Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 3227–3231, June 1983
Biochemistry

Evidence for metabolic and functional discrimination of sterols by *Phytophthora cactorum*

(Oomycetes/Pythiaceae/fucosterol metabolism/reproduction)

W. DAVID NES AND ALLEN E. STAFFORD

Plant Physiology and Chemistry Research Unit, Agricultural Research Service, Western Regional Research Center, U.S. Department of Agriculture, Berkeley, California 94710

Communicated by Linus Pauling, February 28, 1983

ABSTRACT **When fed 10 ppm of one of the following sterols: cholesterol (cholest-5-en-3β-ol), wingsterol (21-isopentylcholesterol), desmosterol [cholesta-5,24(25)-dien-3β-ol], 24-methylenecholesterol [ergosta-5,24(28)-dien-3β-ol], or fucosterol [stigmasta-5,24(28)-dien-3β-ol], the pathogenic fungus *Phytophthora cactorum*, which is naturally unable to epoxidize squalene, accumulated each of the test compounds to similar levels. Fucosterol, the only sterol metabolized, was reduced to yield 24-ethylcholesterol. All the sterols tested induced the formation of sex structures. Fertilization and subsequent maturation of oospores capable of germination occurred only with the naturally occurring sterols. Wingsterol treatments resulted in aborted oospores. None of the sterols tested was inhibitory to growth, measured as changes in the 21-day mycelial dry weight. The results are consistent with the view that the accumulated sterol functions to regulate the life cycle of *P. cactorum*. However, the metabolism and kinds of recognition of the sterol molecule, in terms of uptake and effects on growth and induction of the various sexual events, contrast sharply with what is known for other oomycetous fungi such as *Achlya* and *Saprolegnia*. This implies that the evolutionary histories of the Oomycetes may be different.**

The occurrence, biosynthesis, and metabolism of sterols has been studied in numerous oomycetous fungi [orders Saprolegniales (1–3), Leptomitales (1), Lagenidiales (4), and Peronosporales (5–10)]. The physiological roles of sterols and other polycyclic isopentenoids in effecting growth and reproduction have also been the subject of much investigation (11–14).

Phytophthora cactorum (order Peronosporales, family Pythiaceae) is a soil-borne pathogen of higher plants—e.g., fruit trees (15)—which, unlike many of the other oomycetous fungi, lacks sterol synthetic capabilities (6–10). This deficiency has resulted in a heterotrophic requirement for naturally occurring host sterols to stimulate vegetative growth and an auxotrophic requirement for these sterols to produce functional oospores—i.e., oospores capable of germination. Although *P. cactorum* displays an enzymic preference to metabolize certain nuclear B-ring unsaturated (16), but not saturated (17), sterols to Δ^5 sterols and a strict sterol requirement for oospore production (18, 19), the selectivity for sterols in terms of initial uptake (20), accumulation into logarithmic-phase cultures (17), and derivatization to esters (13, 21) and glycosides (13, 21), and their effects on growth response (17, 22) and induction of sexual structures (23, 24) is less obvious. Nevertheless, the different physiological and biochemical recognitions of sterols exhibited by *P. cactorum* imply a multiplicity of roles that become operational at different times during the life cycle. The purpose of this paper is to examine the metabolic fate and physiological roles of cholesterol (cholest-5-en-3β-ol), desmosterol [cholesta-5,24(25)-dien-3β-ol], wingsterol (21-isopentylcholesterol), 24-methylenecholesterol [ergosta-5,24(28)-dien-3β-ol], and fucosterol [stigmasta-5,24(28)-dien-3β-ol].

MATERIALS AND METHODS

Chemicals. [2,4-^{3}H]Fucosterol and 24-methylene[2,4-^{3}H]-cholesterol were prepared according to Thompson *et al.* (25) and were gifts of J. A. Svoboda. Fucosterol and 24-methylenecholesterol were the gifts of Glenn W. Patterson. Wingsterol prepared by J. M. Joseph (26) was the gift of W. R. Nes. Cholesterol and desmosterol were purchased from Supelco (Bellefonte, PA). Z-17(20)-Dehydrocholesterol, stigmasterol, sitosterol, and other stigmastane and ergostane samples for use as mass spectroscopy (MS) and gas/liquid chromatography (GLC) reference compounds were supplied by H. W. Kircher, D. N. Kirk, and W. R. Nes. The purity of the test sterols was ≥95% as determined by GLC. The radiolabeled substrates were purified by thin-layer chromatography (TLC) prior to use. Organic solvents for high-performance liquid chromatography (HPLC) and other uses were purchased from MCB Labs. TLC plates were from Analtech (adsorption TLC) and Whatman (reversed-phase TLC). Aluminum oxide (neutral) was from ICN.

