

TABLE III

INITIAL RATES OF REACTION IN CATECHOLASE UNITS PER ML. OF ENZYME AT VARIOUS CONCENTRATIONS OF ASCORBIC

Mushroom juice		Prune enzyme	
Ascorbic acid, mg./l.	Rate	Ascorbic acid, mg./l.	Rate
39	2.3	50	4.0
64	2.6	100	4.4
114	2.6	500	4.8
264	2.6	1000	4.4
514	2.5		
1014	2.3		

would be reduced by increasing the ascorbic acid concentration. However, ascorbic acid would not necessarily reduce the concentration of the short-lived enzyme-semiquinone complex that has been proposed to explain reaction-inactivation.⁴

Test for Competitive Inhibition.—In the above experiments there is the small possibility that the inhibition is competitive and the substrate concentration was always too high to detect inhibition. In order to test this possibility the activity of the enzyme was measured as a function of catechol concentration at two different concentrations of ascorbic acid. The results shown in Fig. 1 do not show any competitive inhibition by ascorbic acid even at quite low concentrations of catechol.

TABLE IV

“a” VALUE AT VARIOUS ASCORBIC ACID CONCENTRATIONS^a

Initial ascorbic acid, mg./l.	Mushroom ^b enzyme	Apple ^c enzyme
200	>200	95
300	250	
400	240	95
500	250	
600	230	96
700	280	
800	240	
900	240	
1000	240	

^a In mg. of ascorbic acid. ^b 1.76 mg. of Mann amino enzyme/100 ml. reaction mixture. ^c 0.40 ml. of enzyme/100 ml. reaction mixture.

Experimental

The potato enzyme preparations were prepared by the method of Baruah and Swain.⁶ The prune enzyme was prepared by the same method as the potato enzyme except that frozen depitted prunes were used. The preparation of the apple enzyme⁴ and mushroom juice¹² have been described previously. The *a* values were determined by a method described previously¹² also. Water redistilled through all-glass equipment was used in all experiments.

(12) L. L. Ingraham, *THIS JOURNAL*, **76**, 3777 (1954).

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[CONTRIBUTION FROM THE CLAYTON FOUNDATION FOR RESEARCH, AND THE BIOCHEMICAL INSTITUTE AND DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF TEXAS]

Effects of Some 6-(Substituted)-purines on Regeneration of Hydra

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Adenine has been found to inhibit regeneration of hydra whose tentacles and hypostome have been removed. Sixteen new 6-(substituted)-aminopurines and six new 6-(substituted)-thiopurines have been prepared and characterized. The activity in inhibiting regeneration of “decapitated” hydra has been determined for these new substances and for thirteen other compounds whose syntheses have been reported previously. Certain of these adenine derivatives were found to have more than one thousand fold the activity of adenine in inhibiting regeneration; kinetin has an effect 20 times that of adenine.

The effects of 6-(2-furfuryl)-aminopurine (kinetin) on cell division in tobacco wound callus tissue^{2,3} as well as the effects of a series of 6-(substituted)-aminopurines on development in mosses^{4,5} have been reported. These results, together with the unpublished observations of several investigators, suggest a widespread importance of the 6-(substituted)-aminopurines to processes of cell division and development, at least within the plant kingdom.

Methods for quantitative study of the regeneration of hydra in a chemically defined environment recently have been developed in this Laboratory, and are being published elsewhere.⁶ Since the proc-

ess of regeneration in hydra involves obligatory cell division⁷ and since the process is under investigation as a possible model for development and differentiation in higher animals, a study of the effects of some 6-(substituted)-aminopurines on regeneration of hydra seemed desirable and was undertaken.

