The antibiotic was found to be active *in vitro* against the following test organisms

Organisms	Activity[a]
Bacillus subtilis (CN663)	4+
Proteus vulgaris (CN2770)	+
Streptococcus aureus (CN2)	+
Staphylococcus aureus (CN4108)	2+
Pseudomonas aeruginosa (NCIB8295)	+
E. coli (CN311)	+
Vibrio cholerae	
Ogwa strain	3+
Inaba strain	2+
Paratyphoid B	+

[a] The plus signs indicate the degree of inhibition effect of the antibiotic.

The antibiotic substance in the broth is stable for a long time both at room temperature and under refrigeration. It is stable at acid pH and less stable at alkaline pH. It is quite stable up to 80° and gets partially inactivated at 100°. Copper ions have no effect on it. It is toxic; 100 mg. substance per Kg. body weight of rat, kills the rat when given intraperitoneally.

Structure of the Antibiotic Molecule.—The molecular weight is 256 (Rast method). It contains carbon, hydrogen, and oxygen, but nitrogen, halogens, and phosphorus are absent. The product decolorized acidified potassium permanganate solution and bromine in carbon tetrachloride. It gave a brown color with aqueous ferric chloride and deep blue color (first brown) with sodium nitrite in concentrated sulfuric acid, indicating the presence of phenolic group in the molecule. Carboxyl group was detected by the evolution of carbon dioxide when a few crystals of the substance were added to a solution of sodium bicarbonate.

The physicochemical and antibacterial properties of the compound compared favorably with those of citrinin described (3–5). Both of them have the same functional groups, i. e., phenolic and carboxyl, both are active against *V. cholera* and Gram-positive organisms, and activities of both are completely destroyed by cysteine (6). It was therefore concluded that the new antibiotic might be identical with citrinin. Paper chromatographic studies with the authentic sample of citrinin as a standard (7) and color test for citrinin as described by Tauber, *et al.* (8), showed that the antibiotic is citrinin. In the chromatographic studies, the antibiotics were located by the bioautographic technique using *B. subtilis* as the test organism. It failed to depress the m.p., 165–169°, of the authentic specimen on admixture with it.

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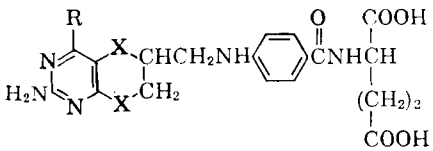
Communications

Analogs of Tetrahydrofolic Acid VI

N-[1-(2-Amino-4-hydroxy-6-methyl-5-pyrimidyl)-3-propyl]-*p*-aminobenzoyl-L-glutamic Acid, an Inhibitor of Folic Reductase

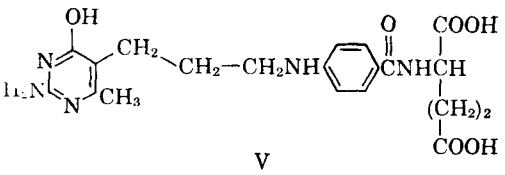
Sir:

Fifteen enzymes utilizing folic acid, tetrahydrofolic acid (I), or derivatives of tetrahydrofolic acid are known (1–3). A number of these enzymes are inhibited by aminopterin (4-amino-4-deoxyfolic acid) (4–7), but nearly as many are not (6, 8–10). 5,6,7,8-Tetrahydroaminopterin (II) can inhibit some of the enzymes not inhibited by aminopterin (10, 11). 5,8-Dideaza-5,6,7,8-tetrahydroaminopterin (IV) has been recently synthesized (12) and found to have inhibitory properties similar to tetrahydroaminopterin (12, 19). In addition, 5,8-dideaza-5,6,7,8-tetrahydrofolic acid (III) (13) has been found to bind to folic reductase eight times stronger than the substrate, folic acid (14).

