TABLE II

BACTERIOSTATIC ACTIVITIES (m.i.c., $1/X \times 10^{-3}$)[a] OF PHENOLIC DERIVATIVES

No.	*M. pyogenes* var. *aureus* (S)	*M. pyogenes* var. *aureus* (R)	*Sarcina lutea*	*Streptococcus faecalis*	*Escherichia coli* No. 198	*Aerobacter aerogenes*	*Salmonella pullorum*	*Pseudomonas aeruginosa*	*Proteus mirabilis*	*Proteus vulgaris*
I	80	80	80	80	10	10	20	20	20	20
II	1280	1280	1280	640	20	20	20	10	10	10
III	1280	1280	2560	1280	10	20	20	10	10	10
IV	2560	640	640	320	80	10	20	10	40	10
V	640	640	640	640	20	20	20	10	20	10
VI	640	1280	2560	1280	10	<10	<10	<10	<10	<10
VII	2560	2560	5120	2560	20	10	160	20	10	10
VIII	320	320	2560	1280	10	<10		10	10	10
IX	80	80	80	80	20	20	160	10	20	10
X	80	160	320	80	20	10	<10	80	20	10
XI	160	160	320	160	20	10	40	10	20	20
XII	640	640	1280	1280	20	10	20	10	10	10
XIII	640	320	640	320	20	10	10	10	10	10
XIV	160	160	160	160	20	10	10	10	20	20
XV	1280	640	640	640	10	10	80	10	20	20
XVI	1280	1280	1280	1280	80	10	<10	10	40	40
XVII	640	640	1280	320	10	<10	40	10	20	10
XVIII	1280	2560	2560	1280	40	20	20	10	40	40
XIX	640	1280	1280	640	160	20	10	10	10	20
XX	2560	2560	2560	2560	10	10	10	20	20	10
XXI	640	320	160	2560	<10	<10	10	10	10	10
XXII[b]	16000	16000	16000	16000	40	40	40	20	20	20

[a] Minimal inhibitory concentration determined by serial tube dilution technique, *e.g.*, the value of 80 is equivalent to a concentration of one part in 80,000. The serial tube dilution technique can give quite wide variations in results and the relative order of activity is more important than the absolute values listed. [b] Hexachlorophene.

(3,4-dichlorobenzyl)-4,6-dichlorophenol, and 2-(3,4-dichlorobenzyl)-4-*n*-octadecylphenol were obtained by the following procedure which describes the preparation of 2-(3,4-dichlorobenzyl)-4-chlorophenol.

3,4-Dichlorobenzyl chloride (39 g., 0.2 mole) was added over a period of 15 min. to a stirred mixture of 4-chlorophenol (154.3 g., 1.2 moles) and fused zinc chloride (2 g., 0.01 mole) at 100°. This mixture was heated further at 150° for 4 hr. Fractional distillation of the reaction product gave 126.1 g. of unchanged 4-chlorophenol, b.p. 80–100° (0.4 mm.), and 50 g. (87%) of 2-(3,4-dichlorobenzyl)-4-chlorophenol, b.p. 176–180° (0.3 mm.); m.p. 69–74°. Recrystallization from petroleum ether (b.p. 60–90°) raised the melting point to 77–78°.

2,6-Di-(3,4-dichlorobenzyl)-4-chlorophenol.—A mixture of 4-chlorophenol (47.6 g. 0.37 mole), 3,4-dichlorobenzyl chloride (77.1 g., 0.4 mole), and zinc chloride (0.5 g., 0.003 mole) was stirred at 100° for 2 hr. The mixture on distillation gave unchanged reactants, b.p. 60–90° (0.4 mm.); yield 43.5 g. and 36.5 g. (34%) of 2-(3,4-dichlorobenzyl)-4-chlorophenol, b.p. 190–200° (0.3 mm.).

The distillation residue was dissolved in chloroform (200 ml.) and the solution was washed with two 100-ml. portions of water. The chloroform solution was dried over anhydrous sodium sulfate and then evaporated *in vacuo*. The oily residue (30.5 g.) was crystallized from benzene and then from petroleum ether. The purified crystals of 2,6-di-(3,4-dichlorobenzyl)-4-chlorophenol melted at 141–142°, yield 8.6 g. (10.4%).

