EFFECT OF CYCLOHEXIMIDE ANALOGS ON THE GROWTH OF MICROORGANISMS

BERNADINE F. PELETZ and M. J. STARZYK
Northern Illinois University, DeKalb

ABSTRACT.—The effect of cycloheximide (Acti-dione) analogs on the growth of bacteria, yeasts and fungi was studied. These analogs had no effect of the bacteria or yeasts tested but did demonstrate variable activity against the fungi. Most notable was the inhibition of the plant pathogen, *Diplodea zeae* by 4-desmethyl dihydroxycycloheximide (test compound no. 6).

The inhibitory action of cycloheximide (β-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-glutarimide) against many plant pathogens is well known. Numerous analogs of this compound have been recently synthesized in a search for chemicals possessing antitumor activity. It was the purpose of this project to test these compounds against representative bacteria, yeasts and molds and determine whether their particular structural features would produce any inhibitory effect.

METHODS AND MATERIALS

Inhibitory activity against bacteria and yeasts was evaluated by the paper disc-seeded agar method. Bacteria were grown in nutrient broth and yeasts were grown in peptone (10 g/liter) dextrose (20 g/liter). After appropriate growth had occurred, 10 ml. of an organism was aseptically pipetted into 100 ml. of melted nutrient agar (bacterial) or peptone dextrose agar (yeasts. The seeded agar was dispensed into Petri dishes (90 mm in diameter) at a volume of 10 ml. per plate. Test solutions (see figure 1) were prepared by dissolving the chemicals in sufficient ethanol to produce a 1000 ug/ml. solution. Paper discs (Whatman assay Discs, 12.7 mm in diameter) were dipped into the test solutions, allowed to dry and then placed upon the seeded agar plates. The test plates were then incubated at 25°C. or 37°C. depending on growth requirements. The plates were examined on each of the following five days for inhibition zones surrounding the treated discs.

Inhibitory activity against molds was evaluated by incorporation of an aqueous solution of the test compound into potato dextrose agar to give a final concentration of 100 ug/ml. in the treated agar. Three plates of this concentration as well as three control plates were prepared for each fungus as well as each compound tested. Approximately 15 ml. of agar was added to each Petri dish and allowed to solidify. Five millimeter sections of fungus mycelium were secured from actively growing fungus cultures by using a sterile #2 cork-borer. Each section was placed in the center of a dish with the mycelium side down on the treated agar. All plates were incubated at room temperature and were examined for growth after 72 hours.

RESULTS AND DISCUSSION

Of the ten analogs tested, none demonstrated any activity against the following yeasts and bacteria: *Saccharomyces pastorianus, Endomycopsis bispora, Endomycopsis fibuliger, Saccharomyces cerevisiae, Hansenula anomala, Citeromyces matritensis, Schizosaccharomyces octosporus, Trichosporon roseus, Rhizopus stolonifer, Escherichia coli, Micrococcus luteus, Proteus vulgaris, Serratia marcescens* and *Aerobacter aerogenes.*

Only six of the analogs were tested against the fungi because of various insolubility problems. From the data in Table 1 it can be seen that compound

