

Mammalian hormones in plants and their roles in plant-peronosporomycete interactions

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ABSTRACT

The major mammalian sex hormones such as 17β -estradiol, androsterone, testosterone, estrone and progesterone, and non-steroidal estrogenic mimics are wide spread in the plant kingdom. Biosynthetic pathways of these hormonal substances in plants are partially known. Although the steroidal sex hormones have shown to enhance growth and reproduction of some plants *in vitro*, their roles in plant physiology is still illusive. Some of these compounds increase abiotic stress tolerance in plants and affect the activities of microorganisms including the peronosporomycete phytopathogens. The peronosporomycetes are fungus-like microorganisms, which are devastating pathogens of plants, animals, fishes, and humans. Mammalian estrogens and other estrogenic compounds display repellent activity against zoospores of the phytopathogenic peronosporomycete, *Aphanomyces cochlioides*. The estrogenic activities of known sex hormones and their repellent activity toward zoospores are likely to be correlated. Some peronosporomycetes also produce characteristic steroidal hormones for their mating for sexual reproduction. This report comprehensively reviews current updates on the occurrence of mammalian sex hormones in plants and discusses their roles in plants and plant-peronosporomycete interactions. Discovery of mating hormones and their functions in sexual reproduction of the peronosporomycetes are also described.

KEYWORDS: sex hormones, 17β -estradiol, testosterone, zoospore chemotaxis, abiotic stress tolerance

1. Introduction

Mammalian sex hormones are structurally steroidal compounds, which play a major role in controlling the key processes of development and reproduction of mammals. They are also engaged in the mineral and protein metabolism. The occurrence of major mammalian sex hormones such as 17β -estradiol, androsterone, testosterone, estrone and progesterone is wide spread in the plant kingdom [1, 2, 3, 4, 5]. Enzymes responsible for their biosynthesis and conversion have been found in plants. The evidence for the presence of estrogen receptor in plants has also been reported [4, 6]. *In vitro* applications of some of these sex hormones have been found to enhance growth [7, 8] and stress tolerance in plants [9, 10, 11]. For example, treatment of plants with sex hormones or their precursors influence plant development, cell division, root and shoot growth, embryo growth, flowering, pollen tube growth, callus proliferation and also enhance tolerance to environmental stresses [5, 7, 8, 10, 11]. The occurrence of mammalian sex hormones and their physiological role in plants have been reviewed by Janeczko and Skoczowski [8]. The metabolic pathways of these hormones have partly been revealed. However, the functions of these sex hormones in plant cells are still illusive.

The peronosporomycetes are fungus-like microorganisms belonging to the kingdom of

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Stramenopila [12]. They are devastating pathogens of plants, fishes, animals, and even humans. Diseases caused by these class of microorganisms result in huge economic losses worldwide [13, 14, 15]. The most notable one is the late blight pathogen of potato, *Phytophthora infestans*, which caused the Great Irish potato famine in mid nineteenth century. In the peronosporomycete *Pythium periplocum* Drechs., 17 β -estradiol prevented sexual reproduction at the concentration of 3.3×10^{-6} M [16]. Islam *et al.* demonstrated that mammalian sex hormones and other non-steroidal estrogenic compounds show repellent activity toward zoospores of the damping-off pathogen of spinach and sugar beet, *Aphanomyces cochlioides* [14]. Interestingly, members of the family Pythiaceae such as *Phytophthora*, *Pythium* etc. which are devastating pathogens of plants cannot produce sterols and hence they sequester these essential compounds from the victim plant hosts [17, 18, 19]. However, some of the peronosporomycete microorganisms also produce steroidal mating hormones for sexual reproduction and thus they are difficult to control by using chemical pesticides [20, 21, 22, 23, 24, 25]. Interestingly, a new steroidal metabolite, terresterol, has recently been isolated from *Saprolegnia terrestris*, which showed immunomodulating activity against bacterial infection [26]. Understanding the roles of sterols and steroids on the biology of peronosporomycetes is needed for the biorational management of the notorious pathogenic species of this unique class of microorganisms.

