ester prepared from 3.0 g. of *p*-methoxyphenacyl chloride as described above except not washed with alkali, was dissolved in 50 cc. of absolute alcohol, saturated with dry hydrogen chloride and refluxed for five hours. After evaporation of the ethanol the residue was dissolved in benzene and washed with dilute sodium hydroxide to remove a small amount of the acid (0.15 g.). The benzene solution was evaporated and the residue recrystallized from methanol, giving 1.95 g. of gray solid; m. p. 64–66°. Evaporative distillation of the filtrate at 140–160° (0.2 mm.) gave an additional 0.80 g. (m. p. 64–66°) bringing the total yield to 65%. The pure compound crystallized from methanol as colorless prisms, m. p. 66.0–67.0°. It gave a light-yellow color with sulfuric acid.

Anal. Calcd. for $C_{15}H_{16}O_4$: C, 69.2; H, 6.2. Found: C, 68.8; H, 6.3.

5-(*p*-Methoxyphenyl)-2-methylfuran-3-carboxylic Acid (IX).—A mixture of 6.0 g. of crude *p*-methoxyphenacylacetoacetic ester, prepared as described before, and 30 cc. of concentrated hydrochloric acid was refluxed for twenty hours, protected from air by means of a mercury trap. After cooling, the mixture was extracted with benzene and washed with water. The crude material, mainly the furan ester, was hydrolyzed by refluxing with 150 cc. of 5% potassium hydroxide for two hours. After extracting some neutral material, the aqueous layer was acidified and filtered. The product was then recrystallized from ethanol to give a total of 2.6 g. (52%) of the furan acid, m. p. 203–205°. Another recrystallization from ethanol raised the m. p. of the colorless prisms to 204.5–206°. The acid also was obtained in 93% yield by hydrolysis of the pure ethyl ester.

Anal. Calcd. for $C_{13}H_{12}O_4$: C, 67.2; H, 5.2. Found: C, 66.9; H, 5.3.

The neutral fraction from above, after evaporative distillation at 0.5 mm. and recrystallization from 90–100° petroleum ether, gave a small amount of 3-(*p*-methoxyphenyl)-cyclopenten-2-one-1, identified by m. p. and mixed m. p.

The **methyl ester** of the acid was prepared in 97% yield using ethereal diazomethane. The ester crystallized from methanol in colorless leaflets, m. p. 81.5–82°.

Anal. Calcd. for $C_{14}H_{14}O_4$: C, 68.3; H, 5.7. Found: C, 68.3; H, 5.8.

5-(*p*-Methoxyphenyl)-2-methylfuran (XI).—The furan acid (0.75 g.) was decarboxylated by refluxing for one and one-half hours with 15 cc. of quinoline and 0.3 g. of copper chromite catalyst. Benzene was then added and the mixture filtered. The benzene was washed thoroughly with dilute acid, alkali and water and the dark residue left after concentration was evaporatively distilled at 100–110° and 0.05 mm. to give 0.44 g. (72%) of the furan with the m. p. 42–44°. One recrystallization from dilute alcohol gave 0.36 g. with the m. p. 44–46°. The pure furan derivative was obtained by further recrystallization as hexagonal plates, m. p. 45.5–46°. The crystals gave a brown color with sulfuric acid and dissolved to give a yellow solution.

Anal. Calcd. for $C_{12}H_{12}O_2$: C, 76.6; H, 6.4. Found: C, 76.1; H, 6.4.

2-Hydroxy-3-acetyl-5-(*p*-methoxyphenyl)-furan (X).—A solution of 0.17 g. of sodium in 5 cc. of absolute alcohol was added to 2 g. of crude *p*-methoxyphenacylacetoacetic ester in 5 cc. of absolute alcohol. After five to ten minutes of gentle warming on the steam-bath, the mixture started to solidify. After twenty minutes the mixture was cooled, and the sodium salt filtered and washed with alcohol. The solid was dissolved in warm water, filtered and the solution acidified with dilute hydrochloric acid; 0.85 g. of crude product was obtained. Recrystallization of the material was accompanied by considerable loss. The compound existed in two polymorphic forms; recrystallization from methanol gave a buff-colored powder with the m. p. 126.5–129°, while recrystallization from 90–100° petroleum ether gave nearly colorless needles with the m. p. 100–102°. A mixture of the two forms melted at nearly the same point as the higher melting form. The analytical sample obtained by recrystallization from absolute ethanol had the m. p. 127–129°. The compound could be evaporatively distilled at 110–140° (0.3 mm.) without decomposition. It did undergo an undetermined type of decomposition upon standing for several weeks at room temperature. The compound gave an initial red-brown color with sulfuric acid, dissolving to a yellow solution; with alcoholic ferric chloride it gave a blue-green color.

