of hydroxyl radical production. Ferric-EDTA stimulates DMSO oxidation (lines 3 and 7, fig. 1). The addition of sodium azide resulted in a further increase (line 7 and 8, fig. 1). Both without ferric EDTA (line 3, fig. 1) and in the presence of sodium azide alone (line 4, fig. 1) the oxidation of DMSO is minimal.
Microsomal NADPH dependent lipid peroxidation is most prominent without any addition at all (line 1, fig. 2). The hydroxyl scavenger, DMSO, did not inhibit lipid peroxidation. Ferric-EDTA resulted in an inhibition of lipid peroxidation. Remarkably, sodium azide decreased lipid peroxidation both in the absence and in the presence of ferric-EDTA.
Comparison of the optimal conditions for microsomal hydroxyl radical formation and lipid peroxidation in this parallel experimental set-up shows: 1) Higher production of hydroxyl radicals, via ferric-EDTA, does not result in an increase in lipid peroxidation. Rather, a decrease in lipid peroxidation is observed. 2) DMSO in a concentration that is able to scavenge hydroxyl radicals (fig. 1) did not inhibit lipid peroxidation (fig. 2). 3) Addition of sodium azide resulted in an increase in hydroxyl radical formation and a concomitant decrease in lipid peroxidation.
Azide is used in order to inhibit endogenous microsomal catalase, thereby leading to a rise in microsomal H_2O_2 concentration. This does not give stimulation of lipid peroxidation, thus confirming the notion that H_2O_2 is not involved in a rate-limiting step in NADPH-dependent lipid peroxidation[2,13]. The observed inhibition by azide may be the result of its scavenging effect of singlet oxygen. Our observations strongly indicate that hydroxyl radicals play no part in NADPH-dependent lipid peroxidation.

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A comparison of cycloartenol and lanosterol biosynthesis and metabolism by the Oomycetes

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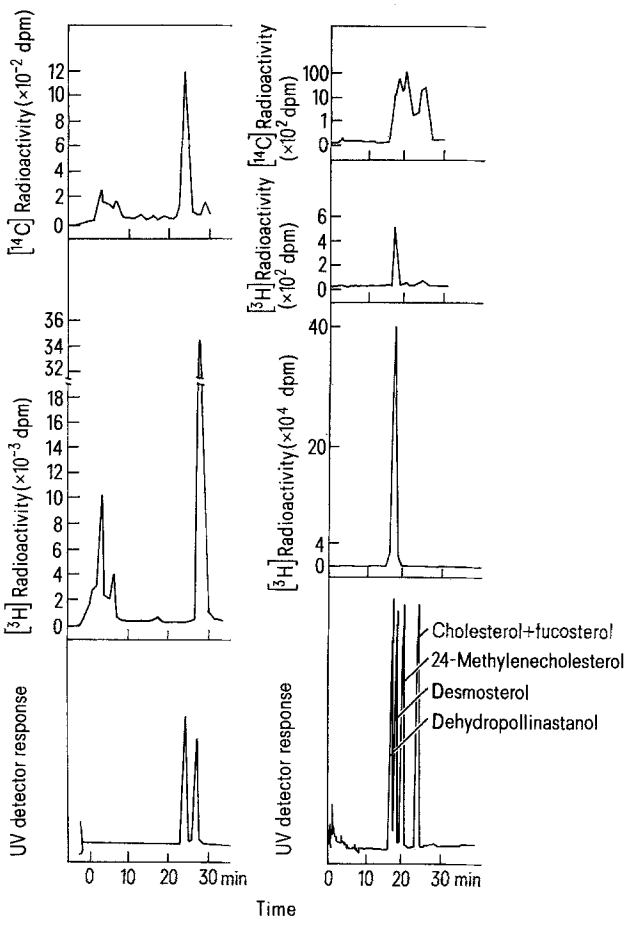
Summary. Representative members in each of the four orders of Oomycetes (*Phytophthora cactorum,* Peronosporales; *Lagenidium callinectes, L. giganteum,* Lagenidiales; *Saprolegnia ferax,* Saprolegniales; *Apodachylella completa,* Leptomitales) have been examined for their ability to synthesize and polycyclize squalene-oxide (SO) to a tetracyclic product and to differentiate between cycloartenol and lanosterol metabolism to sterols. *P. cactorum* and *L. giganteum* failed to synthesize or metabolize SO, cycloartenol or lanosterol. While the other three fungi synthesized sterols via SO and lanosterol, a minor metabolism of added cycloartenol to the 4,4-desmethyl-14α-methylcyclosteroid dehydropollinastanol was observed.
Key words. Oomycetes; squalene-oxide; cycloartenol; lanosterol; sterol metabolism.