Although the occurrence of steroidal sex hormones in plants and their *in vitro* effects on plant growth, stress tolerance of plants as well as their effects on environmental microorganisms including the peronosporomycete phytopathogens have been the subjects of many reports, no comprehensive review has so far been published on the occurrence of steroidal and non-steroidal hormonal substances in plants and their activities in plant-peronosporomycete interactions. This report reviews current updates on the occurrence of mammalian sex hormones in plants and their roles in plant-microbe interactions with special reference to plant-peronosporomycete interactions. The chemistry as well as the biological activities of the steroidal sex hormones in peronosporomycetes are also briefly discussed.

2. Occurrence of mammalian hormones in plants

Mammalian sex hormones such as estrogens, androgens and progesterone belong to steroids, a group of compounds derived from cholesterol, which have a basic sterane carbon skeleton. Cholesterol (**1**) and β -sitosterol (**2**) are ubiquitous in plants. In mammals, the steroid sex hormones play a key role in controlling the process of development and reproduction. These compounds are also engaged in the mineral and protein metabolism in mammals. The major mammalian sex hormones (both androgens and estrogens) such as androstenedione (**3**), androsterone (**4**), testosterone (**5**), 17 α -estradiol (**6**), 17 β -estradiol (**7**), estrone (**8**), estriol (**9**), progesterone (**10**), and pregnenolone (**11**) have been found in many higher plants (Table 1 and Fig. 1) [1-4, 27, 28, 29]. Treatment of plants with sex hormones or their precursors influence plant development, cell division, growth of root, shoot, embryo and pollen tube, flowering and callus proliferation [8, 30]. The occurrence of mammalian sex hormones and their physiological role in plants have been reviewed [8]. Several lines of evidence suggest that these compounds are biosynthesized in plants, and the enzymes responsible for their biosynthesis and conversion were also found in plants. The presence of estrogen receptor in plants has also been found [4, 6]. However, the significance of the occurrence of mammalian sex hormones in plants is still unknown. The metabolic pathways of these hormones in plants have only partly been revealed.

Changes in the concentrations of mammalian sex hormones during physiological processes in plants, or changes in their activity when exogenously applied to plants have been observed by many researchers. But how these steroids are biosynthesized, transported and eventually converted to other metabolites in plants, is an intriguing question. The biosynthesis of steroidal hormones and their conversion to other metabolites in plants have been confirmed by several radio-labeling experiments. Biosynthesis of 17 β -estradiol (**7**) from 2-¹⁴C mevalonic acid was observed in *Phaseolus vulgaris* seedlings [31]. The same authors demonstrated in *P. vulgaris*, interconversion between estrone and 17 β -estradiol (**7**) and the

Table 1. List of mammalian sex hormones found in plants.

Compound	Plant source	Plant organ	Concentration [µg/kg or l]	Reference
17β-estradiol	<i>Phaseolus vulgaris</i>	seed & leaf	100.0	[42]
Estrone	<i>Punica granatum</i> var. <i>nana</i>	seed	4.0	[43]
		root	4.5	
		flower	2.5	
	<i>Phoenix dactylifera</i>	seed	400.0	[44]
		pollen	3300.0	
	<i>Hypaene thebaica</i>	kernel	5130.0-5250.0	[45]
	<i>Zea mays</i>	oil	40.0	[46]
	<i>Olea europa</i>	oil	90.0	
<i>O. europa</i>	kernel	33.75		
<i>Malus pumila</i>	seeds	100.0	[27]	
Estrone ester	<i>Olea europa</i>	oil	90.0	[46]
Estriol	<i>Salix</i> sp.	flower	110.0	[46]
Testosterone	<i>Pinus sylvestris</i>	pollen	80.0	[47]
	<i>P. nigra</i>	pollen	70.0	[48]
Epitestosterone	<i>P. sylvestris</i>	pollen	110.0	[47]
Androsterone	<i>P. nigra</i>	pollen	22.0	[49]
	<i>Triticum aestivum</i>	leaf	140-220	[3]
	<i>Monstera deliciosa</i>	leaf	8400	[3]
	<i>Prunus virginiana</i>	leaf	1300	[3]
	<i>Brassica campestris</i>	leaf	250	[3]
	<i>Crassula arborescens</i>	leaf	1300	[3]
	<i>Daucus carota</i>	stem	310	[3]
	<i>Thlaspi arvense</i>	shoot	190	[3]
Androstenedione	<i>P. nigra</i>	pollen	90.0	[49]
	<i>P. nigra</i>	pollen	80.0	[48]
	<i>P. sylvestris</i>	pollen	590.0	[47]
Androstenone	<i>Pastinaca sativa</i>	root	5.5-11.4	[50]
Progesterone	<i>Pinus nigra</i>	pollen	80.0	[48]
	<i>Hordeum vulgare</i>	leaf	31.0	[3]
	<i>Zea mays</i>	leaf	280.0	[3]
	<i>Crassula arborescens</i>	leaf	130.0	[3]
	<i>Monstera deliciosa</i>	leaf	84.0	[3]
	<i>Urtica dioica</i>	shoot	79.0	[3]
	<i>Thlaspi arvense</i>	shoot	76.0	[3]