Culture and Growth Conditions. *P. cactorum*, strain 51-22, was provided by R. Berman (University of California, Berkeley). The fungus was maintained on supplemented agar plates (27, 28) or cultured on a nonbuffered, liquid (sterol-free) synthetic medium (17). Four types of experimental cultures were used throughout this study: control liquid culture, 2,000 ppm ethanol but no added sterol; sterol-supplemented liquid culture, test sterol added at 10 ppm as an ethanolic solution (2,000 ppm); control solid culture, 2,000 ppm ethanol and 2% Difco agar added to solidify the liquid medium; sterol-supplemented solid culture, test sterol added at 10 ppm as an ethanolic solution (2,000 ppm) to medium solidified with 2% agar. Control solid cultures (5-mm mycelial plugs) served as a source of inoculum for mycelia grown in some liquid and all solid media. Solid media cultures, rather than liquid media cultures, were used to bioassay for sexual structures, oospores, and sterol-induced changes in mycelial dry weight (18, 27, 28) because, in the nonbuffered liquid medium, as mycelia of *P. cactorum* increase in mass, pH changes precipitously (22). The alteration in culture condition prevents sterols from inducing oospore production (unpublished data). Alternatively, no pH changes are apparent in mycelia cultured on solid media (27), and oospores are formed with an appropriate sterol supplement (18). Because sterols are present in Difco agar (see *Results*) an additional method was used for the (nonradioactive) sterol metabolism studies. In these flasks the

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Abbreviations: MS, mass spectroscopy; GLC, gas/liquid chromatography; HPLC, high-performance liquid chromatography; TLC, thin-layer chromatography; RRT, GLC retention time relative to cholesterol.

inocula were derived from hyphal fragments dislodged from the periphery of a 10-day mycelial mat previously cultured in the usual way as a control liquid culture. Mycelia grown in liquid medium were harvested in exponential-phase growth [ca. 12–15 days (17)], whereas mycelia cultured on solid medium were harvested in stationary-phase growth (21 days) after the pathogen had an opportunity to maximally sporulate (24). In every experiment *P. cactorum* was incubated at 20°C in the dark (27).

Analysis of Sterols. Sterols were isolated from the mycelia (without saponification) as described (29). The neutral lipid fraction of mycelia cultured on solid medium was chromatographed on a thin layer of silica gel G (250 μm) developed in benzene/ether, 9:1 (vol/vol). Silica gel from the zone corresponding to cholesterol, R_f 0.3, was scraped from the plate and eluted with ether. The eluted fraction was further analyzed by GLC. Recently we demonstrated that the chromatographic behavior of long-chain fatty alcohols may be somewhat similar to that of sterols, depending on the amount of these materials applied to the TLC plate (30). In order to separate the long-chain fatty alcohols from the 4-desmethyl sterols in the neutral lipid extracts of the liquid-grown cultures, we used a 3% deactivated alumina column (100 g) developed stepwise (10% increments) with increasing amounts (100 ml each) of ether and hexane (30). When labeled sterols were incubated with *P. cactorum*, cholesterol, campesterol, sitosterol, and either 24-methylenecholesterol or fucosterol were added to the extraction thimble at 1 mg each as carrier sterols. The column chromatographic method was employed to recover the radioactive 4-desmethyl sterol fraction. An aliquot of this radioactive sample was dissolved in 1,4-bis[5-phenyl(oxazolyl)]benzene cocktail and its radioactivity was measured in a Packard Tri-Carb liquid scintillation counter with efficiencies of 85% and 26%, respectively, for ^{14}C and ^{3}H. The free sterols recovered from the alumina column were further resolved into individual sterol components by reversed-phase HPLC (31) and in the labeled fucosterol metabolism study by additional chromatography of the acetates on a $AgNO_3$-impregnated silica HPLC column (32). The sterol fractions recovered from the reversed-phase HPLC were, before acetylation, purified by TLC to reduce interference by HPLC bleed. GLC was performed routinely on two packed columns—i.e., 3% SE-30 and 3% OV-17 (both from Applied Science, State College, PA)—which were operated isothermally at 235°C. In the metabolism studies, to verify structure identities and quantities, two additional packed columns were used—i.e., 1% XE-60 and 1% SP-1000 (Alltech Associates, Deerfield, IL, and Supelco, Bellefonte, PA, respectively)—which were operated at 200 and 255°C, respectively. In some cases either 100 μg of stigmasterol [retention time relative to cholesterol (RRT) 1.40 on 3% SE-30] or Z-17(20)-dehydrocholesterol (RRT 0.87 on 3% SE-30) was added to the extraction thimble as an internal standard for sterol quantitation by GLC.