The preparation of most of the 6-(substituted)-aminopurines (Table I) was effected by heating an excess of the appropriate amine with 6-methylthiopurine in a sealed tube, by a procedure similar to that of Elion, Burgi and Hitchings.⁸ This method was suitable for the preparation of 6-dimethylamino- and 6-diethylaminopurine but, with other dialkylamines, the reaction mixture gave oils which did not crystallize; however, a procedure similar to that used by Daly and Christensen⁹ in

- (1) National Science Foundation Predoctoral Fellow.
- (2) C. O. Miller, F. Skoog, M. H. Von Saltza and F. M. Strong, *THIS JOURNAL*, **77**, 1392 (1955).
- (3) C. O. Miller, F. Skoog, F. S. Okumura, H. M. Von Saltza and F. M. Strong, *ibid.*, **77**, 2662 (1955).
- (4) B. S. Gorton, C. G. Skinner and R. E. Eakin, in press, *Arch. Biochem. Biophys.*
- (5) C. G. Skinner and W. Shive, *THIS JOURNAL*, **77**, 6692 (1955).
- (6) R. G. Ham, D. C. Fitzgerald, Jr., and R. E. Eakin, manuscript accepted by *J. Exp. Zool.*, 1956.

(7) F. M. Sturtevant, R. P. Sturtevant and C. L. Turner, *Science*, **114**, 241 (1951).

(8) G. B. Elion, E. Burgi and G. H. Hitchings, *THIS JOURNAL*, **74**, 411 (1952).

(9) J. W. Daly and B. E. Christensen, *J. Org. Chem.*, **21**, 1770 (1956).

TABLE I
 6-(SUBSTITUTED)-AMINOPURINES^a

R	R'	Reaction conditions			Yield, %	M.p., °C., dec.	Empirical formula	Carbon, %		Hydrogen, %	
		Time, hr.	Temp., °C.	Solvent				Calcd.	Found	Calcd.	Found
<i>n</i> -C ₃ H ₇ -	H- ^d	18	140	H ₂ O	66	240-241	C ₈ H ₁₁ N ₅	54.25	53.76	6.26	6.51
<i>n</i> -C ₆ H ₁₁ -	H- ^e	18	140	H ₂ O	44	168-169	C ₁₀ H ₁₅ N ₅	58.51	58.10	7.37	7.40
C ₂ H ₅ -	C ₂ H ₅ - ^{b, e}	18	130	None	64	219-220	C ₉ H ₁₃ N ₅	56.52	56.07	6.85	6.56
<i>n</i> -C ₃ H ₇ -	<i>n</i> -C ₈ H ₁₇ - ^{c, d}	14	90	<i>n</i> -BuOH	66	143-144	C ₁₁ H ₁₇ N ₅	60.25	59.95	7.82	7.77
<i>n</i> -C ₄ H ₉ -	<i>n</i> -C ₄ H ₉ - ^{c, d}	24	90	<i>n</i> -BuOH	72	100-101	C ₁₃ H ₂₁ N ₅	63.12	63.17	8.56	8.86
<i>n</i> -C ₆ H ₁₁ -	<i>n</i> -C ₅ H ₁₁ - ^{c, f}	24	90	<i>n</i> -BuOH	49	113-114	C ₁₅ H ₂₅ N ₅ ·H ₂ O ^g	61.40	61.88	9.29	9.06
CH ₃ -O-C ₃ H ₆ -	H- ^d	18	125	None	67	163-165	C ₉ H ₁₃ N ₅ O	52.16	52.07	6.32	6.17
(CH ₃) ₂ N-C ₃ H ₆ -	H- ^d	18	130	None	45	145	C ₁₀ H ₁₆ N ₆	54.52	54.17	7.32	7.13
(CH ₃) ₂ N-C ₃ H ₆ -	H- ^d	18	125	None	40	140-141	C ₁₂ H ₂₀ N ₆	58.04	57.45	8.12	7.76
C ₆ H ₅ -(CH ₂) ₂ -	H- ^d	12	130	None	69	239-240	C ₁₃ H ₁₃ N ₅ ^h	65.25	65.14	5.47	5.49
C ₆ H ₅ (CH ₂) ₃ -	H- ^d	18	130	None	37	173-175	C ₁₄ H ₁₅ N ₅ ⁱ	66.38	66.19	5.97	5.74
C ₆ H ₅ (CH ₂) ₄ -	H- ^d	18	140	None	42	148-149	C ₁₅ H ₁₇ N ₅ ^j	67.39	67.13	6.41	6.77
C ₆ H ₅ (CH ₂) ₅ -	H- ^d	15	140	None	12	145-147	C ₁₆ H ₁₉ N ₅ ^k	68.35	69.19	6.80	7.15
C ₆ H ₅ (CH ₂) ₇ -	H- ^d	16	140	None	54	110-111	C ₁₈ H ₂₃ N ₅	69.87	69.99	7.49	7.56
α-Naphthyl-CH ₂ CH ₂ -	H- ^d	15	140	None	64	226-227	C ₁₇ H ₁₅ N ₅	70.57	70.11	5.23	5.30
N=CH-CH-(CH ₂) ₂ -	H- ^d	17	125	H ₂ O	49	256	C ₁₀ H ₁₁ N ₇	52.39	51.76	4.84	5.12
[CH=N]											

