Table 1. Effect of arginine on growth of *N. crassa* 1298. The values are averages of triplicate assays, each with 25 ml of minimal medium supplemented with either 4 μmole of uridine or 50 μmole of sodium propionate or aminobutyric acid. The growth periods were 3 days with uridine and 5 days with the other compounds.

L-arginine (mg/25 ml)	Dry mycelia (mg)
Uridine (4 μmole)	
0	45
10.0	47
DL-α-*amino*-n-*butyric acid (50 μmole)*	
0	25
0.001	20
.005	13
.010	0
Sodium propionate (50 μmole)	
0	17
0.001	8
.005	3
.010	0

growth-promoters necessary for 50 percent inhibition was of the order of 1:4000. On the other hand, arginine in concentrations as high as 10 mg/25 ml did not affect growth of the mutant in the presence of uridine. The arginine effect, therefore, must involve a relatively early stage of pyrimidine nucleotide formation.

The use of mycelial fragments for the inoculation process gave results similar to those described above for the usual conidial inoculum. When 1 mg of arginine was added to flasks which had been growing with propionate for 4 days (26 mg of mycelia), the growth in a subsequent 2-day period was only 10 mg of mycelia as compared with 16 mg for the controls. Also, experiments with mixtures of uridine and propionate or aminobutyrate demonstrated that the presence of 10 mg of arginine depressed growth to that obtained with the uridine alone. These results indicate that the arginine effect is not restricted to the germination process, although this may be the most susceptible stage. Higher concentrations of arginine are needed to inhibit growing mycelia, probably because the compound is removed rather rapidly by metabolic processes. With time, growth appears in inhibited cultures concomitantly with the disappearance of arginine from the medium.

Table 2. Comparison of the effect of various amino acids on growth of *N. crassa* 1298 in the presence of 50 μmole of DL-α-amino-*n*-butyric acid per 25 ml of minimal medium. The incubation period was 5 days.

Supplementary compound	Concn. of supplement (μmole/25 ml)	Wt. of dry mycelia (mg)
None		39
Arginine	0.03	11
Ornithine	0.6	17
Citrulline	6.0	30
Aspartic acid	37.0	22
Valine	43.0	12
Isoleucine	38.0	10

Experiments with the other common aliphatic α-amino acids demonstrated that only ornithine, citrulline, aspartic acid, valine, and isoleucine had inhibitory effects in this system (Table 2), although all to a lesser degree than arginine. Ornithine was much more effective than citrulline, contrary to expectation if the action of these compounds required their conversion to arginine. This may reflect differences in membrane permeability or may indicate that ornithine affects growth in a manner different from arginine.

Attempts at this time to explain these effects must be regarded as speculation, for the primary metabolic defect in *N. crassa* 1298 is of unknown nature and the mechanisms by which propionate and aminobutyrate support growth remain to be elucidated. The available evidence suggests (*5*) that these compounds are pyrimidine precursors and that their use for pyrimidine synthesis involves adaptive mechanisms different from those of the common aspartate-orotidylic acid pathway. It seems likely that any such utilization of these aliphatic acids requires the participation of coenzyme A at an early stage. The inhibitory action of valine and isoleucine may simply be the result of competition of their degradation products for this coenzyme.

Should the use of propionate for pyrimidine synthesis require a new transcarbamylation reaction, as is quite possible, then obvious opportunities would exist for aspartic acid and ornithine to inhibit the new transcarbamylase or to compete for the necessary carbamyl phosphate.

The mechanism of the striking inhibitory action of arginine also remains unknown. This amino acid certainly could be simply a powerful inhibitor of some enzyme. However, it may well be that the observed phenomenon is the result of the repression by arginine of the synthesis of an enzyme necessary for the utilization of propionate and aminobutyrate for pyrimidine formation. This mechanism may be of general importance in the metabolic control of pyrimidine biogenesis.