Fungal evolution has classically been assessed through comparisons of specific phenotypic characters and life cycle events coupled with a few biochemical parameters, such as, in the sequencing of intermediates in the sterol pathway[1,2]. Dissociation between a cycloartenol- and a lanosterol-based steroid pathway is known to phylogenetically link organisms having an evolutionary history of photosynthesis, e.g. cycloartenol biosynthesis and metabolism, with those which lacked such an ancestry. Since the fungal progenitors are generally believed to have been, at one time, photosynthetic organisms[3,4] which subsequently lost the chloroplastic system, it seems logical that the Oomycetes, a primitive group believed to be closely allied with the Phaeophyte[5] or Chrysophyte algae[3], may have retained the genes for the cycloartenol route. In fact, there are reports which seemingly support this dogma[6,7]. However, as we now unequivocally show, the Oomycetes either utilize a lanosterol-based pathway or fail to synthesize sterols de novo: no vestigial SO-cycloartenol cyclase or cyclosteroid isomerase was operational in the various mycelia. When the five Oomycetes were examined by GLC and GC-MS, only *L. callinectes, S. ferax,* and *A. completa* produced detectable levels of sterols and lanosterol. While the synthesis of SO and its cyclization are implied in the three latter fungi, detailed studies with *P. cactorum* and *L. giganteum* failed to show mycelial conversion of [2-^{14}C]acetate or [^{3}H]squalene to SO or cyclize [^{14}C]SO to a tetracyclic product. Thus, the ability to synthesize sterols is variable in Oomycetes, as it apparently is in the slime molds[8,9]. In oomycetous fungi which demonstrably produce lanosterol[10,11], the possibility of it having been formed subsequent to cycloartenol synthesis could not be ruled out. Therefore, with *S. ferax, P. cactorum,* and *L. giganteum* we performed on each of the mycelia two sets of additional experiments with ^{14}C and ^{3}H substrates to trap the putative cycloartenol intermediate and to test whether cycloartenol could be directly isomerized to lanosterol.
When 0.056 μCi/flask of [2-^{14}C]acetate and nonradioactive cycloartenol (10 ppm) were fed to *S. ferax,* lanosterol was isolated in radiochemically pure form. While significant mycelial absorption of cycloartenol was evident by GLC of the mycelial extacts, no significant radioactivity eluted with cycloartenol in HPLC. However, as shown in figure 1, an HPLC peak which may have been cycloartenol was evident in the *S. ferax* incubation with [^{14}C]SO (sp. act. 3.6×10^6 dpm/mg; synthesized by incubating the GL-7 yeast sterol auxotroph with [^{14}C]-acetate and purified by HPLC as described in Nes and Heupel[12] and 10 ppm tridemorph (a specific inhibitor of the cyclosteroid isomerase[13]). The HPLC fractions from the latter feed corresponding to lanosterol and cycloartenol, were collected and diluted with nonradioactive carrier to produce a starting sp. act. for lanosterol, 1.42×10^4



dpm/10.0 mg, and for cycloartenol, 866 dpm/12.0 mg. While the specific activity of the cycloartenol sample fell to zero after two recrystallizations from acetone and methanol, the sp. act. of the lanosterol sample remained constant. In the second set of experiments (performed twice) [2-^{3}H] cycloartenol (sp. act. 2.55×10^6 dpm/500 µg/250/ml flask containing 50 ml medium and synthesized by base-catalyzed exchange with tritiated water 1 Ci/ml) was fed to *S. ferax* (fig. 1). Much radioactivity was present in the 4,4-dimethylsterol TLC zone. However, no radioactivity eluted in the HPLC which could be assigned as associated with lanosterol, only that of unmetabolized or autoxidized cycloartenol. Similar experiments, performed with *P. cactorum* and *L. giganteum*, resulted in isolation of unmetabolized and autooxidized cycloartenol. These experiments proved that the sequence, SO→cycloartenol→lanosterol, was not operational in the various fungi. However, cycloartenol may have been metabolized to a 4-desmethyl cyclosteroid as shown in wild type yeast and sterol auxotophic yeast mutants[14,15]. A trace amount of radioactivity was associated with cholesterol (4,4-desmethyl zone) on TLC

