Disassociation of oogonia formation from oospore production in *Phytophthora cactorum* by sterols with varying side chain lengths[1]

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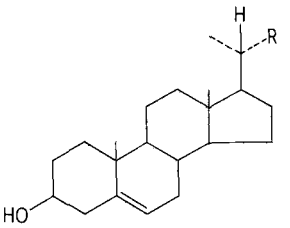
Summary. A series of 20(R)-*n*-alkylpregn-5-en-3β-ols with 0 to 9 (but not with 12) C-atoms in the alkyl group induced formation of oogonia in *Phytophthora cactorum*. However, maturation of the oogonia into germinating oospores occurred only when the alkyl group had 4–7 C-atoms.

Most of the Oomycetes which have been examined possess the ability to synthesize sterols[2]. Two genera in the family Pythiaceae, order Peronosporales which lack this ability are *Phytophthora* and *Pythium*[3]. While these pythiaceous fungi do not require sterols for vegetative growth, there is an auxotrophic requirement for sterols in sexual reproduction[4,5]. Elliott and coworkers demonstrated a number of years ago that a variety of naturally occurring Δ^5-24-desalkyl- and 24-alkylsterols added to the culture medium of *Phytophthora cactorum* will induce the formation of oogonia which after a few days mature into oospores[6]. A *Pythium* sp. (PRL 2142) has been the subject of analogous studies[7]. However, more recently Elliott and Sansome demonstrated with *P. cactorum* in the case of a sterol, 5α-cholestanol, which lacks the Δ^5-bond that, while oogonia will form, abortion occurs before maturation takes place to the oospore stage[8]. We have now found that this phenomenon of induction of oogonia formation with abortion can also be brought about with sterols retaining the Δ^5-bond if the side chain is either somewhat shorter or longer than the natural length.

Mycelia of *P. cactorum* were cultured on a completely synthetic agar medium for 3 weeks in the dark at 20 °C with 8 sterol supplements[9] at a level of 10 ppm. The sterols were cholesterol, pregn-5-en-3β-ol and its 20(R)-*n*-alkyl derivatives (fig. 1) with varying numbers of C-atoms in the alkyl group. The results for hyphal extension and sexual reproduction are shown in tables 1 and 2. The dry weights of the combined mycelia of 5 petri plates from the cultures treated with pregn-5-en-3β-ol and its 20(R)-*n*-alkyl derivatives were not significantly different from that of the cholesterol-treated cells. Four of the sterol-treated cultures (5 Petri dishes each) grown on the 20(R)-*n*-butyl, *n*-pentyl, and *n*-nonyl derivatives, cholesterol, and no sterol were harvest-



R = Pregn-5-en-3β-ol
R = $(CH_2)_3$ CH_3, 20(R)-*n*-Butylpregn-5-en-3β-ol
R = $(CH_2)_4$ CH_3, 20(R)-*n*-Pentylpregn-5-en-3β-ol
R = $(CH_2)_5$ CH_3, 20(R)-*n*-Hexylpregn-5-en-3β-ol
R = $(CH_2)_6$ CH_3, 20(R)-*n*-Heptylpregn-5-en-3β-ol
R = $(CH_2)_8$ CH_3, 20(R)-*n*-Nonylpregn-5-en-3β-ol
R = $(CH_2)_{11}$ CH_3, 20(R)-Dodecylpregn-5-en-3β-ol

Figure 1. Structures of the synthetic sterols used in this study.

Table 1. Sterol induced hyphal extension of *P. cactorum*

No. of C-atoms on C-20[a]	Diameter[b] (mm)
0	23.5
4	41.3
5	39.5
6	40.0
7	37.0
9	34.5
12	34.0
Cholesterol	41.5
No sterol	36.5

[a]No of C-atoms in alkyl group of 20(R)-*n*-alkylpregn-5-en-3β-ol (cf. fig. 1). [b]Data given are mean values for radial diameters in each of 5 (50 mm) petri dishes after 6 days on solid culture. The mean varied by 1.78 mm, based on a least significant difference at $p = 0.05$.

Table 2. The effect of sterols on oogonia and oospore production in *Phytophthora cactorum*[a]

Sterol added	Oogonia	Aborted oogonia	Oogonia with oospores	Germinating[d] oospores	Total
Pregn-5-en-3β-ol	100	150	5	0	255
20(R)-*n*-Butylpregn-5-en-3β-ol	0[c]	0	219	25	244
20(R)-*n*-Pentylpregn-5-en-3β-ol	0[c]	0	413	30	443
20(R)-*n*-Hexylpregn-5-en-3β-ol	0[c]	0	403	46	449
20(R)-*n*-Heptylpregn-5-en-3β-ol	0[c]	0	408	40	448
20(R)-*n*-Nonylpregn-5-en-3β-ol	200	250	8	0	458
20(R)-Dodecylpregn-5-en-3β-ol	0	0	0	0	0
Cholesterol	0[c]	0	326	48	374
Control (sterol-free[b])	5	0	0	0	5