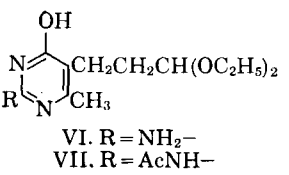


I. R = OH, X = —NH—
II. R = NH_2, X = —NH—
III. R = OH, X = —CH_2—
IV. R = NH_2, X = —CH_2—

The folic cofactor area should be a prime target for utilization of recent developments in nonclassical antimetabolite theory (15–17) since larger differential effects on inhibition of these enzymes might be obtained by the bulk principle of specificity (15), the exo-alkylating irreversible inhibition phenomenon (16), and the bridge principle of specificity (17). In order to use



V



VI. R = NH_2–
VII. R = AcNH–

these three corollaries of nonclassical antimetabolite theory, it would be advisable to have an inhibitor that can be made by a relatively short sequence and the sequence should be one that lends itself to the placing of substituents in a variety of positions. Synthesis of compound V, which satisfies both the inhibitor and synthetic requirements, is the subject of this communication.

Ethyl 2-acetylglutaraldehydate, prepared in 47% yield from Michael addition of ethyl acetoacetate to acrolein (18), was converted in boiling ethanolic ammonium chloride in 63% yield to its diethyl acetal (VIII), b.p. 110–112° (0.2 mm.).

Anal.—Calcd. for $C_{13}H_{24}O_5$: C, 60.1; H, 9.64. Found: C, 60.0; H, 9.70.

Reaction of the keto ester acetal (VIII) with guanidine in boiling absolute ethanol afforded a 76% yield of VI, m.p. 179–180°.

Anal.—Calcd. for $C_{12}H_{21}N_3O_3$: C, 56.6; H, 8.31; N, 16.5. Found: C, 56.5; H, 8.10; N, 16.7.

Treatment of VI with acetic anhydride in pyridine at 85° gave a 57% yield of VII, m.p. 150°.

Anal.—Calcd. for $C_{14}H_{23}N_3O_4$: C, 56.6; H 7.81; N, 14.1. Found: C, 56.8; H, 7.94; N, 14.3.

Hydrolysis of VII by short boiling in water afforded 46% of 2-acetamido-4-hydroxy-6-methyl-5-pyrimidinepropionaldehyde (IX), m.p 159–160°.

Anal.—Calcd. for $C_{10}H_{13}N_3O_3$: C, 53.8; H, 5.87; N, 18.8. Found: C, 53.6; H, 5.95; N, 18.6.

Condensation of the pyrimidinepropionaldehyde (IX) with *p*-aminobenzoyl-L-glutamic acid in boiling alcohol, reduction of the resultant anil (X) with sodium borohydride, and basic hydrolysis of the N-acetyl group gave, after full purification, 15% of V as a white solid,[1] m.p. >250°; $\lambda_{max.}^{pH\ 1}$ 222 (ε 27,800), 270 (ε 19,200), and 303 mμ (ε 11,400); $\lambda_{max.}^{pH\ 8.4}$ 295 mμ (ε 15,100); $\lambda_{max.}^{pH\ 13}$ 284 mμ (ε 20,500).

Anal.—Calcd. for $C_{20}H_{23}N_5O_6$: C, 55.8; H, 5.85; N, 16.3. Found: C, 56.2; H, 5.66; N, 16.5.

Compound V inhibited (14) folic reductase with $K_i = 7 \times 10^{-6}$, about the same as the K_m of folic acid. Since this compound is constructed from ethyl acetoacetate, acrolein, guanidine, and *p*-aminobenzoyl-L-glutamic acid, it is obvious that a variety of derivatives of V can be made by modifying the four components or by transformations of VI; in this way, compounds could be obtained that, by use of nonclassical antimetabolite theory, might selectively inhibit some of the 15 enzymes in the folic acid cofactor area. In addition, considerable information could be obtained about the relative binding and conformational requirements of these substrates to their respective enzymes (19). Such a program is continuing in these laboratories.

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[1] The final product contained about 1% of the anil (X) or *p*-aminobenzoyl-L-glutamic acid as quantitatively determined by the Bratton-Marshall test; the crude yield was 61% of 73% purity.

Received March 8, 1962.
Accepted for publication April 6, 1962.
This work was generously supported by Grant CY-5845 of the National Cancer Institute, U. S. Public Health Service.