Figure 1. Left-top: reversed-phase HPLC radiochromatogram (RPHPLC-RC) of the 4,4-dimethyl fraction (zone corresponding to lanosterol) from the TLC plate of *Saprolegnia ferax* incubated with [^{14}C]squaleneoxide and 10 ppm Tridemorph; left middle: RPHPLC-RC of the 4,4-demethyl fraction from the TLC plate of *S. ferax* incubated with [2-^{3}H]cycloartenol; left bottom: detector response, lanosterol, 24 min and cycloartenol, 27.5 min. Right top: RPHPLC-RC of the 4,4-desmethyl fraction (zone corresponding to cholesterol) from the TLC plate of *S. ferax* incubated with [2-^{14}C]acetate; middle two figures: incubations with [2-^{3}H]cycloartenol and [2-^{3}H]dehydropollinastanol, respectively; bottom right: chromatogram of the four naturally occurring 4-desmethyl sterols of *S. ferax* and dehydropollinastanol. Column, Altex ODS ultrasphere 25 cm × 4.6 mm i.d.; particle size, 5µm; eluant, 4% aq. MeOH; flow rate, 1.6 ml/min; one fraction collected every min; UV detector, 205 nm; recorder speed, 12 cm/h; span, 10 mV.

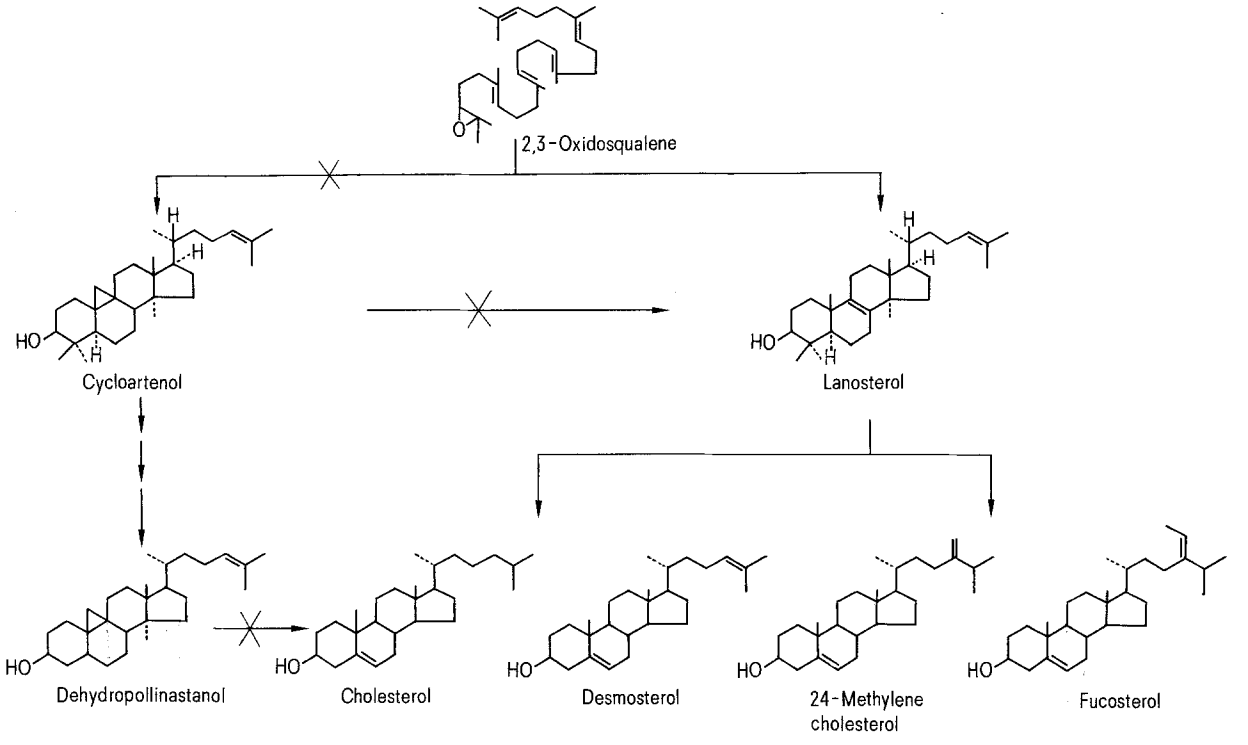


Figure 2. Partial biosynthetic sterol pathway in Oomycetes.