RESULTS

Incubation with Control Media. In control cultures grown in the supposedly sterol-free solid media, a few sex structures were routinely observed, some of which variably matured into oospores. This phenomenon has been reported by others (6). When this mycelium was extracted, the 4-desmethyl sterol band contained, by GLC analysis, at least six major components having RRTs on a 3% SE-30 column of 1.00, 1.40, 1.51, 1.65, 1.89, and 2.00 and on a 3% OV-17 column of 0.75, 1.00, 1.19, 1.35, 1.67, and 1.81. GLC/MS of this material coupled with the cochromatography with authentic samples on four packed columns proved that four of the compounds were the sterols cholesterol, 24-methylcholesterol, 24-ethylcholesterol, and 24-ethylcholesta-5,22-dienol, and two of the compounds were the long-chain fatty alcohols octacosanol and triacontanol. Another compound was tentatively identified as a polycyclic isopentenoid and had a RRT on OV-17 at 235°C of 1.67 (cochromatographs with sitosterol). This material possessed by MS a parent ion, M^+, of 424 (3%) with a characteristic retro-Diels–Alder fragmentation that exhibits a base peak having M^+ of 183 (100%) that is characteristic of pentacyclic triterpenoids (33). The absolute amount of sterol recovered from the control mycelia varied significantly from one experiment to the next, from traces to 0.001% sterol on a dry weight basis. Cholesterol always represented 90% of the sterol mixture. The occurrence of sterols other than the test sterol in control solid and liquid media cultures depended primarily upon the degree to which the agar plugs used to seed the experimental flask had been removed from the mycelium at the time of harvest. When [^{14}C]squalene (27), [^{3}H]lanosterol, or [^{3}H]cycloartenol (28) is incubated with *P. cactorum* cultured on solid media or liquid media, no Δ^5-4-desmethyl sterols are synthesized. Thus, the presence of Δ^5 sterols in the mycelium of control cultures must come from a source other than the fungus. Difco agar was extracted overnight with acetone and the 4-desmethyl sterol TLC band of the neutral lipid fraction was analyzed by GLC and GLC/MS. The same sterols, fatty alcohols, and unknown pentacyclic triterpenoid (M^+ 424), were detected as previously encountered in the control mycelium. There are reports in which sterols, and in particular cholesterol, have been shown to occur (5, 34–36) or presumably are formed by metabolism of some appropriate 4,4,14-trimethylsterol precursor (5, 36). However, the identification in these cases was not supported by the use of radiolabeled acetate, mevalonate, squalene, or lanosterol (8–10, 27, 28). In fact in one of the latter studies the sterol was shown to be present at 2 mg/590 mg (wet weight) of mycelium (9). Because [^{14}C]mevalonate was not converted to labeled sterol the investigators suggested the sterol was "almost certainly due to cholesterol from the original slope of *P. cactorum* being transferred to the liquid cultures." The sterols observed in the former studies (5, 34–36) may arise in a similar fashion from the residual agar, which was coextracted with the harvested mycelium. When the agar was extracted with acetone and then used to solidify the medium, as expected, no oogonia or oospores were observed in 21-day control cultures and no detectable sterols were present in the mycelium.