Anal. Calcd. for $C_{13}H_{12}O_4$: C, 67.2; H, 5.2. Found: C, 67.5; H, 5.1.

Summary

The preparation of 3-(*p*-hydroxyphenyl)-cyclopenten-2-one-1 and its methyl ether was effected from the corresponding *p*-substituted phenacylacetoacetic esters by alkaline cyclization and hydrolysis. By reduction the related cyclopentanone derivatives were obtained.

The cyclization of *p*-methoxyphenacylacetoacetic ester to certain furan derivatives also was carried out.

MADISON, WISCONSIN RECEIVED OCTOBER 2, 1944

[CONTRIBUTION FROM THE STAMFORD RESEARCH LABORATORIES OF THE AMERICAN CYANAMID COMPANY]

Studies in Chemotherapy. VIII. Methionine and Purine Antagonists and their Relation to the Sulfonamides[1]

BY R. O. ROBLIN, JR., J. O. LAMPEN, J. P. ENGLISH, Q. P. COLE AND J. R. VAUGHAN, JR.

It has been suggested[2] that more potent *p*-aminobenzoic acid (PAB) antagonists are not likely to be found among sulfanilamide type compounds. On the other hand, an agent which interfered with another stage in PAB metabolism, when combined with sulfonamides, might be expected to enhance the effectiveness of the latter. Assuming that secondary sulfonamide inhibitors such as methionine[3] and the purines[4] may bear some metabolic relationship to PAB[3,4,5] we have attempted to prepare antagonists of these metabolites applying the general concept proposed by Fildes.[6]

(1) Presented in part before the Division of Medicinal Chemistry, New York meeting of the American Chemical Society, September 15, 1944.

(2) Bell and Roblin, THIS JOURNAL, **64**, 2905 (1942).

(3) Bliss and Long, *Bull. Johns Hopkins Hosp.*, **69**, 14 (1941); Harris and Kohn, *J. Pharmacol.*, **73**, 383 (1941); Straus, Dingle and Finland, *J. Immunol.*, **42**, 313, 331 (1941).

(4) Harris and Kohn, *J. Biol. Chem.*, **141**, 989 (1941); Snell and Mitchell, *Arch. Biochem.*, **1**, 93 (1942); Kohn and Harris, *J. Pharmacol.*, **77**, 1 (1943).

(5) Kohn, *Ann. N. Y. Acad. Sci.*, **44**, 503 (1943).

(6) Fildes, *Lancet*, *I*, 955 (1940); *cf.* Reviews by McIlwain, *Lancet*, *I*, 412 (1942); *Nature*, **151**, 270 (1943); Kuhn, *Die Chemie*, **55**, 1 (1942); Wagner-Jauregg, *Naturwissenschaften*, **31**, 335 (1943).

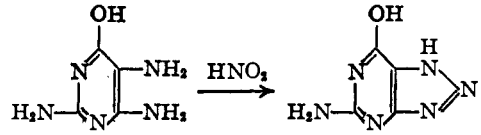
dl-α-Amino-γ-methoxybutyric acid (methoxinine), the oxygen analog of methionine, was prepared through β-methoxyethyl bromide[7] and phthalimidomalonic ester.[8] In the course of this work, an improved method for the preparation of the bromide was devised.

The growth of various microörganisms such as *E. coli* and *Staph. aureus* in synthetic media was prevented by methoxinine. This antibacterial action was reversed by *l*-methionine, but not by the *d*-isomer. One mole of *dl*-methionine interfered with the bacteriostatic effect of 500 to 1000 moles of *dl*-methoxinine. No reversing action was observed with PAB or choline alone, although together they appeared to produce a partial reversal.

In combination with sulfonamides approximately one-fourth the minimum effective concentration of methoxinine together with one-fourth the m. e. c. of sulfanilamide, sulfapyridine, sulfathiazole or sulfadiazine produced complete bacteriostasis. Similar results were obtained with *dl*-ethionine[9] (S-ethylhomocysteine), confirming the observations of Harris and Kohn.[3]

Because the activity of ethionine was not reversed by PAB, Harris and Kohn[3] suggested that methionine was probably related to PAB in some subsequent metabolic process. The results reported here might also be accounted for by assuming that methionine serves as a precursor of PAB. Failure of the latter substance to reverse the growth inhibition due to methoxinine and ethionine may not be conclusive, since methionine is probably involved in several metabolic processes.

