the origin. At 505 mμ, concentrations from 1.0 to 3.5 mcg. gave a straight line which did not pass through the origin. In this latter case a standard curve was used to calculate the results. The mean recoveries at different levels were 100.4 ± 2.7% for 7 determinations with the first method and 100.1 ± 2.5% for 6 determinations with the second method.

Reaction of Related Compounds.—To test the specificity of the methods, compounds most likely to react similarly to nikethamide were assayed at the 3-mcg. level for the regular method and at 2 mcg. for the rapid method. Results are reported in Table I in terms of percentage of absorbance of related compound in comparison to nikethamide. For the regular method, the highest percentage was 1.2% for pyridine-3-sulfonic acid and nicotinic acid ethylamide; other substances tested gave no absorbance. For the rapid method, the highest percentages were 9.1% for nicotinic acid dimethylamide and 7.4% for niacinamide; others were below 4%. The fact that nikethamide is practically the only compound giving a colored product seems to be due (*a*) to the different rate of reaction of various pyridine derivatives with cyanogen bromide and (*b*) to the difference in stability of the colored compound formed upon reaction of the pyridinium derivative with barbituric acid unsubstituted in the 5 position.

It may be concluded that the regular method is more specific than the rapid method although the latter would be quite satisfactory for most routine checking purposes.

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Citrinin from *Penicillium steckii* Zaleski

By A. JABBAR and A. RAHIM

The antibiotic substance isolated from cultured *Penicillium steckii* Zaleski was identified as citrinin.

PENICILLIUM STECKII Zaleski[1] was shown to have antibiotic activity against *Staph. aureus* and *Ps. pyocyanes* (1). It was later found that the same strain formed a penicillin-like antibiotic (2). In this paper the isolation, characterization, and crystallization of the antibiotic is described and it is confirmed that the antibiotic is citrinin.

The organism *Penicillium steckii* Zaleski was cultivated in Czapek-Dox solution containing 0.25% yeast extract; the active principle from the potent greenish culture broth was isolated by chloroform, and purified by adsorption chromatography on acid-washed alumina. The antibiotic was crystallized in prismatic needles and in plates from chloroform and petroleum ether mixture. The crystalline sample, melting at 165–169° (with decompn.), is readily soluble in chloroform and acetone. LD_{50} is 100 mg. The determination of its functional groups and the studies of its physicochemical properties show that the antibiotic is citrinin.

EXPERIMENTAL

Culture Media.—Czapek-Dox solution; sucrose, 20 Gm.; sodium nitrate 2.0 Gm.; potassium dihydrogen phosphate, 1 Gm.; potassium chloride, 0.5 Gm.; magnesium sulfate, heptahydrate, 0.5 Gm.; ferrous sulfate, heptahydrate, 0.01 Gm.; water to 1 L. The only source of nitrogen in the medium is sodium nitrate which corresponds to 0.3296 Gm. of nitrogen per L. Czapek-agar medium was the best for sporulation.

Production and Isolation of the Antibiotic on Czapek-Dox Solution.—For the production of the antibiotic, the surface culture was found to be better than the shake culture. Of the different carbon sources such as glucose, sucrose, lactose, and galactose, sucrose was the best. Grain extract, beef extract, and yeast extract were found to have a stimulatory effect both on the growth of the organism and on the production of the antibiotic.

Czapek-Dox solution containing 0.25% yeast extract was distributed in 50-ml. amounts in 250-ml. conical flasks which were plugged with cotton and sterilized at 15 lb. pressure for 20 minutes. The contents of the flask were then inoculated with spore-suspension in water, prepared from Czapek-agar slopes of *Penicillium steckii*, one flask being sown from each slope. The flasks were incubated at room temperature for 4 days. The culture fluids were separated from the mycelia by decantation. The filtrates were then extracted with an equal volume of chloroform. For the isolation of the antibiotic present in the mycelia, the thoroughly washed mycelia were crushed with sand and extracted with alcohol. The extracts were evaporated to dryness at 50° under reduced pressure, taken into chloroform, and chromatographed on acid-washed alumina to remove the coloring matter. The antibiotic was crystallized from chloroform and petroleum ether mixture. Repeated crystallization gave lemon-yellow prismatic needles, sometimes lemon-yellow plates, melting at 165–169° with decomposition.

The purified product is readily soluble in chloroform and acetone, very sparingly soluble in water. It is soluble in alcohol, aqueous alkali, less soluble in ether, still less in petroleum ether.

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[1] *Penicillium steckii* Zaleski was isolated from local soils and has been identified by Dr. A. H. S. Brown and deposited in Herb IMI as 61422.

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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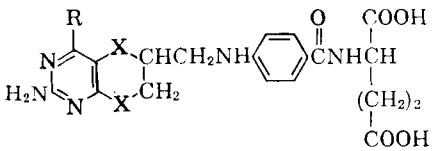
Communications

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