Incubation with Cholesterol and Wingsterol. There was no significant difference in the accumulation of sterol (free sterol was *ca.* 0.01% of the mycelium on a dry weight basis), dry weight, or effects on sexual structure production after incubation with cholesterol or wingsterol (Fig. 1). We have previously shown that, when the incubation temperature is maintained at 20°C, cholesterol stimulates mycelial growth relative to a control, measured as changes in dry weight (27) and hyphal extension (13), and stimulates the formation of great numbers of oospores (13, 18).

In the present experiments, cholesterol produced oospores (*ca.* 320 oospores per transect per Petri plate), most of which germinated after another 2 weeks of incubation (data not shown), whereas wingsterol induced the oospores (*ca.* 230) to abort (Fig. 2). Four major peaks previously identified as components in the agar (Fig. 3) as well as the peak corresponding to wingsterol (M^+ 456) were discernible in the total ion current chromatogram of the wingsterol-treated solid-grown cultures. By GLC quantitation on the packed columns, cholesterol relative to wingsterol was present at 0.01%. It is unlikely this low amount of cholesterol played a significant contributory role in the wingsterol effects on sexual reproduction. By selected ion monitoring we could detect other trace amounts of 24-methylcholesterol, 24-ethylcholesterol, and 24-ethylcholesta-5,22-dienol.

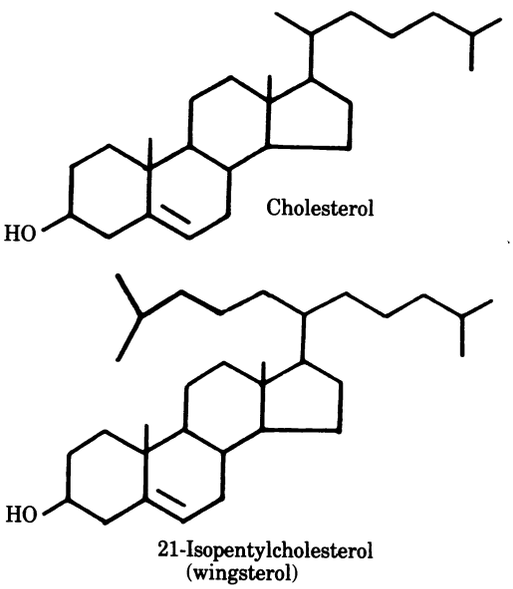

FIG. 1. Structures of two sterols used to supplement culture media for *P. cactorum*.

Incubation with Desmosterol and 24-Methylenecholesterol. Desmosterol and 24-methylenecholesterol were accumulated to similar levels and stimulated growth as cholesterol did. Sexual structures were evident by 12 days and by 21 days they had begun to mature into functional oospores (*ca.* 350 oospores with both test sterols). Because of sterol contamination from the agar, the assessment of whether alkylation and reduction of the double bond ($\Delta^{24(25)}$ or $\Delta^{24(28)}$) in the sterol side chain of desmosterol or 24-methylenecholesterol had occurred was equivocal. Thus an additional set of cultures was grown in liquid media with the respective test sterol and the flasks were inoculated with mycelial fragments rather than mycelial plugs. In the desmosterol incubation, no sterol other than the test sterol was detectable in the GLC chromatograms (four packed columns) at a level $\leq 0.01\%$ relative to the level of desmosterol. Similarly, from the mycelium of the 24-methylenecholesterol treatment, only the substrate was isolated; GLC RRTs 1.28 (3% SE-30) and 1.40 (1% SP-1000), M^+ 398. The spectrum corresponding to 24-methylenecholesterol was composed of mass-to-charge ratio m/z (assignment, relative intensity) 398 (M^+, 10%), 383 ($M^+ - CH_3$, 11%), 365 ($M^+ - CH_3 - H_2O$, 8%), 314 ($M^+ - C_6H_{12}$, 80% brought about by allylic cleavage of the C-22—C-23 bond), 299 ($M^+ - C_6H_{12} - CH_3$, 30%), 296 ($M^+ - C_6H_{12} - H_2O$, 18%), 281 ($M^+ - C_6H_{12} - CH_3 - H_2O$, 21%), 271 ($M^+ - C_9H_{19}$, 35% from the side chain), 253 (M^+ − side chain − H_2O, 10%), 229 (M^+ − side chain − $H_2O - C_3H_8$, 22%), 211 (M^+ − side chain − $H_2O - C_3H_8 - H_2O$, 18%). The absence of 24-methylenecholesterol metabolism was confirmed by incubation with 24-methylene-[2,4-^{3}H]cholesterol (1.0×10^6 cpm/250 μg). The mycelium (15 mg dry weight) was harvested after 10 days of incubation. The neutral lipid extract (1.61×10^4 cpm/7.0 mg) was separated into 4-desmethyl sterols by column chromatography and a sterol mixture was obtained (2.00×10^4 cpm/3 mg, composed of 24-methylenecholesterol and carrier sterols). An aliquot was chromatographed on reversed-phase HPLC (Fig. 4). No significant amount of radioactivity eluted in fractions other than the fraction corresponding to 24-methylenecholesterol.