Purine antagonists in the 1-*v*-triazolo[*d*]pyrimidine[10] series were obtained by diazotization of the requisite 4,5-diaminopyrimidines as illustrated.



Analogs of guanine, adenine, xanthine and hypoxanthine, in which a nitrogen replaced a carbon atom of the corresponding purine, were prepared by this general method. The pyrimidine intermediate required for the synthesis of the hypoxanthine analog was obtained by refluxing an aqueous solution of 2-thio-4,5-diamino-6-hydroxypyrimidine with Raney nickel catalyst.[11] An attempt to prepare this intermediate by the action of nitric acid on the 2-thiopyrimidine was unsuccessful. This procedure for the removal of thio groups from heterocyclic rings[12] led to the anomalous[13] formation of the 2-hydroxy derivative. An analysis of the triazolopyrimidine derived from it confirmed the structure of the intermediate pyrimidine. Furthermore, the ultraviolet absorption spectrum[14] of this triazolopyrimidine was identical with 5,7-dihydroxy-1-*v*-triazolo[*d*]pyrimidine prepared by another method. It is interesting that the position of the ultraviolet absorption maxima and the type of shift with *p*H for all the triazolopyrimidines were very similar to the values for their purine counterparts. These data are presented in Table I.

TABLE I

ULTRAVIOLET ABSORPTION MAXIMA OF TRIAZOLOPYRIMIDINES AND PURINES[a]

Compound[b]	Ref.	Absorption maxima in Å. *p*H 3	7	11
Adenine	[c]		2610	2700[d]
I		2700	2750	2750
Hypoxanthine	[e]	2500	2480	2600
II		2535	2585	2675
Guanine	[f]	2480[g]	2450[h]	
		2505	2730	
		2750	2780	
III		2475	2475	2400–
		2650–		2500
		2700	2770	2800
Xanthine	[e]	2660	2670	2410
				2780
IV-A		2650	2650	2350
				2850
IV-B[i]		2650	2650	2350
				2850

[a] Beckman Spectrophotometer Model DU; range 3000–2200 Å., 7 × 10^{-5} *M* solutions. [b] See Table II. [c] Loofbourow and Stimson, *J. Chem. Soc.*, 844 (1940). [d] *p*H 12.25. [e] Stimson and Reuter, THIS JOURNAL, **65**, 153 (1943). [f] Heyroth and Loofbourow, *ibid.*, **56**, 1728 (1934). [g] *p*H 2.6. [h] *p*H 6.6. [i] Prepared from the product obtained by treatment of 2-thio-4,5-diamino-6-hydroxypyrimidine with nitric acid.

Woolley[15] has reported that the bacteriostatic action of benzimidazole on various microörganisms was reversed by the purines, adenine and guanine. Xanthine, hypoxanthine and, with one exception, uracil were ineffective in this respect. Benzimidazole completely inhibited the growth of our strain of *E. coli* at 128 mg. per 100 cc., but under these conditions, adenine, guanine and other purines and pyrimidines did not reverse this effect at concentrations which were not in themselves bacteriostatic.

(7) Karvonen, *Chem. Zentr.*, **83**, II, 1269 (1912).

(8) *Cf.* Sorensen, *Z. physiol. Chem.*, **44**, 448 (1905); "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, 1943, p. 384.

(9) We are indebted to Dr. V. du Vigneaud, Cornell University Medical College, New York, N. Y., for a generous supply of *dl*-ethionine and *d*- and *l*-S-benzylhomocysteine from which *d*- and *l*-methionine were prepared by the method of du Vigneaud, Dyer and Harmon, *J. Biol. Chem.*, **101**, 719 (1933).

(10) This name conforms with Patterson and Capell, "The Ring Index," Reinhold Publishing Corp., New York, N. Y., 1940, system No. 702. The older literature refers to compounds of this type as aziminopyrimidines; *cf.* Gabriel and Coleman, *Ber.*, **34**, 1249 (1901).

(11) Mozingo, Wolf, Harris and Folkers, THIS JOURNAL, **65**, 1013 (1943).

(12) (a) Houben, "Die Methoden der organischen Chemie," vol. 2, p. 197, 3rd ed., Georg Thieme, Leipzig, 1925; (b) Traube, *Ann.*, **331**, 73 (1904).

(13) *Cf.* Wheeler and Bristol, THIS JOURNAL, **33**, 437 (1905).