[a]Mean oogonia and oospore count with *Phytophthora cactorum* for 4 radial transects in each of 5 petri plates. Mycelia were cultured for 3 weeks in the dark on agar media at 20 °C. Confidence limits for most mean values based on a t-distribution are at the 95% confidence level. Sterols were added at a level of 10 ppm (wt/vol.) dissolved in 2 ppm of warm ethanol (vol./vol.). [b]Contained traces of cholesterol. [c]Oogonia have matured into oogonia containing oospores. [d]Germinating oospores had morphologically distinct germ-tubes and germ-sporangia (no attempt was made to differentiate these structures for purposes of quantitation) analogous to those depicted in the papers by Kaosiri, T., Zentmyer, G.A., and Erwin, D.A., Mycologia *72* (1980) *888*, and Nes, W.D., in: Biochemistry and function of isopentenoids in plants. Eds W.D. Nes, G. Fuller and L. Tsai. Marcel Dekker, New York 1982, in press.

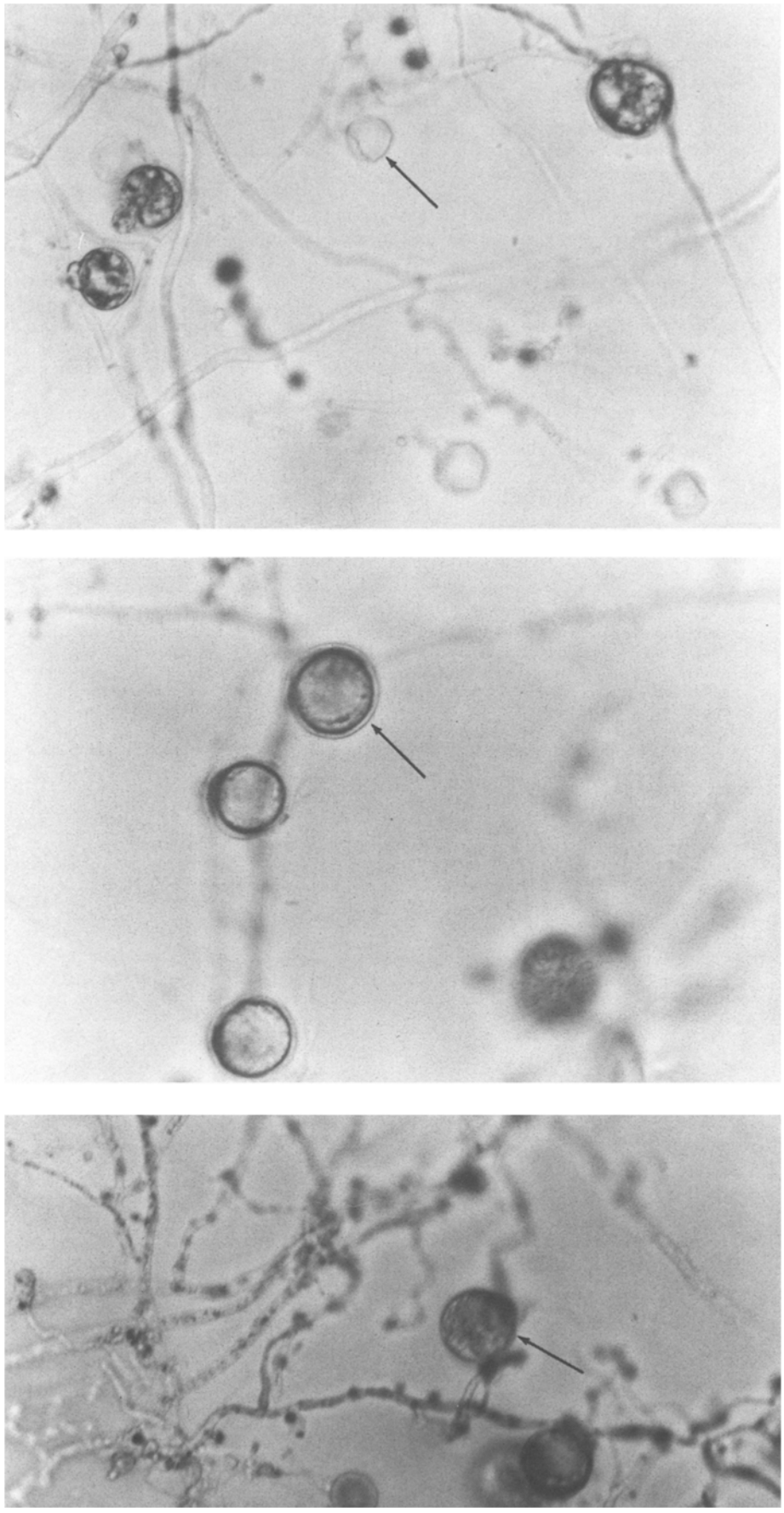


Figure 2. Phase-contrast micrographs (×20) of cultures of *P. cactorum* incubated for 21 days on chemically defined agar medium at 20 °C in the dark with 10 ppm (top) pregn-5-en-3β-ol, (middle) cholesterol, or (bottom) 20(R)-*n*-nonylpregn-5-en-3β-ol. Single-walled cells are oogonia (bottom). Hollow-'cells' are aborted oogonia (top). Double-walled cells are oospores (middle).

ed after the other observations were made. In each case the sterol added to the culture was found in the mycelium[9]. Peak area in GLC for the various free sterols (isolated without saponification) were similar. The amounts were near 0.01% of each sterol on a dry wt basis (wt/wt).