Incubation with Fucosterol. In previous studies, we (18) and others (37) demonstrated that fucosterol stimulates formation of about twice as many oospores as are formed with cholesterol under similar culture conditions. Similar results were obtained in this study. The free sterol isolated by extraction and TLC of mycelia cultured on solid medium amounted to *ca.* 0.01% on the basis of dry weight of mycelium, analogous to the other treatments. Some 24-ethylcholesterol was expected to be present as an agar component, but the amount of 24-ethylcholesterol relative to fucosterol by GLC on columns packed with 3% OV-17 and 1% SP-

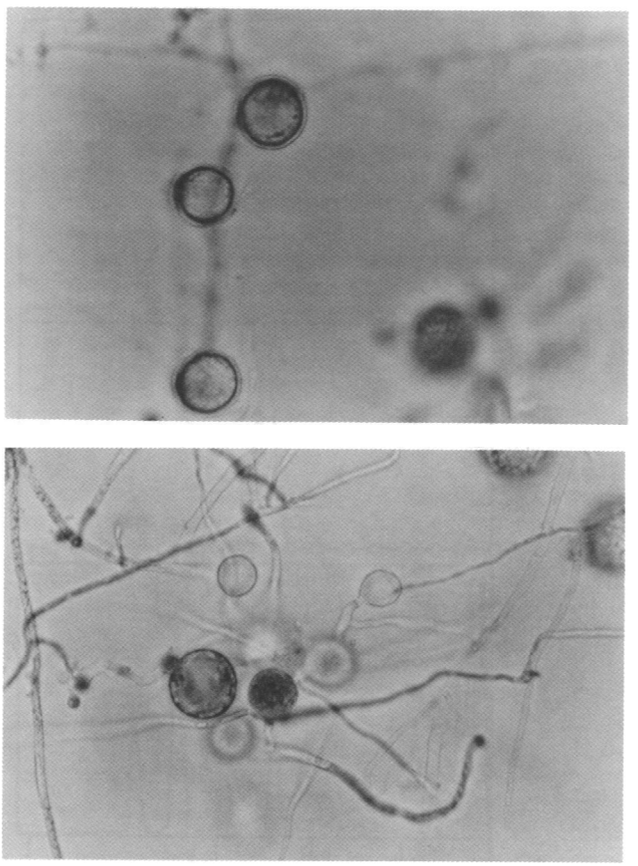

FIG. 2. Photomicrograph of cultures of *P. cactorum* incubated for 21 days on chemically defined agar medium at 20°C in the dark with cholesterol (*Upper*) or wingsterol (*Lower*). Single-walled spores are oogonia, and hollow spores are the aborted oogonia in the wingsterol treatment. The double-walled spores in the cholesterol treatment are oospores. (×6.)