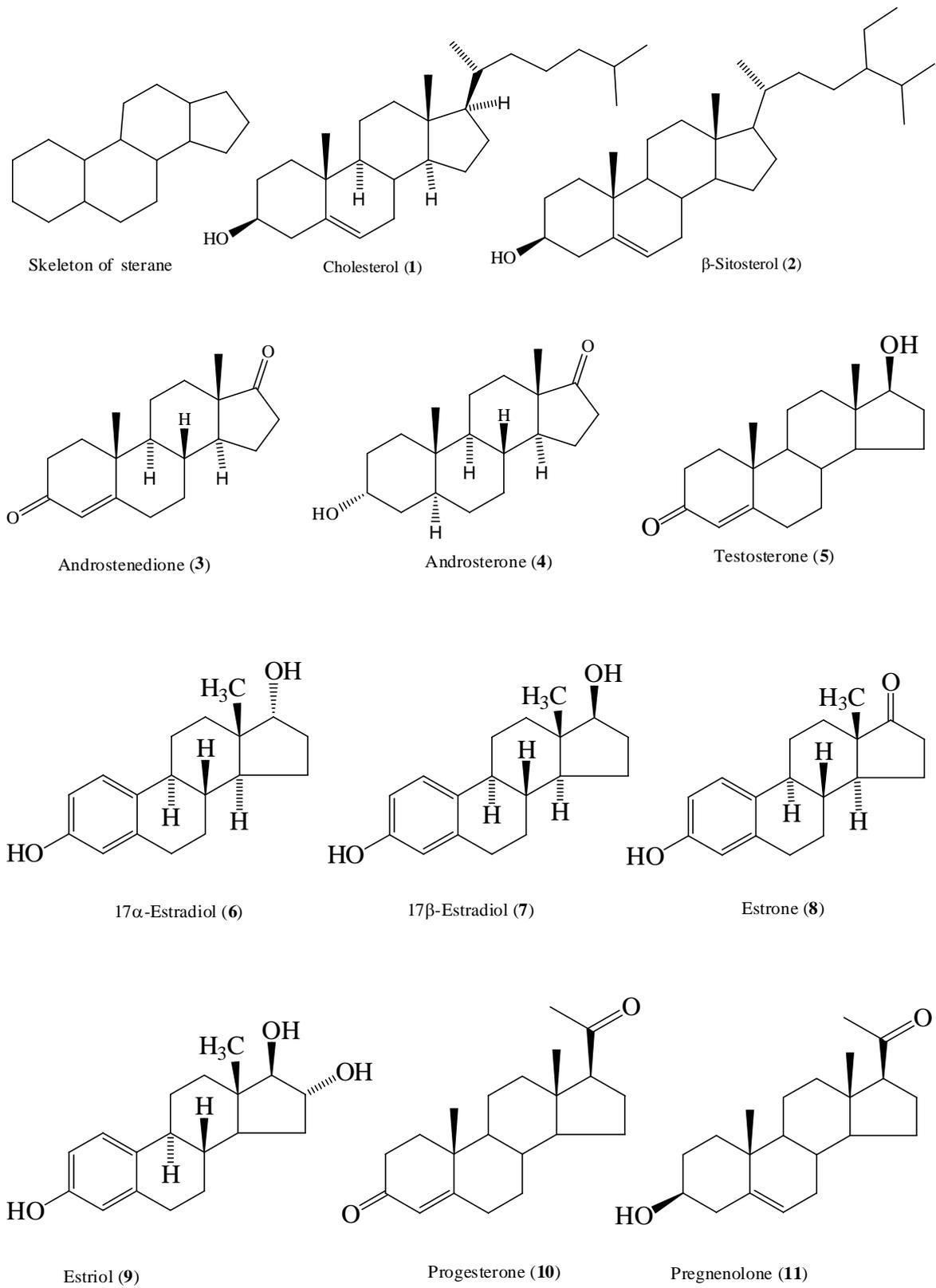


Fig. 1. Structures of mammalian sterols and steroid sex hormones found in plants.

