and picolinic acids. Cyclodehydration of the 2-(β-hydroxyalkyl)nicotinic, isonicotinic, and picolinic acid hydrazides, containing either a primary, secondary, or tertiary hydroxyl group, to substituted 5,6-dihydro-2-(2-, 3-, and 4-pyridyl)-4H-1,3,4-oxadiazines was accomplished utilizing the four following methods: (1) concentrated H_2SO_4, (2) hydrogen bromide in acetic acid, (3) O-tosylation followed by solvolysis, and (4) replacement of OH by Cl followed by NaOH dehydrochlorination. All 2-(4-pyridyl)oxadiazines in this series antagonized the effects of tremorine in mice. The 2-(2- and 3-pyridyl) isomers were inactive in this test. It is suggested that the 2-(4-pyridyl)oxadiazines interfere with the metabolic conversion of tremorine since they did not antagonize its active metabolite oxotremorine.

As part of a continuing exploratory research program in heterocyclic syntheses, we turned our attention to the substituted 5,6-dihydro-2-(2-, 3-, and 4-pyridyl)-4H-1,3,4-oxadiazines. Previously,[2] we had discovered that the acid-catalyzed dehydration of certain 2-(β-hydroxyalkyl)carboxylic acid hydrazides proceeds *via* neighboring-group participation with concomitant formation of a substituted 5,6-dihydro-4H-1,3,4-oxadiazine. For example, treatment of *erythro*-(−)-2-methyl-2-(α-methyl-β-hydroxy-β-phenethyl)benzoic acid hydrazide (I) with gaseous hydrogen bromide in glacial acetic acid (HBr–AcOH) at ambient temperature gave *cis*-(−)-4,5-dimethyl-2,6-diphenyl-5,6-dihydro-4H-1,3,4-oxadiazine hydrobromide (II) in 87% yield.[2d]



(1) Presented in part before the Division of Medicinal Chemistry at the 150th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept. 1965.

(2) (a) D. L. Trepanier, V. Spranemanis, and K. G. Wiggs, *J. Org. Chem.*, **29**, 668 (1964); (b) D. L. Trepanier and V. Spranemanis, *ibid.*, **29**, 673 (1964); (c) *ibid.*, **29**, 2151 (1964); (d) D. L. Trepanier, V. Spranemanis, D. S. Tharpe, and P. E. Krieger, *J. Heterocyclic Chem.*, in press.