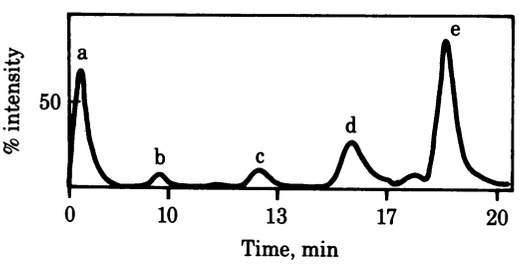

FIG. 3. Total ion current chromatogram of extract from wingsterol-treated culture. Peaks a, b, c, d, and e are, respectively, octacosanol, cholesterol, triacontanol, unknown triterpenoid, and wingsterol. A 2-m spiral column packed with 3% OV-17 was fitted via on-column injection to a VG-micromass 70/70-HS mass spectrometer. A temperature rather than isothermal program was employed. The initial column temperature was 240°C; this temperature was maintained for 12 min, then programmed at 3°C/min to 270°C. Reference sterols cholesterol, campesterol, stigmasterol, sitosterol, and fucosterol and a mixture of long-chain fatty alcohols (octacosanol and triacontanol) were chromatographed and their mass spectra were obtained with the GLC/MS (performed by W. F. Haddon) under conditions analogous to those used to identify the test sterols isolated from the fungus.

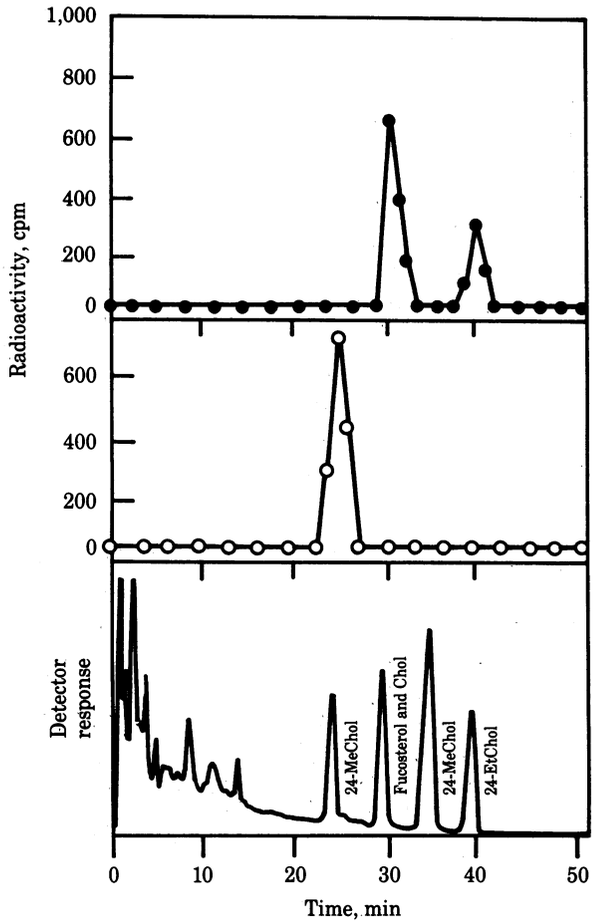

FIG. 4. (*Top* and *Middle*) Reversed-phase HPLC radiochromatogram of the 4-desmethyl sterol fraction from the alumina column of *P. cactorum* incubated with [2,4-^{3}H]fucosterol (*Top*) and 24-methylene[2,4-^{3}H]cholesterol (*Middle*). (*Bottom*) Elution profile of cholesterol (Chol), 24-methylcholesterol (24-MeChol), 24-methylenecholesterol (24-CH_2Chol), 24-ethylcholesterol (24-EtChol), and fucosterol (24-ethylidenecholesterol). Column, Altex octadecylsilane ultraspheres 25 cm × 4.6 mm inside diameter; particle size, 5 μm; eluant, 96% aqueous MeOH; flow rate, 1.6 ml/min; one fraction collected every min; UV detector, 205 nm; recorder speed, 12 cm/hr; span, 10 mV.