2-(3,4-Dichlorobenzyl)-4,6-dichlorophenol.—A mixture of 2,4-dichlorophenol (196 g., 1.2 mole) and anhydrous aluminum chloride (5 g.) was stirred at 150° for 1 hr. until hydrogen chloride evolution had ceased. 3,4-Dichlorobenzyl chloride (117 g., 0.6 mole) was added to this stirred mixture at 150° over a period of 30 min. and the heating was continued for an additional 3 hr. The cooled mixture was dissolved in chloroform (500 ml.) and the chloroform solution was washed with 5 *N* hydrochloric acid (500 ml.) and water (500 ml.). This solution was dried over anhydrous sodium sulfate and the chloroform was removed by evaporation. Fractional distillation of the residue gave 100 g. of unchanged 2,4-dichlorophenol, b.p. 60–80° (0.2 mm.), and 135 g. (70%) of 2-(3,4-dichlorobenzyl)-4,6-dichlorophenol, b.p. 170–180° (0.2 mm.); m.p. 80–88°. This product was recrystallized from petroleum ether to a constant melting point of 91–92°, yield 97 g. (50%).

2-(3,4-Dichlorobenzyl)-4-*n*-octadecylphenol.—4-*n*-Octadecylphenol (10.4 g., 0.03 mole) and fused zinc chloride (0.1 g.) were heated to 160° and 3,4-dichlorobenzyl chloride (1.96 g., 0.01 mole) was added dropwise with stirring. The reaction was held at 150–160° for 1 hr. after which the cooled product was dissolved in ether (75 ml.). The ether solution was washed with water (50 ml.) and dried. After the ether was removed by evaporation, the unchanged reactants [b.p. 212–220° (0.3 mm.), yield 6 g.] were recovered by distillation. The distillation residue was dissolved in benzene (100 ml.) and passed through a silica gel column. The column was eluted with benzene in 200-ml. portions. Fractions I, II, and V on evaporation gave oils while fractions III and IV gave the desired product, yield 3.71 g. (74%). The melting point was raised from 56–68° to a constant value of 68–69° by recrystallizing from petroleum ether.

Some 2-Substituted Aminopurines and Purine Analogs[1]

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Received July 11, 1963

Cresswell and Strauss[2] have reported the activating effect of a 5-nitroso group on the nucleophilic displacement of the 2-methylmercapto group in pyrimidines (I → II). From the several 2-substituted amino pyrimidines (III, a = 6-OH; b = 6-NH_2) thus obtained,[2] a number of 8-mercaptopurines (IV), *v*-triazolo[*d*]-pyrimidines (V), and purines (VI) have now been prepared. Their properties are given in Table I.

None of these has yet shown significant tumor inhibitory activity.[3] It is of interest that the 6-amino-8-

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, Public Health Service (Grant No. CY-3190-07), and from the Atomic Energy Commission (Contract No. AT(30-1)-910).

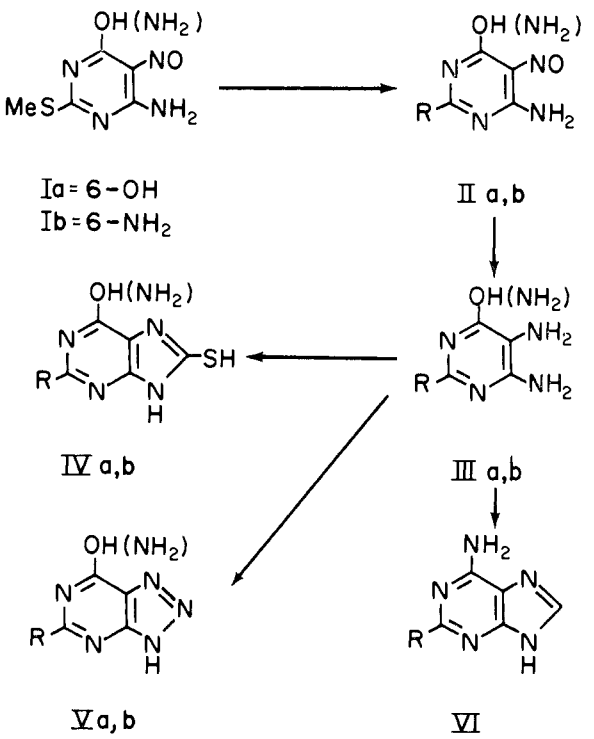
(2) R. M. Cresswell and T. Strauss, *J. Org. Chem.*, **28**, 2563 (1963).