Although naturally occurring sterols display considerable variation in structure, there is a rather narrow distribution of chain lengths attached to C-20 (other than C-21). Only 4–6 C-atoms in a linear array are found even though the total number may be expanded by branches, as in the case of campesterol with a 5-carbon array and 2 methyl branches at C-24 and C-25. With *P. cactorum* both hyphal extension (table 1) and development of oogonia into oospores (table 2) were influenced most by sterols with or very nearly with the natural range. Thus, for hyphal extension maximal stimulation occurred with chains on C-20 of 4–6 C-atoms, and it was chains of 4–7 C-atoms which yielded germinating oospores. However, either a moderate increase (to a 9-carbon chain as in 20(R)-*n*-nonylpregn-5-3β-ol) or a decrease (to a 0-carbon chain as in pregn-5-en-3β-ol) in this distribution of chain lengths remained consistent with production of oogonia (table 2), although maturation was nearly abolished (fig. 2), since most of the oogonia aborted before becoming oospores. The striking retention of regulatory activity (induction of oogonia formation) despite drastic reduction in the size of the side chain is unusual[10] for a sterol but not unknown. For instance, as a mammalian component of the diet pregn-5-en-3β-ol acts qualitatively and quantitatively as cholesterol does to inhibit hepatic sterol synthesis[11]. Androst-5-en-3β-ol is also though somewhat less active[10].

In view of the ability of pregn-5-en-3β-ol to induce formation of oogonia, it seems unlikely that this sterol is normally converted in *P. cactorum* to a hormone with an oxygenated side chain of the sort (the oogoniols)[12] which arises in *Achlya* sp. An alternative regulatory role is for the sterol to modulate membrane structure and function by incorporation into the lipid bilayer of hyphae and spores. The size and shape of the side chain or the nature of unsaturation could then play roles of their own without the occurrence of any metabolism. If such were the case in the pythiaceous fungi, the reproductive similarity of these organisms with those in the genus *Achlya* may be a resultant of an evolutionary history which was convergent with respect to acquisition of oomycetous character. Had the organisms arisen by parallel evolution, one might also have expected the regulatory phenomena to be similar.

1 W.R.N. thanks the National Institutes of Health for support through grant No. AM-12172. We also appreciate the help of Mr A. Stafford and of Dr W. Haddon of the USDA, Berkeley, in obtaining the mass spectral and some of the chromatographic data. Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

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9 All of the sterols used in this study except cholesterol were prepared by J.M. Joseph, Ph.D. Dissertation, 1980, Drexel University. The chromatographic and mass spectral properties of the sterols reisolated from *P. cactorum* were similar to those reported by Joseph. See also Nes, W.R., Joseph, J.M., Landrey, J.R., and Conner, R.L., J. biol. Chem. *255* (1980) 11815. Procedures for isolation and quantitation are to be found in Nes, W.D., Sanders, G.A., and Heftmann, E., Lipids *16* (1981) 744. The control cultures were found to contain small amounts of cholesterol by GLC and GLC-MS as was the Difco agar (derived from red algae which synthesize cholesterol). Thus, the occurrence of a few oogonia in the control is presumably due to the remnants of cholesterol left in the agar after its industrial processing.
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Comparison of circulating lipoprotein lipase activity in Zucker *fa/fa* and *Fa/–*rats

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Summary. Lipoprotein lipase activity was determined in Zucker rats by assaying VLDL radioactivity. Animals were i.v. injected with $^3H_2$-oleic acid and ^{14}C-glycerol with or without Triton WR 1339. This enzymatic activity was higher in *fa/fa* rats than in non-obese *Fa/–* rats.

It is relatively well established[1–5] that hyperlipoproteinemia of the *fa/fa* Zucker rat is due to an increased synthesis of very low density lipoproteins (VLDL)[6]. An eventual decrease of lipid clearance, however, has not been proven. It is known[7,8] that fat cell lipoprotein lipase activity of the *fa/fa* rat is higher than that of the *Fa/–* rat.

We have studied the total activity of circulating lipases with Triton WR 1339 which has the property of inhibiting these lipases[9,10]. VLDL catabolism is suppressed by the action of Triton and lipoproteins accumulate in the circulatory system[11–13]. The quantity of VLDL formed can be determined by the use of radioactive precursors (^{14}C-1-glycerol and $^3H_2$-9,10- oleic acid). The radioactivity of circulating VLDL in *fa/fa* Zucker rats can be compared with that of the *Fa/–*