^a The following compounds were prepared and characterized according to the procedure and data of Elion, Burgi and Hitchings (reference *b*) where R = CH₃—, CH₃CH₂— and C₄H₉— and R' = H—; also, R = CH₃— and R' = CH₃—. The 6-benzylaminopurine has been previously reported by C. G. Skinner and W. Shive, *THIS JOURNAL*, **77**, 6692 (1955). ^b This compound previously prepared and reported as a hydrochloride salt by G. B. Elion, E. Burgi and G. H. Hitchings, *ibid.*, **74**, 412 (1952). ^c Prepared through 6-chloropurine and the appropriate amine by the procedure of J. W. Daly and B. E. Christensen, *J. Org. Chem.*, **21**, 177 (1956). ^d Recrystallized from ethanol. ^e Recrystallized from alcohol-water. ^f Recrystallized from water at pH 2–3. ^g *Anal.* Calcd. for C₁₅H₂₅N₅·H₂O: N, 23.87. Found: N, 23.68. ^h *Anal.* Calcd. for C₁₃H₁₃N₅: N, 29.28. Found: N, 29.77. ⁱ *Anal.* Calcd. for C₁₄H₁₅N₅: N, 27.65. Found: N, 27.59. ^j *Anal.* Calcd. for C₁₅H₁₇N₅: N, 26.20. Found: N, 26.27. ^k *Anal.* Calcd. for C₁₆H₁₉N₅: N, 24.90. Found: N, 24.71.

which the appropriate dialkylamine is condensed with 6-chloropurine gave good yields of 6-dipropyl-, 6-dibutyl- and 6-dipentylaminopurine.

During the course of the above work the intermediates used in the preparation of the 6-(substituted)-aminopurines were assayed for ability to inhibit hydra regeneration, and 6-methylthiopurine was found to have approximately the same inhibitory activity as kinetin. To study further the structural basis for this activity, all of the 6-*n*-alkylthiopurines from ethyl through *n*-pentyl (Table II) were prepared by condensing the appropriate halide with 6-mercaptapurine in alkaline solution.

The addition of adenine to the solution used in hydra regeneration tests results in an inhibition of regeneration roughly proportional to the amount added. By extrapolation of data for several concentrations, it is possible to determine quite accurately the minimum concentration necessary for complete inhibition of visible tentacle formation at 18 hours. This minimum concentration for complete inhibition has been adopted as a standard unit for evaluating the relative effectiveness of the various compounds reported here. The activity of adenine in producing this inhibition has also been used as an arbitrary standard for comparing the activities of other compounds.