1000 was disproportionately high. That suggested that the side chain of fucosterol may have been metabolized to some degree. 24-Ethylcholesterol and fucosterol cochromatograph on 3% SE-30 (RRT 1.63), suggesting that the inability of others to detect fucosterol reduction to 24-ethylcholesterol may be due to their use of a 3% SE-30 column for routine sterol analysis (5). In order to confirm that fucosterol was in fact metabolized, 10 flasks were inoculated with mycelial fragments and this sterol. From 297 mg (dry weight) of mycelium, 12.3 mg of neutral lipid was obtained. This material was chromatographed on an alumina column and the 4-desmethyl sterol band (325 μg by gravimetric determination) was analyzed by GLC. Two sterol components with RRTs of 1.51 and 1.67 (1% SP-1000) were present in the ratio of 1:9. GLC/MS of this sterol mixture confirmed the minor component as 24-ethylcholesterol (M^+ 414) and the major component as 24-ethylidenecholesterol (M^+ 412 with the characteristic peak at m/z 314). The aqueous medium contained only the test sterol, as found by extraction with ether and analysis of the organic layer by GLC. Incubation of one experimental flask (inoculated with a mycelial mat plug) with [2,4-^{3}H]fucosterol (1.0×10^6 cpm/550 μg) for 13 days produced mycelia with a dry weight of 71 mg. The neutral lipid fraction, which contained carrier sterols (5.0×10^4 cpm/9.0 mg), was separated into sterol derivatives (esters and glycosides) and free sterols by chromatography on an alumina column. On the basis of the radioactivity corresponding to appropriate fractions, the mole ratio of steryl esters to glycosides to free sterols was 25:9:66. Formation of steryl esters and glycosides *in vivo* with cholesterol and sitosterol as substrates has been reported in studies with various pythiaceous fungi (refs. 13, 21, and 29 and references cited therein).

An aliquot of the 4-desmethyl sterol band was chromatographed on reversed-phase HPLC. Two radioactive components corresponding in elution time to fucosterol and sitosterol were evident (Fig. 4). Because our reversed-phase HPLC system fails to separate fucosterol from cholesterol, this radioactive fraction (after preparative HPLC) was acetylated and an aliquot of the sample (1.03×10^4 cpm/772 μg—the mass represented two sterols: fucosterol and cholesterol added as carrier) was rechromatographed on a $AgNO_3$ HPLC column. As shown in Fig. 5, no radioactivity was associated with cholesteryl acetate (sitosteryl acetate and cholesteryl acetate cochromatograph on this column), which confirms the inability of fucosterol to be dealkylated by *P. cactorum*. The radioactive material from the fractions corresponding to sitosterol showed a single peak of mass on

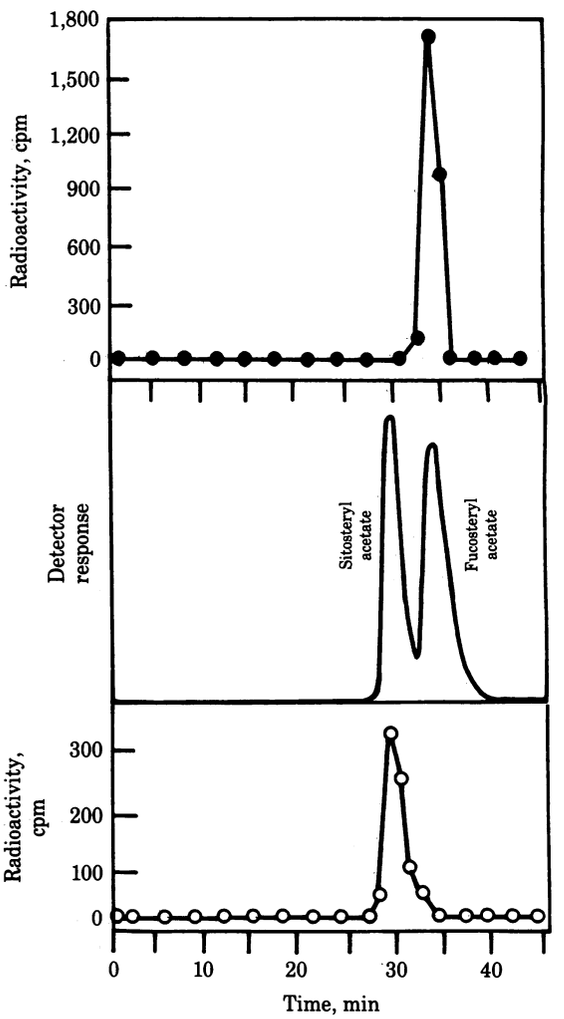

FIG. 5. (*Top* and *Bottom*) $AgNO_3$ HPLC radiochromatogram of an aliquot of the acetylated reversed-phase HPLC fractions (cf. Fig. 4) corresponding to fucosterol (*Top*) and sitosterol (*Bottom*). (*Middle*) Elution profile of sitosteryl acetate and fucosteryl acetate. Column, $AgNO_3$ (0.50% $AgNO_3$ in methanol)-impregnated Zorbax BP-SIL 25 cm × 4.6 mm inside diameter; particle size, 7–8 μm; eluant, 0.1% ethanol in hexane; flow rate, 0.4 ml/min; one fraction collected every min; UV detector, 205 nm; recorder speed, 12 cm/hr; span, 10 mV.