(3) In tests carried out in the Division of Experimental Chemotherapy of this Institute.

TABLE I

Compound	R	Recrystallization solvent	Yield, %	M.p., °C.	R_f in solvent (A)	R_f in solvent (B)	λ_{max}, mμ ($\alpha_M \times 10^{-3}$ in parentheses)[a] in H_2O at pH given	
IVb	Morpholino	Ethanol	68	>300°	0.66	0.23	223 (25.8) 312 (17.2)[b]	pH 12.3
							273 (25.8) 329 (15.6)	pH 1.4
IVb	Piperidino	Ethanol	76	>300°[b]	.81	.22	223 (22.3) 316 (15.0)[b]	pH 12.3
							274 (21.2) 332 (11.5)	pH 1.3
IVb	Pyrrolidino	"	66	>300°	.67	.12	225 (26.2) 318 (17.2)[b]	pH 12.3
							273 (23.6) 332 (13.8)	pH 1.3
IVa	Morpholino	Water	60	>300°	.47	.35	238 (21.2) 303 (18.0)[b]	pH 12.4
							278 (24.9) 305 (16.3)	pH 1.2
IVa	Piperidino	"	79	>300°	.17	.30	236 (21.4) 304 (19.7)[b]	pH 12.3
							267 (21.7) 308 (11.5)	pH 1.3
IVa	Pyrrolidino	"	58	>300°	.08	.24	235 sh (22.4) 307 (15.9)[b]	pH 12.3
							275 (18.0) 310 (10.5)	pH 1.3
Vb	Morpholino	"	41	297–298°	.60	.39	228 (32.8) 299 (7.4)	pH 12.2
							220 sh (16.2) 262 (17.0) 290 sh (12.0)	pH 1.3
Vb	Piperidino	"	64	>300°	.78	.37	231 (31.6) 305 (6.6)	pH 12.2
							224 sh (17.1) 261 (16.3) 295 sh (11.3)	pH 1.1
Vb	Pyrrolidino	Ethanol	71	291–292°	.61	.27	228 (35.3) 310 (7.4)	pH 12.2
							220 sh (18.6) 260 (16.0) 292 sh (10.9)	pH 1.1
Va	Morpholino	"	55	270–271°	.56	.58	227 (29.8) 285 (8.6)	pH 12.4
							210 (20.7) 257 (19.5)	pH 1.2
Va	Piperidino	"	72	>300°	.83	.56	230 (28.2) 292 (7.8)	pH 12.4
							215 (19.6) 261 (16.0)	pH 1.2
Va	Pyrrolidino	"	58	276–277°	.63	.48	228 (33.0) 297 (8.5)	pH 12.2
							214 (25.5) 258 (11.0)	pH 1.2
VI	Morpholino	Water	38	>300°	.57	.39	227 (28.4) 288 (7.7)	pH 12.4
							234 (20.0) 290 (9.4)	pH 1.1
VI	β-Hydroxyethylamino	Water		>300°	.15	.51	263 sh (9.1) 285 (10.0)	pH 12.4
							249 (11.7) 298 (6.5)	pH 1.1