 TABLE II
 6-(SUBSTITUTED)-THIOPURINES

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6-(SUBSTITUTED)-THIOPURINES

R	Yield, %	M.p., °C.	Empirical formula	Analyses, %			
				Carbon		Hydrogen	
				Calcd.	Found	Calcd.	Found
C ₂ H ₅ —	55	185	C ₇ H ₉ N ₄ S	46.65	46.47	4.47	4.57
<i>n</i> -C ₃ H ₇ — ^a	81	163–165	C ₈ H ₁₀ N ₄ S ^c	49.46	49.54	5.19	5.28
<i>n</i> -C ₄ H ₉ — ^a	33	126–127	C ₉ H ₁₂ N ₄ S	51.89	52.06	5.81	6.01
<i>n</i> -C ₅ H ₁₁ — ^a	52	78–79	C ₁₀ H ₁₄ N ₄ S	54.02	53.70	6.35	6.26
C ₆ H ₅ —CH ₂ —	82	178–180	C ₁₂ H ₁₀ N ₄ S ^d	59.48	59.45	4.16	3.96

^a Alcohol was added to the reaction mixture to increase the reaction rate through solubility of the alkyl halide.

^b These compounds burned with difficulty on analysis.

^c *Anal.* Calcd. for C₈H₁₀N₄S: N, 28.84. Found: N, 29.08.

^d *Anal.* Calcd. for C₁₂H₁₀N₄S: N, 23.12. Found: N, 23.10.

^a Alcohol was added to the reaction mixture to increase the reaction rate through solubility of the alkyl halide.

^b These compounds burned with difficulty on analysis.

^c *Anal.* Calcd. for C₈H₁₀N₄S: N, 28.84. Found: N, 29.08.

^d *Anal.* Calcd. for C₁₂H₁₀N₄S: N, 23.12. Found: N, 23.10.

The plant callus cell division factor kinetin (6-(2-furfuryl)-aminopurine) has an activity of 20 when compared to adenine. A variety of other aromatic and heterocyclic substitutions on the amino group of 6-aminopurine give compounds with activities as high as, or frequently much higher than, that of the furfuryl derivative (Table III, A). One series in particular, the 6-(*ω*-phenyl-alkyl)-aminopurines is extremely active; the 6-(7-

phenylheptyl)-aminopurine reaches an activity of 5000 times that of adenine.¹⁰ The only non-active compounds thus far observed in these classifications are those which contain one or more basic nitrogen atoms in the substituted group.

Similarly, 6-alkylaminopurines have activities comparable to or higher than that of kinetin (Table III,B). Substitution in the alkyl group decreases the activity in the case of 3-methoxy-*n*-propyl-, and certain compounds containing a basic nitrogen in the side chain are not appreciably active. Aliphatic disubstitution on the amino group of 6-aminopurine produces compounds whose activities are somewhat higher than the corresponding monosubstituted derivatives (Table III,C).

In all cases tested thus far, S-substituted 6-thiopurines have given inhibitions of regeneration roughly comparable to the analogous nitrogen derivatives (Table III,D).

Available data indicate that the effects of N-substituted 6-aminopurines upon regenerating hydra are not comparable with the effect of "kinens" in various developing plant systems. The relative activities of the compounds show different patterns when tested in moss than they do in hydra.¹¹ This is particularly apparent in the disubstituted series which is highly active in inhibiting hydra regeneration and is practically inactive in initiating budding in mosses. Differences also appear to exist between the activities in plant callus tissues and in hydra; 6-methylaminopurine, which is twice as effective as kinetin on hydra, is reported to have no activity in the callus.¹² Although these series are not complete enough for a thorough analysis of the structural basis of activity, certain trends can be observed: (1) increasing the separation of an aromatic ring from the purine nucleus causes a sharp increase in activity; (2) increase in chain length of aliphatic substitutions causes a definite increase in activity, particularly in the disubstituted series; (3) the presence of a basic group in the 6-substituent greatly decreases activity; and (4) replacement of the 6-amino nitrogen atom with a sulfur atom does not extensively alter the effect on rate of regeneration.