JAMES L. FAIRLEY
A. BIRK ADAMS
Department of Chemistry, Michigan State University, East Lansing

References and Notes

1. M. B. Mitchell and H. K. Mitchell, *Proc. Natl. Acad. Sci. U.S.* **38**, 205 (1952); R. H. Davis, *ibid.* **46**, 677 (1960); ———, *Science* **134**, 470 (1961); E. J. Miller and J. S. Harrison, *Nature* **166**, 1035 (1950).
2. J. L. Fairley, *J. Biol. Chem.* **210**, 347 (1954).
3. J. L. Fairley, R. L. Herrmann, J. M. Boyd, *ibid.* **234**, 3229 (1959).
4. This work was supported in part by contract No. (11-1)-289, U.S. Atomic Energy Commission, and in part by research grant No. C-5097, National Institutes of Health.
5. J. M. Boyd and J. L. Fairley, *J. Biol. Chem.* **234**, 3232 (1959).

24 April 1961

Antifungal Agent

Abstract. From the soil of the San Joaquin Valley a fungus has been isolated, an extract of which inhibits the growth of *Coccidioides immitis* on Sabouraud's medium. An acute toxicity study in mice indicates a certain tolerance of the extract. Tentative identification indicates that the fungus is a penicillium.

Early in 1958 certain attributes of a green fungus seen occasionally in the course of our soil survey study of *Coccidioides immitis* caused one of us (M.C.E.) to isolate it and study its effect upon other fungi. It was found to overgrow and displace *C. immitis* on modified Sabouraud's medium.

It was planted on 3-day-old cultures of *C. immitis* and 1 week later washings of this combined growth were injected intraperitoneally into 12 mice. At the same time equal amounts of a much lighter suspension of pure *C. immitis* were injected intraperitoneally into 12 other mice. All of the mice that received the pure *C. immitis* culture died of coccidioidomycosis within 3 months. Only three of the mice that received the combined suspensions died.

Further studies pointed to the need for making an extract of the fungus for determining its effect upon *C. immitis*. The 8-day-old cultures of fungus grown on modified Sabouraud's medium were heated in the autoclave at 250°F for 5 min, and the mat and substrate were extracted with chloroform. The chloroform was boiled off under distilled water, and when only a tarry residue remained at the bottom, the water with

Table 1. Results of acute toxicity study.

Mice (No.)	Amount of extract (ml)	Deaths (No.)
10	0.1	none
21	0.2	3
19	0.3	18
10	0.5	10

that part of the chloroform extract that it had taken up during the boiling was pipetted off. This distilled water extract was used for measuring the zone of inhibition of growth of *C. immitis* and for toxicity studies in mice.

One-tenth of a milliliter of this aqueous extract was placed on each of six testing disks and when dried these were placed in the center of 1-day-old cultures of two different strains of *C. immitis* growing on Sabouraud's medium. The average diameter of the zone of inhibition in the six cultures was 4.5 cm. The acute toxicity study performed in young mice averaging 21 g in weight gave the results shown in Table 1.

The extract has since been tried against *Microsporum gypseum* and a species of Trichophyton with moderate zones of inhibition; it is being tested against other fungi.

This green fungus has been tentatively identified by Chester Emmons at the National Institutes of Health as *Penicillium janthinellum* (*1*).

MARGARET C. EGEBERG
ANN F. ELCONIN
ROGER O. EGEBERG
Los Angeles County Hospital and
University of Southern California School of Medicine, Los Angeles

Note

1. This work was supported by grants from the National Institutes of Health, Bethesda, Md., and the Attending Staff Association of the Los Angeles County Hospital, Los Angeles, Calif.

6 April 1961

Significance of Some Fossil Wood from California

In June 1952, N. L. Taliaferro, R. Taylor, and I found fragments of wood and gastropod shells in a sandstone boulder 600 feet above sea level in Angus Canyon, a tributary to Capay Valley, about 40 miles west-northwest of Sacramento, Calif. The wood was later identified (by L. H. Daugherty) as *Cupressinoxylon*, and the gastropods (by J. W. Durham) as *Cophocara*.

Although the boulder is firmly embedded in a conglomerate near the base of the Eocene Capay formation, it is dated as upper Cretaceous by its gastropods and by lithologic comparison with nearby upper Cretaceous sandstone exposures. Analyses were made on parts of the dark-brown wood fragment shown in Fig. 1.

