Fungal Transformations of Triparanol

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Fungal transformations of triparanol, a hypercholesterolemic drug, were studied in Lagenidium giganteum and Lagenidium callinectes. The products were identified by combined gas chromatography-mass spectrometry. Two metabolites were observed from each organism; only one of the metabolites was found in both organisms.

The hypercholesterolemic drug triparanol, 4-chloro-α-[4-[[2-(diethylamino)ethoxy]phenyl]-α-(4-methylphenyl)benzeneethanol, has been used in vertebrates to block conversion of desmosterol to cholesterol (2). Triparanol also prevents transformation of sitosterol to cholesterol in an insect (6) and a nematode (3). Patterson et al. (5) have shown that it inhibits the growth of three species of Chlorella and facilitates the accumulation of several new sterols. The drug primarily inhibits the Δ24 sterol reductase (2, 5), although effects have also been observed on Δ22 reduction, 14α-desmethylation, and 24-methyl methylation (5).

We are investigating sterol transformations in Lagenidium giganteum, a fungal pathogen of mosquito larvae. It is incapable of de novo sterol synthesis and must be supplied with exogenous sterols or mevalonic acid to produce the infective zoospores (4). In contrast, Lagenidium callinectes, a parasite of blue crab ova, is capable of sterol synthesis (7). Neither organism converts lanosterol to other sterols, but both metabolize cycloartenol (7). In an attempt to delineate their sterol pathways by analysis of intermediates, we administered a variety of inhibitors of sterol biosynthesis, including SKF-7332-A3, SKF-7997-A3 (Smith Kline & French Laboratories, Philadelphia, Pa.), AY-9944 (Ayerst, McKenna, and Harris Research Laboratories, Montreal, Canada), and triparanol (Merrell National Laboratories, Cincinnati, Ohio), to cultures of both organisms. None of these compounds inhibited growth of either Lagenidium. Sterol synthesis was modified only in L. callinectes after growth with AY-9944, which caused the accumulation of ergosterol (S. Warner, unpublished data). Both organisms, however, metabolized triparanol.

L. giganteum ATCC 36492 (American Type Culture Collection, Rockville, Md.) and L. callinectes L3b, obtained from C. E. Bland, Department of Biology, East Carolina University, Greenville, N.C., were cultured in peptone-yeast extract-glucose broth as described previously (7). Ultraclean glassware and "distilled-in-glass" solvents were employed throughout this study (8). Cultures of both organisms were supplemented with 100 μg of mevalonolactone per ml (Sigma Chemical Co., St. Louis, Mo.). Triparanol (1 to 10 μg/ml final concentration) was added in 95% ethanol to the hot, autoclaved culture broth. Cultures were grown for 7 days at 25°C in a Gyrotory incubator (New Brunswick Scientific Co., New Brunswick, N.J.) under continuous and variable illumination.

The sterols were extracted from the mycelia as described previously (7). Triparanol and its metabolites were also extracted by these methods. The extracts were analyzed by gas chromatography (GC)-mass spectrometry with a Finigan (Sunnyvale, Calif.) 4023 instrument in the electron impact (70 eV) mode. A 14-m flexible fused silica capillary column was programmed from 160 to 260°C at 10°C/min with initial 3-min and final 15-min hold. The injector temperature was 260°C with helium as the carrier gas.

Triparanol underwent chemical decomposition either as a pure compound injected into the GC or when recovered from the autoclaved uninoculated control media that had otherwise been maintained and extracted as for control cultures. The chemical decomposition of triparanol yielded two GC peaks (Fig. 1) with molecular ions at m/e 419. Unaltered triparanol accounted for 8% of the reisolated material after chromatography. Peaks A and B, at relative retention times (RRT) to triparanol of 0.93 and 0.96, accounted for 37 and 55%, respectively, of this material. Since the molecular weight of triparanol is 437, these results suggest that the two peaks arise from thermal dehydration of the parent compound to yield cis/trans isomers. The mass spectra of the peaks were identical (Fig. 2). Attempts to eliminate this decomposition by reducing the injection port temperature or intro-
FIG. 1. GC chromatograms of triparanol that was extracted from an uninoculated control culture.

FIG. 2. Mass spectrum of the dehydration product of triparanol.
FIG. 3. Mass spectrum of the RRT = 0.77 triparanol metabolite.

FIG. 4. Mass spectrum of the RRT = 0.71 triparanol metabolite.
ducing the samples into the mass spectrometer by a direct-insertion probe were unsuccessful.

Extracts from mycelia of both fungi exhibited not only the chemical decomposition peaks due to thermal dehydration above but also peaks for other compounds not present in cultures which were not administered triparanol, indicating that the organisms were metabolizing triparanol by a series of enzymatic reactions heretofore unsuspected. Thus, both fungi had a peak (RRT = 0.77) with a molecular ion of m/e 320, suggesting a metabolic loss of triethylamine plus thermal dehydration from the parent compound (Fig. 3). A peak (RRT = 0.71) with a molecular ion of m/e 348, corresponding to a metabolic loss of diethylamine plus thermal dehydration (Fig. 4), was observed only in extracts from L. giganteum. A peak (RRT = 0.73) with a molecular ion of m/e 207, suggesting a metabolic loss of aryl chloride plus thermal dehydration (Fig. 5), was observed only in extracts from L. callinectes. It is of note that only a single peak is observed for each metabolite, possibly due to the particular GC liquid phase that was employed in this study.

The catabolism of triparanol may be related to the enzyme-mediated penetration of the host cuticle by the infective zoospores of these fungi. Arthropod cuticle contains a vast array of phenolic structures, such as the diityrosyl residues of the structural protein resilin (1), and the ability to degrade triparanol may occur due to the enzymes that function to degrade these compounds.

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LITERATURE CITED

FIG. 5. Mass spectrum of the RRT = 0.73 triparanol metabolite.