(14) We are indebted to Dr. P. H. Bell and Mr. J. F. Bone for the ultraviolet absorption data.

(15) Woolley, *J. Biol. Chem.*, **152**, 225 (1944).

In the triazolopyrimidine series the adenine and guanine analogs in particular exhibited a specific antibacterial action (Table II).

TABLE II

BACTERIOSTATIC ACTIVITY AND MOLECULAR INHIBITION RATIOS OF TRIAZOLOPYRIMIDINES

	Compound	M. E. C.[a] *E. coli*	M. E. C.[a] *S. aureus*	Inhibition[b] By	Inhibition[b] Ratio
I	7-Amino-1-*v*-triazolo[*d*]-pyrimidine	8	>128	A H	640 640[d]
II	7-Hydroxy-1-*v*-triazolo-[*d*]pyrimidine[c]	64	16	A H	6400 6400
III	5-Amino-7-hydroxy-1-*v*-triazolo[*d*]pyrimidine	64	4	G X	128 35
IV	5,7-Dihydroxy-1-*v*-triazolo[*d*]pyrimidine	>128	>128	..	..

[a] M. E. C., Minimum effective concentration in mg./100 cc. preventing visible growth for forty-eight hours. [b] With *E. coli;* A, adenine; H, hypoxanthine; G, guanine; X, xanthine. Inhibition ratio represents the number of moles of antagonist required to inhibit the effect of one mole of purine. [c] Results at sixteen hours, M. E. C. >128 mg./100 cc. at forty-eight hours. (See Experimental.) [d] The action of hypoxanthine was less rapid; no reversal was apparent even at a ratio of 3:1 for the first sixteen hours.

When several m. e. c.'s were employed, the action of I on *E. coli* was reversed by adenine and hypoxanthine but not by guanine, xanthine, uracil, cytosine or thymine. Similarly, the activity of the guanine analog, III, was prevented by guanine, less effectively by xanthine and not by the other purines and pyrimidines. If only the minimum effective concentrations of the antagonists were used, the reversing action of the purines was less specific. Thus, over short periods of time (sixteen hours) compound II produced a bacteriostasis which was reversible by all four purines. Much higher concentrations were required to inhibit growth during a longer interval, and the purines no longer prevented this bacteriostatic action. The xanthine analog, IV, showed a similar non-specific growth inhibition in concentrations greater than 128 mg./100 cc.

Compound III, in combination with the sulfonamides, displayed a synergistic effect similar to methoxinine with both *E. coli* and *Staph. aureus*. In contrast to this result, no such effect was observed with the other triazolopyrimidines. Since sulfonamide-fast organisms were no more resistant to III than the parent strains, a more complete investigation of this compound, both alone and in combination with the sulfonamides, may be of particular interest.

Experimental[16]

β-Methoxyethyl Bromide.—The procedure of Karvonen[7] for the preparation of this compound gave poor yields (15–20%). Better results were obtained by substituting ethylene bromohydrin in a modification of the method of Jones and Powers[17] for the synthesis of β-methoxyethyl chloride from ethylene chlorohydrin and dimethyl sulfate.

One hundred twenty-five grams (1 mole) of ethylene bromohydrin, 126 g. (1 mole) of dimethyl sulfate and 110 g. (1.1 moles) of calcium carbonate were heated and stirred with a Hershberg stirrer. The bath temperature was held at 120–124° until distillation practically ceased. The bath was then heated slowly to 150° and maintained at that temperature to the end of the distillation. By reducing the pressure to 500 mm. additional distillate was obtained—total 107 g. After washing with 5 *N* sodium hydroxide, the product was dried over anhydrous sodium sulfate and distilled. The yield of β-methoxyethyl bromide boiling at 109.5–111.5° was 66.5 g. (48%).

***dl*-α-Amino-γ-methoxybutyric Acid (Methoxinine).**—This compound was prepared according to the directions for the synthesis of methionine.[8] Thirty-seven grams of β-methoxyethyl bromide and 57 g. of sodiophthalimidomalonic ester gave 38 g. (65%) of once recrystallized (95% ethanol) phthalimido-β-methoxyethylmalonic ester—m. p. 62–65°. Hydrolysis of 31 g. of this ester with sodium hydroxide yielded 21 g. (76%) of phthalamido-β-methoxyethylmalonic acid—m. p. 116–117° (effervescence). This acid (21 g.) was smoothly hydrolyzed and decarboxylated by hydrochloric acid. Methoxinine was too soluble to be isolated from the water–alcohol mixture suggested for methionine, and it was necessary to use absolute alcohol in this step. Pyridine precipitated the amino acid as glistening flakes which were collected and dried. The yield was 6.3 g. (73%). It was purified by continuous extraction with absolute alcohol, which gave 4.6 g. (53%) of *dl*-methoxinine m. p. 253° (dec. with effervescence).