Experimental¹³

Starting Materials.—All of the starting materials were obtained from standard commercial sources unless otherwise stated.

3-Phenylpropylamine, 4-phenylbutylamine, 5-phenylpentylamine and 2- α -naphthylethylamine were prepared from the corresponding nitrile¹⁴ by hydrogenation at 3.4 atm. pressure in the presence of approximately 3 parts of 95% alcohol and 1 part of ammonium hydroxide with Raney

(10) R. G. Ham, R. E. Eakin, C. G. Skinner and W. Shive, *THIS JOURNAL*, **78**, 264 (1956).

(11) B. S. Gorton and R. E. Eakin, unpublished observations.

(12) F. M. Strong, American Chemical Society Southwestern Regional Meeting, December 1, 1955, Houston, Texas.

(13) All of the melting point data were taken on a Fisher-Johns melting point block. The organic analyses were made by Mr. J. Russell Claybrook of the Biochemical Institute, The University of Texas. Ultraviolet absorption spectra were determined at a concentration of 10 γ /ml. in 95% ethyl alcohol using a Beckman model DK-2 ratio-recording spectrophotometer.

(14) 5-Phenylvaleronitrile was prepared from the corresponding acid. The acid was treated with thionyl chloride to form the acid chloride which was then transformed to the amide with ammonium hydroxide and finally the nitrile was recovered by dry distillation of the amide mixed with phosphorus pentoxide.

TABLE III
INHIBITION OF HYDRA REGENERATION BY 6-(SUBSTITUTED)-PURINES

Purine	Minimum concn. for full inhibition, μ mole/ml.	Activity in terms of adenine
A. 6-(Aromatic and heterocyclic)-amino-		
6-Amino- (Adenine)	5.0	1
6-Furfurylamino- (Kinetin)	0.25	20
6-Benzylamino- ^a	.18	30
6-(2-Phenylethyl)-amino-	.04	130
6-(3-Phenylpropyl)-amino-	.02	250
6-(4-Phenylbutyl)-amino-	.003	1700
6-(5-Phenylpentyl)-amino-	.003	1700
6-(7-Phenylheptyl)-amino-	.001	5000
6-(2- α -Naphthylethyl)-amino-	.003	1700
6-(2-pyridylmethyl)-amino- ^a	1.1	4.5
6-(3-Pyridylmethyl)-amino- ^a	1.3	3.8
6-(4-Pyridylmethyl)-amino- ^a	1.5	3.3
6-(2-Thienyl)-amino- ^a	0.1	50
6-Histamino-	.. ^b
B. 6-(Alkyl and substituted alkyl)-amino-		
6-Methylamino-	0.2	25
6-Ethylamino-	.12	40
6- <i>n</i> -Propylamino-	.08	60
6- <i>n</i> -Butylamino-	.06	80
6- <i>n</i> -Pentylamino-	.06	80
6-(3-Methoxy- <i>n</i> -propyl)-amino-	.5	10
6-(3-Dimethylamino- <i>n</i> -propyl)-amino-	.. ^b
6-(3-Diethylamino- <i>n</i> -propyl)-amino-	.. ^b
C. 6-(Di- <i>n</i> -alkyl)-amino-		
6-Dimethylamino-	0.12	40
6-Diethylamino-	.06	80
6-Di- <i>n</i> -propylamino-	.04	130
6-Di- <i>n</i> -butylamino-	.03	170
6-Di- <i>n</i> -pentylamino-	.01	500
D. 6-(Substituted)-thio-		
6-Mercapto-	.. ^b
6-Methylthio-	0.36	14
6-Ethylthio-	.30 ^c	17
6-Propylthio-	.20	25
6-Butylthio-	.07	70
6-Pentylthio-	.02	250
6-Benzylthio-	.05	100
2-(6-Thiopurine)-succinic acid	.. ^b