GLC, with RRTs of 1.63, 1.68, 1.89, and 1.51 on columns packed with SE-30, OV-17, XE-60, and SP-1000, respectively. [^{3}H]Sitosterol obtained by preparative HPLC (4.75×10^3 cpm/320 μg) was acetylated and an aliquot was chromatographed on a $AgNO_3$ HPLC column (Fig. 5). Of the applied sample 90% of the radioactivity was associated with the eluate corresponding to the monoene. The latter proves that the radioactivity associated with the fraction having an α_c of 1.32 (cf. Fig. 4) was in fact due to labeled 24-ethylcholesterol and not unmetabolized [^{3}H]fucosterol. [$\alpha_c = k'$ of sample/k' for cholesterol in HPLC, in which k' is (elution volume − dead volume)/dead volume.]

DISCUSSION

The regulation of the isopentenoid pathway to produce linear and cyclized products for hormonal and structural purposes has been demonstrated in the fungi [as reviewed in depth elsewhere (24)]. In Oomycetes that are taxonomically related to *Phytophthora*—e.g., *Achlya* and *Saprolegnia*—a functional link between structure and biosynthesis has been observed (24, 38). For instance, the demonstrable transfer of a CH_3 group to the C-24 position of a $\Delta^{24(25)}$ and $\Delta^{24(28)}$ sterol (2, 3) has been shown to be a prerequisite for the synthesis of the oxygenated steroid hormones—namely, antheridiol and oogoniol—which induce male and female sexual structure formation, respectively (38). These steroids have retained, as a molecular feature for function, an intact side chain that is alkylated at C-24. Presumably, the reduction of the $\Delta^{24(25)}$ bond to produce 24-desalkyl sterols is primarily associated with their role in the construction of mycelial membranes. The fact that *Achlya* is unable to accumulate significant quantities of dietary sterol (39) may have its functional origin in the operation of an isopentenoid pathway that is capable of providing a continued supply of this compound for physiological and biochemical functions.

In *P. cactorum* sterol availability is a limiting factor in the disease cycle; however, no obligatory requirement for sterol metabolism has been observed for function. Furthermore, unlike *Achyla* (39), *P. cactorum* can accumulate significant amounts of cholesterol and sitosterol and utilize these and other Δ^5 sterols as mycelial membrane components (20). The ability of cholesterol, fucosterol, as well as wingsterol and a series of other synthetic sterols—e.g., pregn-5-en-3β-ol, Z-17(20)-dehydrocholesterol, and 20-epicholesterol (24)—to induce oogonia formation, some of which (those induced by the synthetic sterols) abort at the oospore stage of maturation, indicates that recognition of the sterol side chain is of little importance to the initiation of (sterol-induced) sexual reproduction. In further contrast to *P. cactorum*, *Achlya* and related Oomycetes (3–5, 40) synthesize fucosterol from various tetracylcic steroids, presumably via 24-methylenecholesterol. The reverse metabolism, to dealkylate fucosterol by one (5) and two carbon units (3, 5), has also been shown in some of these fungi. None of these metabolic events are demonstrable in *P. cactorum* once the background level of sterol contaminant is accounted for. Thus sterols accumulated by *Phytophthora* are not likely converted to a hormone with an oxygenated side chain of the sort (the oogoniols) produced from fucosterol by *Achlya* (38). This implies that regulation of sexual structure formation in *Phytophthora* is controlled by different mechanisms than those in *Achlya*. Had the oomycetous fungi evolved as a homogeneous group, we would have expected that not only the morphogenic properties but also the regulatory and metabolic phenomena should be similar. Because this apparently is not the case, the Oomycetes may have a polyphyletic ancestry. Thus characters typical of this class of fungi—e.g., production of spores—may have evolved in certain groups such as the Pythiaceae by convergent rather than parallel evolution.

The support of Drs. A. I. Morgan, Jr., and Glenn Fuller throughout the course of this investigation is gratefully acknowledged.

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