Anal.[18] Calcd. for $C_5H_{11}O_3N$: C, 45.1; H, 8.3; N, 10.5. Found: C, 44.8, 45.1; H, 8.1, 8.1; N, 10.6, 10.2.

I. 7-Amino-1-*v*-triazolo[*d*]pyrimidine.—To a well-cooled solution of 0.75 g. (0.006 mole) of 4,5,6-triaminopyrimidine[19] in 75 cc. of water containing 5 cc. of glacial acetic acid was added, with stirring, a solution of 0.42 g. (0.006 mole) of sodium nitrite in 5 cc. of water. A colorless precipitate of the product separated almost immediately. The reaction mixture was then heated for twenty minutes on the steam-bath, cooled, and the triazolopyrimidine removed by filtration. A dilute aqueous ammonia solution of the product was purified by treatment with charcoal. The product obtained by neutralization of the ammonia solution with acetic acid was dried at 85°. The yield was 0.65 g. (80%) of colorless material which decomposed without melting above 310°.

Anal. Calcd. for $C_4H_4N_6$: C, 35.3; H, 3.0; N, 61.8. Found: C, 35.4, 35.7; H, 3.0, 3.4; N, 61.6, 61.4.

4,5-Diamino-6-hydroxypyrimidine.—Five grams of 2-thio-4,5-diamino-6-hydroxypyrimidine hydrochloride[12b] was dissolved in 100 cc. of water containing 1.86 g. of sodium carbonate, and the resulting 0.5% carbonate solution of the free pyrimidine base heated under reflux with 13–15 g. of Raney nickel for two hours.[20] After cooling, the reaction mixture was filtered and the light yellow filtrate was partially decolorized with charcoal. It was then acidified with hydrochloric acid and treated twice again with charcoal. The almost colorless solution so obtained was concentrated to 10–15 cc. on the steam-bath and cooled to crystallize the hydrochloride of the desired product as light red needles. These were removed, washed well with alcohol and dried *in vacuo* at room temperature; yield, 3.06 g. (73%), m. p. 245–255° (dec.). On standing, an additional 0.66 g. (16%) of product separated

(16) All melting points are corrected. Microanalyses were carried out in these Laboratories under the direction of Dr. J. A. Kuck to whom we are indebted for these data.

(17) Jones and Powers, THIS JOURNAL, **46**, 2531 (1924).

(18) We are indebted to Dr. G. L. Royer, Calco Chemical Division, American Cyanamid Company, for these results.

(19) Baddiley, Lythgoe and Todd, *J. Chem. Soc.*, 386 (1943).

(20) Dethiolations using Raney nickel have been reported by Bougault, Cattelain and Chabrier, *Bull. soc. chim.*, [5] **7**, 781 (1940); du Vigneaud, Melville, Folkers, Wolf, Mozingo, Keresztesy and Harris, *J. Biol. Chem.*, **146**, 475 (1942); Melville, Dittmer, Brown and du Vigneaud, *Science*, **98**, 494 (1943); Mozingo, Wolf, Harris and Folkers (Ref. 11).

from the alcohol washings. After two additional crystallizations from 50% alcohol, the hydrochloride of 4,5-diamino-6-hydroxypyrimidine was obtained as well-formed colorless needles; m. p. 251–252° (dec.).

Anal. Calcd. for $C_4H_5ON_4 \cdot HCl$: C, 29.6; H, 4.3; N, 34.5. Found: C, 29.8, 30.1; H, 4.4, 4.6; N, 34.7, 34.6.

II. **7-Hydroxy-1-v-triazolo[*d*]pyrimidine.**—To a cooled solution of 0.5 g. of 4,5-diamino-6-hydroxypyrimidine hydrochloride in 8 cc. of water was added a second solution containing 0.3 g. of sodium nitrite (50% excess) in 2 cc. of water. A product separated almost instantly as light orange, crystalline needles. After ten to fifteen minutes, this was removed and recrystallized from 10 cc. of water, decolorizing with charcoal, to yield colorless needles of a product containing approximately 6.5% sodium[21] and melting sharply at 332° (dec.). Recrystallization of this material from 8 cc. of 5% hydrochloric acid followed by a second recrystallization from 6 cc. of water gave 0.21 g. (50%) of the desired product as colorless, crystalline needles. The material darkened slowly above 260° and exploded sharply at 308°.