The specific gravity, determined by immersing fine splinters of the wood in heavy liquids, ranges from about 2.3 to 2.7. X-ray analysis of a splinter (by Adolf Pabst) showed it to be chiefly calcite. Ignition of a small piece showed the ash content to be 51.6 percent by weight. This ash was unaffected by an Alnico magnet, suggesting that no magnetic iron was present, and hence that no pyrite was present in the original unburned sample (*1*). The ash, x-rayed by Pabst, was found to be calcium oxide with a trace of hydrated calcium oxide.

If, as the x-ray analysis suggested, the ash was wholly calcium oxide, calcined from calcite, one can easily calculate the weight percentage of calcite in the original—namely, 92.1 percent. This explains, in part, the high specific gravity of the wood.

A transverse thin section (Fig. 2) shows that the original vegetal structure is excellently preserved. Evidently the calcite permineralization occurred before decomposition had progressed far enough to weaken the cell walls. Indeed, in view of Goldberg and Parker's reconstruction of phosphatization of wood (*2*), it appears likely that calcification was synchronous with oxidation of the tissue.

With crossed nicols, both transverse and tangential thin sections show that the lumina are filled with calcite, but opaque cell walls make up almost half the apparent volume. In view of the high specific gravity, the cell walls, as well as the lumina, must be impregnated with calcite.

Transverse sections also show that much of the calcite is fine-granular, unlike that in the calcified wood described by Greenland and Wherry (*3*); individual grains are generally smaller than the lumina they fill, so that many cells contain more than one individual. C-axes of several of these, measured on a Universal stage, appear to be randomly oriented. The relatively fine granularity of the calcite suggests that crystallization began at closely spaced centers and proceeded rather uniformly and rapidly. This suggestion supports the inference that permineralization was synchronous with initial decomposition, presumably soon after deposition. Also supporting this idea is the fact that the wood, both micro- and macroscopically, appears to have been but little deformed and flattened by the weight of overlying sediments before calcification.

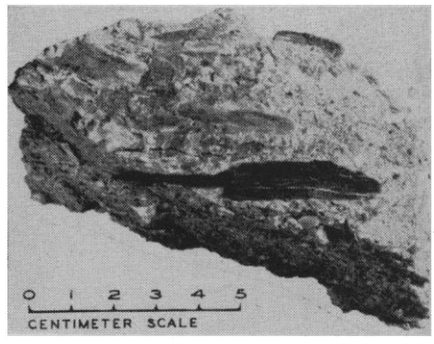

Fig. 1. Rock fragment showing piece of dark calcified *Cupressinoxylon* wood. Streaky gray fragments are other calcified wood chips; white fragments are shells of *Cophocara*, a gastropod with upper Cretaceous affinities.

The presence of *Cophocara* shells shows that the wood was deposited in a marine environment; coarse granularity of the sandstone suggests relatively strong local currents; absence of pyrite suggests relatively free circulation of the water. All of this points to an environment unlike that in which the wood of the Carboniferous coal balls was calcified—a restricted, anaerobic, lagoon or flood-plain environment.

The environment, though marine, was also different from that of the 410-m terrace, in the Gulf of Tehuantepec, from which Goldberg and Parker dredged phosphatized wood (*2*). They and others have suggested that, since the hydrogen-ion concentration requirements are similar for precipitation of calcium carbonate and calcium phosphate, the relative concentration of carbonate and phosphate ions in the water will determine whether calcite or apatite will be deposited.

Presumably, then, if the phosphate content of the Central Valley Cretaceous geosyncline was relatively low,

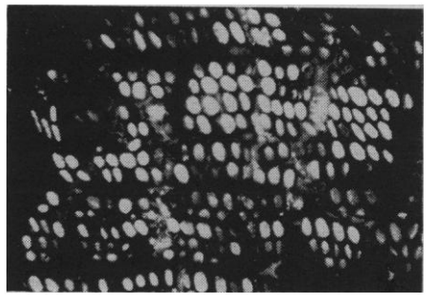

Fig. 2. Photomicrograph of transverse section of the wood fragment shown in Fig. 1. Crossed nicols. Width of view is approximately 0.45 mm.