ability to hydrolyze estrone sulphate to free estrone [32]. Enzymes capable of converting estrone to estradiol are present or inducible in leaf tissue [31, 32]. In *Pisum sativum* seedlings, radioactive 4-androstene-3,17-dione [$4\text{-}^{14}\text{C}$] when added to leaves was reduced to testosterone [33]. In cultured cells of *Nicotiana tabacum*, testosterone was transformed to Δ 4-androstene-3,17-dione, 5 α -androstane-17 β -ol-3-one and 5 α -androstane-3 β ,17 β -diol [34]. *Cucumis sativus* L. plant transformed 4-androstene-3,17-dione [$4\text{-}^{14}\text{C}$] to testosterone and to other androstane derivatives [33]. Androstane derivatives were found in plants as free steroids and also as conjugated forms such as fatty acid esters and diesters. In the process of conversion, enzymes from the group of reductases could also be involved.

The conversions of female hormone, progesterone, are relatively well known. Leaf homogenate of *Cheiranthus cheiri* L., however, converted 20 α -hydroxycholesterol-7-3H to pregnenolone (**11**) and progesterone (**10**) [35]. Progesterone was modified to different derivatives of pregnane by suspension cultures of *Dioscorea deltoidea* Wall. and *Digitalis purpurea* L. [36, 37]. Furuya *et al.* proposed the scheme of biotransformation of progesterone in *Digitalis purpurea* L. callus culture and suggested that 20 α -hydroxysteroid dehydrogenase may be involved in this process [37]. It is well known that progesterone is an intermediate in cardenolide pathways and is present in plants producing cardenolides such as the aforementioned *Digitalis purpurea* L. and *Cheiranthus cheiri* L. The enzymes participating in the conversions of compounds along this pathway were described by Seitz and Gärtner [38], Lindemann and Luckner [39] and Finsterbusch *et al.* [40]. In mammals, hydroxysteroid dehydrogenase- and steroid 5 α -reductase-type enzymes participate in the biosynthesis and control of steroid hormone activity, including the activity of sex hormones. Hence, similar enzymes were searched in plants. It seems interesting that in brassinosteroid-deficient *Arabidopsis* plants lacking steroid 5 α -reductase activity, the introduction of the human type of steroid 5 α -reductase, enzyme restored normal plant growth without exogenous brassinosteroid application [41]. The plant steroidal hormone, brassinosteroid also shows diverse activities on

various organisms such as insects, fungi, fishes and protozoa at concentration comparable with those for plants.

The molecular mechanism of the activity of mammalian sex hormones in plants has not been elucidated precisely and thus requires further investigations. There are two papers reporting on the presence of estrogen receptors in plant cells, which may be involved in steroid actions. The endogenous receptor for 17 β -estradiol (**7**) has been found in the ovules of *Gladiolus primulinus* Bak. in 1984 by Janik and Adler [6]. According to these investigators, the receptor passed from cytoplasm to the nucleus upon activation by 17 β -estradiol (**7**), estriol (**9**) and diethylstilbestrol (**12**) (Figs. 1 and 2). Recently, Milanesi *et al.* proved the presence of estrogen receptors in the callus of *Solanum glaucophyllum* Desf. [4]. The authors also found the proteins, which gave positive response as estrogen binders in ligand blot experiments when 17 β -estradiol (**7**) derivatives were used as ligands.

In addition to the steroidal sex hormones, several plant secondary metabolites such as flavonoids [mainly isoflavonoids such as formononetin (**13**), equol (**14**), medicarpin (**15**), genistein, diadzein, naringenin (**16**), 3'-prenylated naringenin (**17**), 8-prenylated naringenin (**18**) and 6-prenylated naringenin (**19**)] and lignans, and some environmental pollutants or endocrine disruptors such as bisphenol A (**20**) and diethylstilbestrol (**12**) mimic estrogenic activity in higher animals (Fig. 2). Plant products having estrogenic activity are known as phytoestrogens. Phytoestrogens such as diadzein and genistein from soybean have economic importance as therapeutic foods. Biological activity of phytoestrogens in mammalian systems has been a subject of many investigations. Endocrine disruptors in the environment pose a serious threat to the health of human and animals. The environmental compounds showing estrogenic mimic are known as xenoestrogens. All these hormonal mimics are known to bind to the receptor as do the natural mammalian sex hormones. The role of plant secondary metabolites in the symbiotic and antagonistic relationship between plants and other animals are well established. However, our knowledge on the role of plant steroidal sex hormones in the symbiotic