Anal. Calcd. for $C_4H_3ON_5$: C, 35.0; H, 2.2; N, 51.1. Found: C, 35.2, 35.1; H, 2.6, 2.5; N, 50.7, 50.8.

III. **5-Amino-7-hydroxy-1-v-triazolo[*d*]pyrimidine.**—Seven grams of 2,4,5-triamino-6-hydroxypyrimidine sulfate[22] was suspended in 100 cc. of water and dissolved by the addition of a small amount of sodium hydroxide. Sodium nitrite (5.0 g.) was added, the solution acidified by the dropwise addition of glacial acetic acid, and heated on the steam-bath for forty-five minutes. After cooling overnight, the crude product crystallized slowly—yield 3.4 g. (77%). A 3.0-g. sample of this material was purified by dissolving it in 40 cc. of cold, concentrated nitric acid and reprecipitating as the colorless silver salt by the addition of an excess of silver nitrate (4.0 g.) in 15 cc. of water. This salt was removed, washed well with water, resuspended in 100 cc. of cold water, and the silver precipitated as silver sulfide by passing hydrogen sulfide through the suspension for ten to fifteen minutes. The reaction mixture was then heated to boiling for thirty minutes to remove excess hydrogen sulfide, made distinctly alkaline with sodium hydroxide, and filtered. On acidifying the filtrate with acetic acid and cooling, the product crystallized out slowly. It was removed, redissolved in dilute sodium hydroxide, decolorized with charcoal and filtered. When this solution was acidified and cooled as before, the product separated slowly as colorless, microscopic needles. After drying for twenty-four hours at 0.1–0.15 mm. pressure over phosphorus pentoxide at room temperature, the yield was 1.62 g. of material decomposing without melting above 300°.

Anal. Calcd. for $C_4H_4ON_6$: C, 31.6; H, 2.7; N, 55.3. Found: C, 31.4, 31.4; H, 2.6, 2.9; N, (54.4).[23]

IV-A. 5,7-Dihydroxy-1-v-triazolo[*d*]pyrimidine, from III.[24]—A 0.55 g. (0.0036 mole) sample of 5-amino-7-hydroxy-1-γ-triazolo[*d*]pyrimidine was dissolved in 10 cc. of boiling 15% sulfuric acid, and the solution cooled to 60–70°. A solution of 0.5 g. (0.0072 mole) of sodium nitrite in 2 cc. of water was then added in small portions, with vigorous shaking. After addition was complete and gas was no longer evolved, the reaction solution was cooled for two hours to allow the product to crystallize; yield 0.46 g. (74%). It was recrystallized from 15 cc. of water to yield 0.29 g. (47%) of the colorless, crystalline monohydrate of 5,7-dihydroxy-1-v-triazolo[*d*]pyrimidine. This material decomposed without melting above 270°.

Anal. Calcd. for $C_4H_3O_2N_5 \cdot H_2O$: C, 28.1; H, 3.0; N, 40.9. Found: C, 28.2, 28.1[25]; H, 3.2; N, 41.5, 41.6.

IV-B. From 2-Thio-4,5-diamino-6-hydroxypyrimidine.—A 4.0-g. sample of 2-thio-4,5-diamino-6-hydroxypyrimidine hydrochloride[12b] was heated on the steam-bath with 100 cc. of 25% nitric acid until solution was complete and there was no further evolution of gas. The solution was then cooled and the product precipitated as the colorless silver salt by addition of an excess of silver nitrate solution. This salt was well washed with water, resuspended in 100 cc. of boiling water, and the silver precipitated as silver sulfide by passing hydrogen sulfide into the hot reaction mixture for five to ten minutes. The mixture was filtered and the light yellow filtrate evaporated to dryness to yield 0.85 g. (24%) of crude product as the residue. Recrystallization of this material from 25 cc. of water yielded 0.60 g. (17%) of pure material which separated as the monohydrate and decomposed without melting above 270°.

Anal. Calcd. for $C_4H_3O_2N_5 \cdot H_2O$: C, 28.1; H, 3.0; N, 40.9. Found: C, 28.3, 28.3; H, 3.1, 3.3; N, 40.8, 40.6.

