

needles of the adduct are stable in air for short periods of time, but the odor of the amine soon became appreciable above the solid. In warm sunlight the decomposition is rapid and a brown gum is produced.

In attempts to purify the brown, partly decomposed crystals, vacuum sublimation at 70–80° and 10⁻³ mm. was employed. Under these experimental conditions only small amounts of sublimate were observed. The residue consisted of an apparently polymerized material which no longer possessed the solubility characteristics of the addition compound.

When (C₄H₉)₃N:BF₃ was dissolved in anhydrous methanol and an excess of a saturated methanol solution of anhydrous ammonia was added, a white crystalline solid was produced. This material could be recrystallized from chloroform and

yielded crystals melting at 161–163° (literature value for the melting point of H₃N:BF₃, 163°²). This observation is indicative of the greater acid strength of boron trifluoride with respect to ammonia than with respect to tri-*n*-butylamine.

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(2) A. W. Laubengayer and G. F. Condiak, *THIS JOURNAL*, **70**, 2274 (1948).

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COMMUNICATIONS TO THE EDITOR

COPROGEN, A NEW GROWTH FACTOR FOR COPROPHILIC FUNGI

Sir:

Members of the genus *Pilobolus* are strictly dung inhabiting fungi. Several investigators^{1,2} who have studied this genus in culture have found it necessary to use dung or a dung extract as a necessary component of their culture medium.

On a basal medium adjusted to pH 7.6 and containing the following amounts of ingredients per 500 ml.: acid hydrolyzed casein 25 ml.,³ L-cystine 50 mg., DL-tryptophan 100 mg., uracil 5 mg., thymine 5 mg., adenine 5 mg., guanine 5 mg., xanthine 5 mg., thiamine 0.5 mg., pyridoxine 0.1 mg., calcium pantothenate 0.1 mg., riboflavin 0.2 mg., nicotinic acid 0.5 mg., choline 0.5 mg., inositol 0.2 mg., *p*-aminobenzoic acid 0.2 mg., pteroylglutamic acid 12 μg, biotin 1.2 μg, vitamin B₁₂ 2.4 μg, and inorganic salts A and B,⁴ the dung extracts could be replaced by the fermentation liquors of a number of species of bacteria and fungi.

From such source materials a brick red crystalline biologically active compound has been isolated. The isolation was effected by solvent extraction, adsorption on florasil and partition chromatography on a Filter-cel column. After several crystallizations from ethanol, the compound evidenced no sharp melting point but darkened and decomposed from 205°. Elemental analyses indicated the following percentage composition: C, 50.96; H, 6.88; N, 10.26; Fe, 6.61. The compound has a broad ultraviolet absorption maximum at 440 mμ in 50% ethanol, E_{1cm}^{1%} = 36.6.

The name "Coprogen" is proposed for this new compound.

The assays were carried out in 125-ml. erlenmeyer flasks containing 20 ml. of medium. The inoculum consisted of uniform amounts of actively

growing mycelium in dung agar blocks. After incubation at 25° for five days the pads were harvested by freeing the mycelium from the small agar block and then drying the mycelium pad at 105° for 16 hours. Weights of the pads were used as a measure of growth.

The addition of 5 millimicrograms of Coprogen per ml. of medium allowed for growth and fruiting of *P. kleinii* with no loss of vigor through ten serial transfers. The compound was equally effective in all the species of *Pilobolus* studied.

Page⁵ has recently reported that hemin can partially replace the usual dung extracts in the nutrition of *Pilobolus*; however, the growth was much less vigorous. Under our experimental conditions hemin exhibited only one one-thousandth the growth promoting activity of Coprogen on a weight basis.

(5) R. M. Page, paper presented at the Am. Inst. of Biol. Sci. Meetings, U. of Minn., Sept. 1951.

CONTRIBUTION FROM THE
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THE VALENCE OF PRECIPITATING ANTIBODIES

Sir:

Following the studies of Pappenheimer, Lundgren and Williams,¹ a number of investigators^{2,3,4} have recently applied the techniques of electrophoretic and sedimentation analysis to the problem of antibody valence.

These studies and ours indicate the dependence of the molecular composition of antigen-antibody complexes upon the relative amount of antigen

(1) A. H. R. Buller, "Researches on Fungi," Vol. VI, Longmans' Green and Co., New York, N. Y., 1934.

(2) E. A. Bessey, *Michigan Academy of Sciences, Arts and Letters*, **32**, 15 (1948).

(3) 1 ml. is equivalent to 100 mg. of casein.

(4) E. E. Snell and F. M. Strong, *Ind. Eng. Chem.*, **11**, 346 (1939).

(1) A. M. Pappenheimer, Jr., H. P. Lundgren and J. W. Williams, *J. Exp. Med.*, **71**, 247 (1940).

(2) J. R. Marrack and H. Hoch, *Brit. J. Exp. Path.*, **32**, 212 (1951).

(3) S. J. Singer and D. H. Campbell, *THIS JOURNAL*, **73**, 3543 (1951), "Communication."

(4) J. L. Oncley and D. Gitlin, *J. Phys. Chem.*, **56**, 85 (1952).