[a] Based upon single determinations. [b] When determined immediately. [c] Compound dissolved in dilute NH_4OH and reprecipitated by



mercapto-2-piperidinopurine (IVb, R = piperidino), which was shown to be ineffective against sarcoma S180 by Dr. H. C. Reilly and against B82A and P815 leukemias by Dr. J. H. Burchenal, was found by Dr. D. A. Karnofsky to inhibit profoundly cleavage in Sand Dollar embryos at levels of 0.2 μg./ml.[4] The remarkably low toxicity of this compound in mice, LD_{50} > 500 mg./kg. with repeated daily i.p. dosage, is of interest when it is compared to that of 6-amino-8-mercaptopurine, 16–32 mg./kg.,[5] and of 2,6-diamino-8-mercaptopurine, *ca.* 150 mg./kg.[3]

Experimental

Ring Closure of Pyrimidines to 8-Mercaptopurines.—Four grams of the appropriate 2-substituted, amino-4,5-diamino-6-hydroxy- (or 6-amino-) pyrimidine[1] was suspended in pyridine (32 ml.) containing 50% aqueous potassium hydroxide (1.2 vol.) and treated with carbon disulfide (12 ml.). The mixture was refluxed for 2 hr., then poured into water (200 ml.), and neutralized with glacial acetic acid. The precipitate was collected, dissolved in 0.1 *N* NaOH, filtered to remove sulfur, and reprecipitated by addition of glacial acetic acid. These 8-mercaptopurines are stable in acid and neutral solutions, but the spectra change slowly in alkali.

Ring Closure to 8-Azapurine Derivatives.—The 4,5-diaminopyrimidine (4 g.) suspended in 2 *N* acetic acid (80 ml.) was treated at 0° with a solution of sodium nitrite (4 g.) in water (120 ml.). The mixture was stirred at room temperature for 2–3 hr. The azapurine crystallized when the solution was cooled; the crystals were collected and washed.

6-Amino-2-morpholinopurine.—4,5,6-Triamino-2-morpholinopyrimidine (1 g.) in 98% formic acid (5 ml.) was heated at 90° for 1 hr. Addition of ethanol and then ether to the cooled reaction mixture gave a white precipitate (0.8 g.) which was collected, suspended in formamide (8 ml.), and heated at 170° for 1 hr. Crystals were collected after cooling the solution and were recrystallized from water containing a few drops of ammonium hydroxide to give the product (0.4 g., 38%) as white plates.

6-Amino-2-β-hydroxyethylaminopurine.—4,6-Diamino-2-β-hydroxyethylamino-5-nitrosopyrimidine (0.3 g.) in 98% formic acid (20 ml.) was heated to 90° and treated with zinc dust (0.5 g.). After 30 min. at 90°, the dark red color of the starting material was lost, and the zinc residue was removed by filtration from the hot reaction mixture. Addition of ethanol (25 ml.), followed by ether (200 ml.) gave a white solid (0.2 g.) which was

(4) D. A. Karnofsky, to be described.

(5) C. C. Stock, Ed., *Cancer Res.*, Suppl. No. 2, 193 (1955).

Molecular composition	Calcd., %				Found, %			
	C	H	N	S	C	H	N	S
$C_9H_{12}N_6OS \cdot C_2H_5OH$	44.3	6.1	28.2	10.7	43.9	6.0	27.8	11.0
$C_{10}H_{14}N_6S$	48.0	5.6	33.6	12.8	47.8	5.8	33.5	13.0
$C_9H_{12}N_6S$	45.7	5.1	35.6	13.6	45.6	5.3	35.3	13.7
$C_9H_{11}N_5O_2S$	42.7	4.4	27.7	12.7	42.3	4.4	27.3	12.3
$C_{10}H_{13}N_5OS \cdot C_2H_5OH$	48.5	6.4	23.6	10.8	48.3	6.5	23.6	11.1
$C_9H_{11}N_5OS$	45.6	4.7	29.5	13.5	45.5	5.1	29.4	13.4
$C_8H_{11}N_7O$	43.4	5.0	44.3		43.8	5.4	44.3	
$C_9H_{13}N_7$	49.3	6.0	44.7		49.0	6.2	45.0	
$C_8H_{11}N_7$	47.8	5.4	46.8		47.3	5.7	46.4	
$C_8H_{10}N_6O_2$	42.5	4.5	37.2		42.7	4.8	37.4	
$C_9H_{12}N_6O$	49.1	5.5	38.2		49.4	5.5	38.0	
$C_8H_{10}N_6O$	46.6	4.9	40.8		47.0	5.1	40.4	
$C_9H_{12}N_6O$	49.1	5.5	38.2		49.4	5.8	38.6	
$C_7H_{10}N_6O$	43.3	5.2	43.3		43.0	5.2	42.8	

addition of glacial acetic acid to pH 7. [d] Compound recrystallized from boiling H_2O with addition of sufficient ethanol to effect solution.