^a These compounds reported by C. G. Skinner and W. Shive, *THIS JOURNAL*, **77**, 6692 (1955). ^b No inhibition at 250 γ /ml. ^c This preparation was quite toxic to Hydra. Regeneration data for it are somewhat inconsistent.

nickel as a catalyst. The alcohol solution and washing from the catalyst were acidified to pH 1 and evaporated to remove the alcohol. The aqueous phase was made alkaline with sodium hydroxide in an ice-bath and then extracted with ether. The ether phase was dried over calcium sulfate, and the amine recovered by distillation under reduced pressure. The yields and boiling points of the amines were: 51%, 104–105° (15 mm.); 71%, 114–115° (14 mm.); 55%, 135–136° (10 mm.); and 54%, 170–173° (16 mm.), respectively. This compares with reported boiling points for the amines of 75–80° (1 mm.);¹⁵ 123–124° (17 mm.);¹⁶ 131° (15 mm.);¹⁸ and 170–173° (16 mm.),¹⁷ respectively, for samples prepared by different methods. 7-Phenylheptylamine was kindly supplied by Dr. P. D. Gardner, unpublished data.

(15) French Patent 751,712, Sept. 8, 1933 (*C. A.*, **28**, 778 (1934)).

(16) J. V. Braun, *Ber.*, **43**, 2837 (1910).

(17) F. Mayer and A. Sieglitz, *ibid.*, **55**, 1847 (1922).

6-(Substituted)-aminopurines.—The appropriate amines were condensed by the same general procedure whereby one part of 6-methylthiopurine¹⁸ was mixed with 2 to 5 parts of the corresponding amine, and the reaction mixture was sealed in a micro Carius tube and heated at 130 to 140° for 15 to 18 hours. The lower aliphatic amines were used in aqueous solution. In condensing some amines, as noted in Table I, a diluent was used. At the conclusion of the heating period, the cooled bomb was carefully opened with an oxygen torch. The liberated methyl mercaptan was immediately evident as a foul smelling by-product. In several instances a precipitate was present at this stage which could be washed with cold alcohol, dried and analyzed directly. In other experiments the solvent had first to be removed under reduced pressure to yield a mass of crystals which, after treating with charcoal and cooling, yielded the desired product. The yields varied considerably among the different amines; however, this may have been due more to solubility factors than to extent of the reaction, since the more soluble products were difficult to isolate in the small scale experiments that were conducted. Specific reaction conditions are itemized in Table I for each of the amines studied. The extent of purity of the reaction product readily could be ascertained by determining its ultraviolet absorption spectrum. 6-Methylthiopurine has a characteristic double peak at about 283 and 290 $m\mu$ (10 γ /ml. in 95% ethanol) which is absent in the 6-(substituted)-aminopurines. The 6-(substituted)-aminopurine spectra were almost identical in possessing a λ_{max} from about 267 to 271 $m\mu$ when examined at concentrations of 10 γ /ml. in 95% ethyl alcohol.

The 6-(di-*n*-propyl)-, 6-(di-*n*-butyl)- and 6-(di-*n*-pentyl)-aminopurines were prepared through the condensation of 6-chloropurine with the appropriate amine in the presence of butanol in a procedure similar to that of Daly and Christensen.⁹ One part of 6-chloropurine and about five parts of the appropriate amine, in the presence of 20 parts of *n*-butyl alcohol, were sealed in a glass tube and heated at 90° for varying periods of time as noted in Table I. After cooling, the solvent was removed under reduced pressure, and the residue recrystallized from alcohol-water or hot water acidified to effect solution.