Biochemical Results.—The antimetabolite activity of the various analogs was tested on *Escherichia coli* and on four *Staphylococcus aureus* cultures.[26] The *Staph. aureus* strains were parent 7, sulfonamide-resistant 7, parent 14, and sulfonamide-resistant 14.[27] Data in Table II were obtained with the parent strain 7. Stock cultures of *E. coli* were kept on Nutrient Agar (Difco), and Blood Base Agar (Baltimore Biological Laboratories) was used for *Staph. aureus*.

To prepare an inoculum the culture was transferred into A. C. Broth (Difco) and allowed to grow for six to eight hours. The culture was then centrifuged, washed twice with a saline-phosphate buffer and resuspended in buffer. The inoculum for the *E. coli* series was one drop of a 10^{-4} dilution for each 10-cc. tube and one drop of a 10^{-5} dilution when 1-cc. volumes were employed. One drop of a 10^{-4} dilution per 1-cc. tube was customary with the *Staph. aureus* strains.

The basal medium of MacLeod[28] was used for measuring the activity of the analogs against *E. coli*. For staphylococci the basal was essentially that of Porter and Pelczar.[29] However, the amino acids and the potassium dihydrogen phosphate were autoclaved together and the glucose, vitamins and other salts subsequently added as sterile solutions. Methionine was omitted from the basal medium for experiments on methoxinine. Good growth was obtained without methionine, but its addition did cause some stimulation at sixteen hours. Therefore, it was included for the testing of the purine analogs.

The compounds to be studied were dissolved in the basal media before sterilization and diluted in two-fold steps. A comparison of methoxinine and ethionine is given in Table III. They are of approximately equal activity, and inhibition ratios of 500–1000 were obtained for each in several series. Methoxinine bacteriostasis was reversed

TABLE III

GROWTH INHIBITION BY METHIONINE ANTAGONISTS

Organism	Antagonist	Added methionine, mg./100 cc.	M. E. C., mg./100 cc. 24 hrs.	48 hrs.	72 hrs.
E. coli	Ethionine	..	12.5	25	50
E. coli	Ethionine	0.05	50	100	100
E. coli	Methoxinine	..	12.5	25	50
E. coli	Methoxinine	1.0	500	1000	1000
Staph. aureus (parent 14)	Methoxinine	..	25	50	200

(21) The normal sodium salt of 7-hydroxy-1-v-triazolo[*d*]pyrimidine would contain 14.5% sodium.

(22) Traube, *Ber.*, **33**, 1371 (1900).

(23) Good duplicate nitrogen values were not obtained by the micro Dumas method.

(24) The method used was that of Fischer, *Ann.*, **215**, 253 (1882), for converting guanine to xanthine.

(25) Micro Van Slyke wet combustion.

(26) The *Staph. aureus* cultures were generously furnished by Dr. Maurice Landy of the Army Medical Center, Washington, D. C.

(27) Landy, Larkum, Oswald and Streightoff, *Science*, **97**, 265 (1943).

(28) MacLeod, *J. Expll. Med.*, **72**, 217 (1940).

(29) Porter and Pelczar, *J. Bact.*, **41**, 173 (1941).

by *l*-methionine but not by the *d*-isomer. Thus, 250 mg. of *dl*-methoxinine per 100 cc. was reversed by 10 mg. per 100 cc. of *l*-methionine at sixteen hours, 1 mg. per 100 cc. at twenty-four hours and 0.1 mg. per 100 cc. at sixty-four hours, but was not reversed by 10 mg. per 100 cc. of *d*-methionine at sixty-four hours.

Methionine is known to function as a biological methylating agent,[30] and a PAB-methionine interrelation has been demonstrated.[8] In order to assess the importance of these systems in the growth inhibition, the reversal of methoxinine by choline and by PAB was checked (Table IV). Neither substance alone produced any significant effect; however, with the combination a partial reversal resulted.

TABLE IV

REVERSAL OF METHOXININE INHIBITION OF *E. coli*

Supplement	M. E. C. methoxinine, mg./100 cc. 24 hrs.	48 hrs.	72 hrs.
...	12.5	50	100
1-Methionine, 1 mg./100 cc.	>250		
PAB, 0.05 mg./100 cc.	12.5	100	100
Choline, 0.4 mg./100 cc.	12.5	100	100
PAB + Choline	100	100	400

In Tables V and VI are presented typical experiments illustrating the synergistic[31] action of methoxinine or of the guanine analog, III, when used in combination with the sulfonamides. Similar results were obtained with sulfapyridine and sulfadiazine. In these particular tests one-eighth the m. e. c. of the analog and one-fourth the m. e. c. of the sulfonamide (or *vice versa*) produced complete inhibition. One-fourth and one-fourth of the respective m. e. c.'s was a more customary end-point. Approximately the same synergistic effects were shown at twenty-four or seventy-two hours.