dissolved in formamide (3 ml.) and heated at 170° for 1 hr. Addition of ethanol and ether gave a formyl derivative (0.16 g.), m.p. above 300°.

Anal. Calcd. for $C_8H_{10}N_6O_2$: C, 43.2; H, 4.5; N, 37.8. Found: C, 42.9; H, 4.7; N, 38.1.

The formyl compound (0.1 g.) was deformylated by solution in water and treatment with a few drops of NH_4OH. A solid soon separated which was collected and recrystallized from water to yield white needles (0.065 g.).

Hydroxymethylglyoxal Bisguanylhydrazone[1]

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Received May 21, 1963

Several derivatives of guanylhydrazine have been reported to be active in inhibiting animal and human tumors.[3a-c] One of the most active compounds of this group, hydroxymethylglyoxal bisguanylhydrazone, was purportedly prepared by an osazone type of reaction between 1 mole of hydroxyacetone and 3 moles of aminoguanidine sulfate in aqueous acetic acid.[3b] The detailed synthetic procedure and the characterization of the compound have so far not been published. From available chemical, physicochemical, and biological evidence[4] it soon became apparent that the presumed hydroxymethylglyoxal bisguanylhydrazone was actually methylglyoxal bisguanylhydrazone instead. In view of the ready isomerization of dihydroxyacetone into methylglyoxal[5a-d] and the possible failure of the osazone reaction we decided to prepare the hydroxymethylglyoxal bisguanylhydrazone by the direct condensation of aminoguanidine with hydroxymethylglyoxal freshly prepared by the mild oxidation[6] of dihydroxyacetone. This proved to be successful, and the product so obtained was significantly different from the compound previously reported.[3b] Because elementary analysis cannot differentiate between methylglyoxal bisguanylhydrazone dihydrochloride monohydrate ($C_5H_{16}Cl_2N_8O$) and hydroxymethylglyoxal bisguanylhydrazone dihydrochloride ($C_5H_{14}Cl_2N_8O$) it was necessary to resort to n.m.r. spectroscopy. The data reported below are consistent with the conclusion that the present condensation product is indeed hydroxymethylglyoxal bisguanylhydrazone.

Experimental[7]

Hydroxymethylglyoxal.—This was prepared according to the published procedure[6] from dihydroxyacetone by oxidation either

(1) The correct chemical name is 1,1-[(hydroxymethyl)ethanediylidine dinitrilo]diguanidine.

(2) The work at Riker Laboratories, Inc., was supported by Contract SA-43-ph-3764 from the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service.

(3) (a) B. L. Freedlander and F. A. French, *Cancer Res.*, **18**, 360 (1958); (b) B. L. Freedlander and F. A. French, *ibid.*, **18**, 1286 (1958); (c) J. F. Holland, E. Milhich, B. Bryant, and A. I. Mulhern, *ibid.*, **21**, (1961),

(4) J. D. Davidson, R. R. Engle, and R. W. Mancuso, *Cancer Chemotherapy Rept.*, in press.

(5) (a) K. Bernhauer and B. Görlich, *Biochem. Z.*, **212**, 462 (1929); (b) H. O. L. Fischer and L. Feldmann, *Ber.*, **62**, 863 (1929); (c) H. O. L. Fischer and C. Traube, *ibid.*, **57**, 1502 (1924); (d) G. Pinkus, *ibid.*, **31**, 36 (1898).

(6) G. Hesse, F. Ramisch, and K. Renner, *ibid.*, **89**, 2137 (1956).

(7) All melting points are corrected.