6-Alkylthiopurines.—A solution, prepared by dissolving a given weight of 6-mercaptapurine in one equivalent of dilute sodium hydroxide, was treated with one equivalent of the corresponding alkyl halide using vigorous stirring. After an hour or so the oily halide phase disappeared, and a precipitate of the corresponding 6-alkylthiopurine formed. This product was separated, washed with water, and, if necessary due to a yellow color denoting unreacted 6-mercaptapurine, recrystallized from hot water. The presence of unreacted starting material was also noted by ultraviolet spectra. The 6-mercaptapurine absorbed at about 328 $m\mu$ whereas the alkylated products did not. Thus, the reaction products were purified until this absorption at 328 $m\mu$ in 95% alcohol was absent. The ultraviolet absorption spectra of these compounds are very similar in that all have characteristic double maxima of about the same intensity

between 281 and 292 $m\mu$ at concentrations of 10 γ /ml. in 95% ethanol.

The experimental data on these preparations, and the properties of the isolated products, are presented in Table II.

Using the same conditions stated above, benzyl chloride was condensed with 6-mercaptapurine in alkaline solution, and the data for this preparation are recorded in Table II.

DL-2-Chlorosuccinic Acid.¹⁹—The lack of specific preparative data in the literature leads us to outline this synthesis in some detail. Twenty ml. of glacial acetic acid was saturated at 0° with dry hydrogen chloride. To this solution, contained in a small glass bomb, was added 2.0 g. of fumaric acid and the bomb carefully sealed. The reaction mixture was then heated at 100° for about 16 hours with intermittent shaking. Complete solution was effected at the reaction temperature. The cooled bomb was opened and excess HCl and glacial acetic acid removed under reduced pressure. The recovered solid was filtered and recrystallized from ether-chloroform to yield 1.7 g. (66%) of product, m.p. 150–152°.²⁰

The DL-2-bromosuccinic acid was prepared in exactly the same manner wherein 2.0 g. of fumaric acid yielded 1.9 g. (56%) of product, m.p. 157–158°.²¹

2-(6-Thiopurine)-succinic Acid.—One gram of 6-mercaptapurine was dissolved with warming in 45 ml. of water containing one equivalent of sodium hydroxide. To this well-stirred solution was added over a period of 75 minutes 1.2 g. of DL-2-bromosuccinic acid dissolved in 20 ml. of water containing two equivalents of sodium hydroxide. The pH of the reaction mixture dropped from 10–11 to ca. 7 at the conclusion of the addition. After an additional 2 hours of stirring the solution was taken to pH 3 with dilute hydrochloric acid and reduced in volume to yield 0.6 g. of product (38% of theory) which starts decomposing at 195° and melts at 210–215° dec.

Anal. Calcd. for $C_6H_8N_4O_4S$: C, 40.3; H, 3.01; N, 20.9. Found: C, 39.31; H, 3.04; N, 21.20.

Biological Testing.—The hypostome and tentacles of the hydra are cut away and the “decapitated” bodies washed and placed in the depressions of the Pyrex spot plates containing the solutions to be tested. All compounds are tested in a phosphate buffered inorganic solution of the following composition: $CaCl_2$ 3×10^{-4} M, KCl 1×10^{-4} M, NaH_2PO_4 1.8×10^{-4} M, Na_2HPO_4 4.2×10^{-4} M, Na_2 Versenate 1×10^{-6} M. After 18 hours incubation at 27°, degree of regeneration is determined visually from the length to width ratio of the regenerated tentacles. The minimum concentration required for complete inhibition is then determined by extrapolation of a plot of degree of regeneration against concentration. A detailed account of the method of culturing and testing is being published elsewhere.⁶

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(19) R. Anschütz and C. Bennert, *Ber.*, **15**, 542 (1882), reported m.p. 151.5–152.0°.

(20) R. Anschütz and C. Bennert, ref. 19, reported m.p. 151.5–152.0°.

(21) R. Anschütz and C. Bennert, ref. 19, reported m.p. 160°.

(18) 6-Methylthiopurine was prepared by the method of G. B. Eliot, E. Burgi and G. H. Hitchings, *THIS JOURNAL*, **74**, 413 (1952).