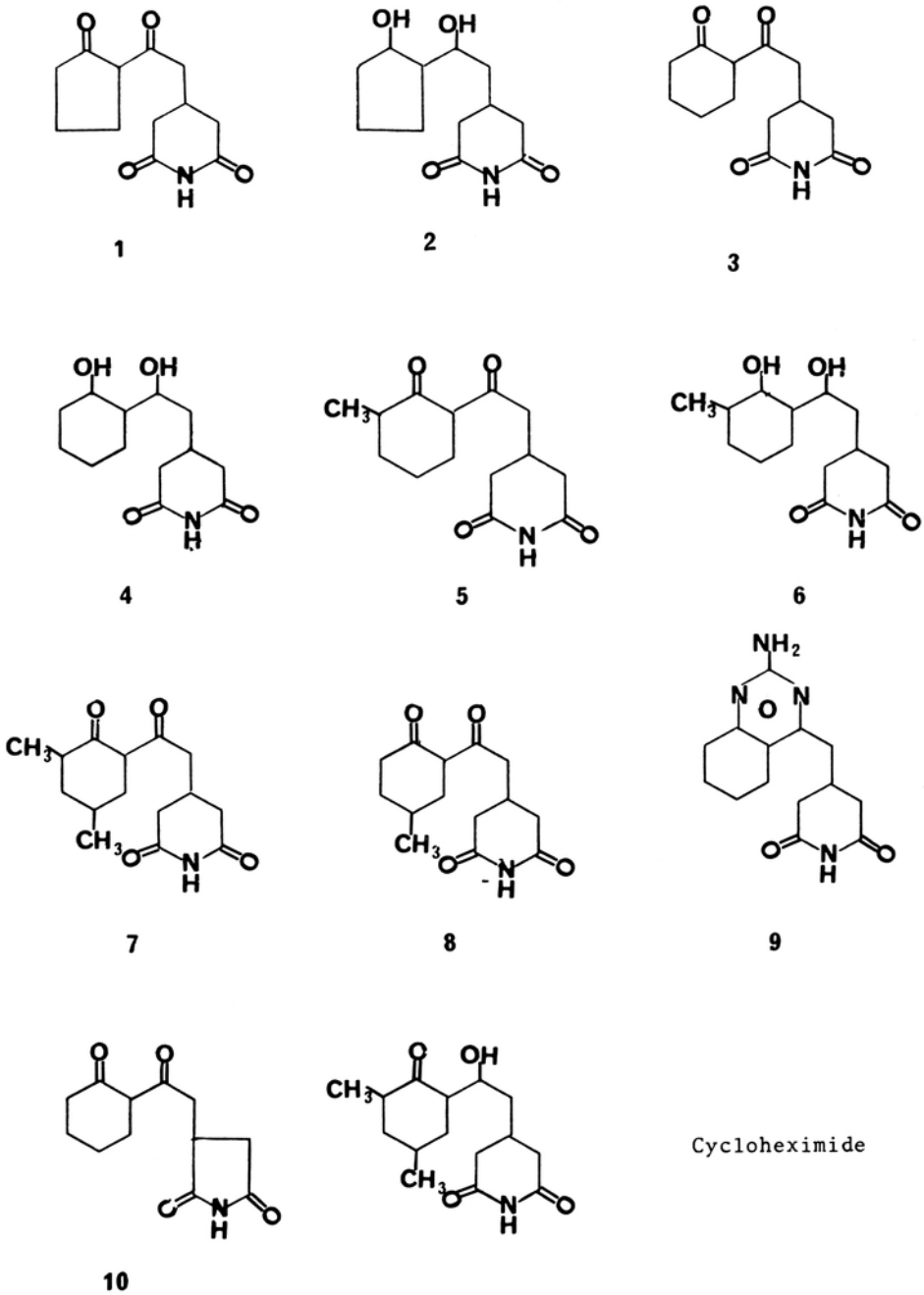

Figure 1. Analogs of Cycloheximide

TABLE 1. Percent inhibition of growth at 25°C. in relation to controls.

Fungus	Compound tested						
	1	3	4	5	6	10	control
Sclerotinia sclerotiorum	20.2	19.5	24.2	29.0	20.9	23.7	0
Curvularia sp.	7.1	13.9	6.1	28.9	33.8	10.2	0
Rhizoctonia solani	4.8	3.1	2.9	24.0	29.8	25.3	0
Alternaria solani	4.3	2.2	4.9	16.0	17.3	15.4	0
Diplodia zeae	9.8	1.9	7.0	39.4	70.4	34.5	0
Chaetomium coprinum	27.2	17.6	2.1	12.1	7.9	12.1	0
Cerotocystis fagacearum	13.4	9.4	7.6	9.9	15.2	4.7	0
Colletotrichum circans	14.2	25.3	15.8	20.0	2.6	12.1	0
Ceratocystis ulmi*	0	0	0	0	0	0	0

* For *Ceratocystis ulmi* there was a slight stimulatory response in growth to all the test chemicals in relation to the control. Since percent inhibition is recorded, no value other than 0 is appropriate here.

no. 6 (4-desmethyl dihydroxycyclohexiimide) was definitely active against *Diplodia zeae*. Except in the case of *Ceratocystis ulmi*, all compounds did have a slight inhibitory effect on the filamentous fungi tested.

ACKNOWLEDGMENTS

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