from the [2-^{3}H]cycloartenol and [2-^{3}H]lanosterol (sp. act. 2.55×10^6/dpm/500 µg/flask) feeds to *P. cactorum* and *L. giganteum*. However, the ^{3}H sample, eluted from the plates and diluted with cholesterol and a mixture of phytosterols, was found to elute much earlier then with the carrier sterols on HPLC. When similar experiments were performed with *S. ferax* significant activity was evident in the 4,4-desmethyl TLC band. In other work on the sterol composition of pollen we had recently found that certain cyclosteroids, e.g. dehydropollinastanol, co-chromatographed with 24-methylenecholesterol on GLC (3% SE-30) and may co-chromatograph with 24-methylenecholesterol, as the acetates when chromatographed on silver nitrate impregnated AL_2O_3 columns but eluted earlier on HPLC than either desmosterol or 24-methylenecholesterol. The radioactive material from the TLC 4,4-desmethyl zone injected into the HPLC from the [2-^{3}H]cycloartenol feed coincided with an authentic sample of dehydropollinastanol (fig. 1). In order to verify cycloartenol metabolism by *S. ferax*, we performed another feed on a large scale. From this latter feed we isolated a few µg of radioactive compound which corresponded to dehydropollinastanol. This material was diluted with 10 mg of dehydropollinastanol producing an initial sp. act. of 471 dpm/mg: recrystallization from acetone-water, methanol-water, hexane-dichloromethane, yielded a compound having a sp. act. of 332 dpm/mg, 323 dpm/mg and 319 dpm/mg. When 1.0×10^7 dpm/500 µg/flask [2-^{3}H]dehydropollinastanol was incubated with *S. ferax*, a single radioactive 4,4-desmethylsterol was recovered from the mycelia (fig. 1). While no metabolism of the dehydropollinastanol was obvious, some ($< 1\%$) esterification occurred. The other fungal groups, many of which synthesize ergosterol[2], presumably metabolize lanosterol and cyclosteroids in a similar manner. We conclude that the Oomycetes may have evolved in a polyphyletic manner[16,17] with respect to the other fungi. Their lack of having a cycloartenol-based pathway (fig. 2) is consistent with the view[18] previously advanced that the Oomycetes may have evolved through a nonphotosynthetic, lanosterol-biosynthesizing lineage which has its origins with the prokaryotes, rather than a cycloartenol-biosynthesizing photosynthetic eukaryote, such as, the brown or golden-brown algae.

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A new selective insecticidal uncoupler of oxidative phosphorylation

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Summary. A new aryl hydrazone structure with high insecticidal activity against the Australian sheep blowfly, *Lucilia cuprina*, was shown to have a higher activity as an uncoupler of oxidative phosphorylation in insect compared to mammalian mitochondrial preparations. This compound possesses the requirements of other uncouplers in its measured pKa and lipid solubility. However, when compared to a closely related structure with similar physicochemical properties, its insecticidal and insect mitochondrial uncoupling activities are greater and it exhibits decreased mammalian toxicity corresponding to this differential biochemical selectivity.
Key words. Lucilia cuprina; sheep blowfly; insecticides; aryl hydrazone; oxidative phosphorylation; mitochondrial uncoupling.

Mitochondrial oxidative phosphorylation which produces the energy storage molecule ATP, is a ubiquitous process throughout the animal kingdom. Any attempt to use uncouplers or inhibitors of this process as insecticidal agents must take into account that high activity of any active structures in insects may also be accompanied by high toxicity in the mammal[3]. We therefore report the finding of a selective new aryl hydrazone (**I**), with high uncoupling and insecticidal activity against the Australian sheep blowfly *Lucilia cuprina* (Wiedemann), but with a lower uncoupling activity in rat liver mitochondrial preparations.

A wide variety of structures are known which uncouple oxidative phosphorylation and these compounds generally possess a readily dissociable proton and are highly lipid soluble structures[4,5]. Some of the most active uncouplers previously reported are cyano-alkoxy carbonyl derivatives of phenylhydrazones and these agents are usually highly active in mammalian systems[5,6]. We synthesized a series of novel arylhydrazones[7] following on earlier work on phenyltriazole[8] uncouplers of oxidative phosphorylation in rat liver and yeast cell mitochondria. Structurally, the new compounds contain a phenyl ring substituted by electron withdrawing groups, NO_2 and/or CF_3. These groups were used to obtain an increase in the ionization of the proton attached to the N^1 nitrogen of the hydrazone, thus increasing the acidity of the compounds as measured by their pKa. This in-