Methoxinine at 1.56 mg. per 100 cc. produced partial reversal of sulfathiazole. This apparent stimulation by sub-inhibitory levels was noted in a number of experiments with methoxinine, III, or the sulfonamides. The sulfonamide resistant strains of *Staph. aureus* were no more resistant to III than were the parent cultures, all being inhibited for forty-eight hours by 4 mg. of III per 100 cc.

While methoxinine, III, and the sulfonamides all produced complete inhibition at two to four times the amounts required for partial activity, I caused a gradual decrease in growth over about a two hundred-fold range. Thus, while 8 mg. per 100 cc. was required for complete stasis of *E. coli*, $^1/_{32}$ mg. per 100 cc. usually produced partial inhibition. I had no synergistic action with the sulfonamides. Sulfanilamide concentrations causing temporary inhibition were partially reversed by sub-inhibitory levels of I.

In determining the inhibition ratios given in Table II, four times the m. e. c. of the analog was used when possible. Under these conditions the adenine analog (I) was reversed only by adenine and hypoxanthine, the guanine analog (III) only by guanine and xanthine. If just the m. e. c. was added the analogs were reversed by any of the four purines. II produced only temporary stasis and reversals had to be tested under borderline conditions. Here too the reversal was non-specific. The guanine and xanthine inhibition ratios were of the same magnitude as those given for adenine and hypoxanthine (Table II).

(30) Du Vigneaud, Cohn, Chandler, Schenck and Simmonds, *J. Biol. Chem.*, **140**, 625 (1941).

(31) The term synergistic is used to signify a greater than additive effect. Thus, if one-half the m. e. c. of each antagonist were required, the action would be regarded as merely additive. Since the limit of error in the bacteriostatic tests was ± one dilution, only consistent values of no more than one-fourth the m. e. c. of each substance were considered to represent a synergistic effect.

TABLE V

EFFECT OF SULFATHIAZOLE–METHOXININE COMBINATIONS ON THE GROWTH OF *E. coli*

Methoxinine mg./100 cc.	ST, mg./100 cc. $^1/_8$	$^1/_{16}$	$^1/_{32}$	$^1/_{64}$	$^1/_{128}$	$^1/_{256}$	$^1/_{512}$	$^1/_{1024}$	None
50	–[a]	–	–	–	–	–	–	–	–
25	–	–	–	–	–	–	–	+	+
12.5	–	–	–	–	–	–	+	+	+
6.25	–	–	–	–	–	+	+	+	+
3.13	–	–	–	–	+	+	+	+	+
1.56	–	+	+	+	+	+	+	+	+
None	–	–	–	+	+	+	+	+	+

[a] Visual turbidity at forty-eight hours.

TABLE VI

EFFECT OF SULFANILAMIDE 5-AMINO-7-HYDROXY-1-*v*-TRIAZOLO[*d*]PYRIMIDINE COMBINATIONS ON THE GROWTH OF *Staph. aureus* (P #7)

III mg./100 cc.	SA, mg./100 cc. 2	1	$^1/_2$	$^1/_4$	$^1/_8$	$^1/_{16}$	$^1/_{32}$	None
4	–[a]	–	–	–	–	–	–	–
2	–	–	–	–	–	+	–	+
1	–	–	–	–	–	+	+	+
$^1/_2$	–	–	–	–	–	+	+	+
$^1/_4$	–	–	–	+	+	+	+	+
$^1/_8$	–	–	+	+	+	+	+	+
$^1/_{16}$	–	+	+	+	+	+	+	+
None	–	–	+	+	+	+	+	+

[a] Visual turbidity at forty-eight hours.

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Summary

Methoxinine, the oxygen analog of methionine, has been prepared, and its antagonistic effect toward methionine investigated. The bacteriostatic action of *dl*-methoxinine is reversed by *l*-methionine but not by the *d*-isomer.

Analogs of adenine, hypoxanthine, guanine and xanthine in the triazolopyrimidine series have been synthesized. A comparison of the ultraviolet absorption maxima of these analogs and their purine counterparts is recorded. The growth inhibiting action of the adenine and guanine antagonists is effectively reversed only by the most closely related purines.

In combination with the sulfonamides, a synergistic effect is produced by either methoxinine or the guanine analog. Under these conditions considerably less of each substance is required to obtain the same degree of bacteriostasis.

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