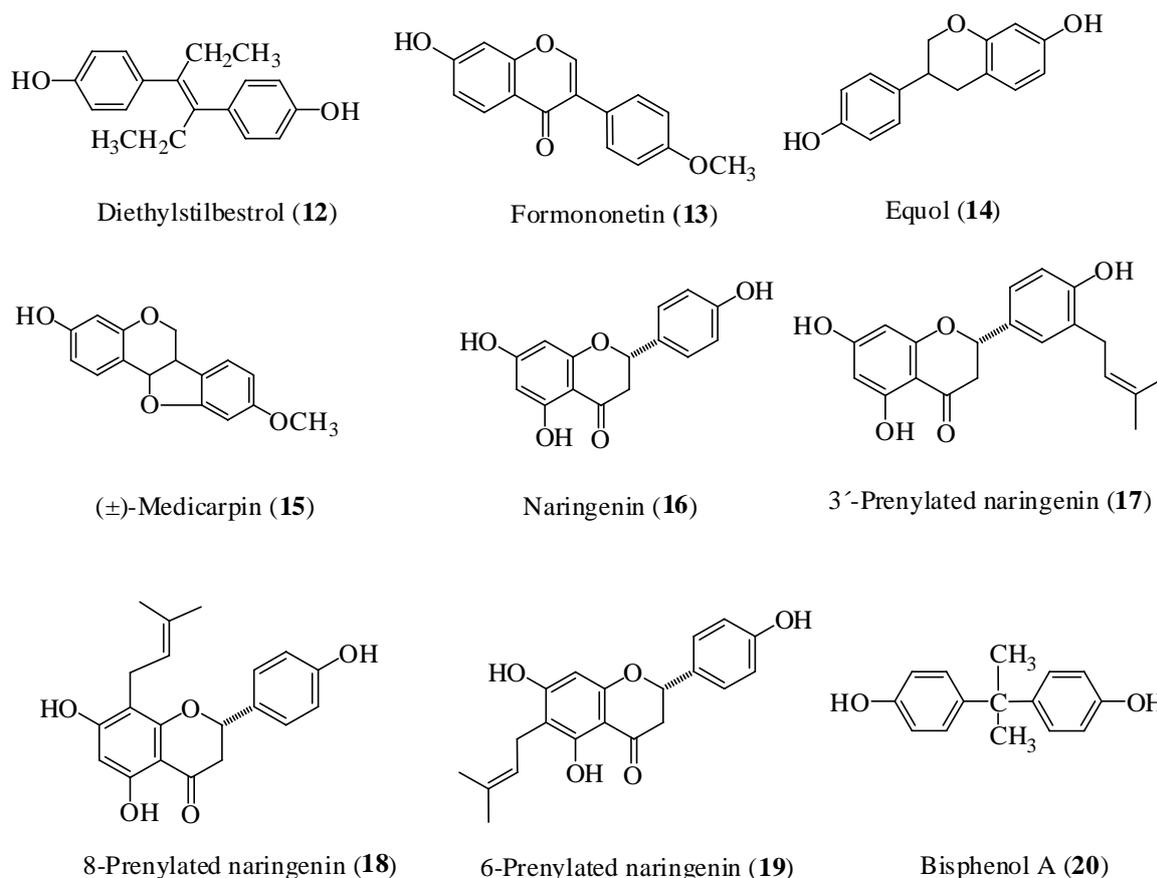


Fig. 2. Structures of phytoestrogen and xenoestrogens that show repellent activity towards *Aphaomyces cochlioides* zoospores.

or antagonistic interactions between plants and microorganisms is limited.

3. Activities of mammalian sex hormones in plant growth and tolerance to stresses

The presence of mammalian sex hormones of both natural and anthropogenic origins is widespread in the environment. Production of these compounds by plants is also wide spread. 17β -estradiol (7) improves embryo growth and alleviates cadmium and copper toxicity during germination of lentil (*Lens culinaris* Medik) seeds [10]. Addition of 1×10^{-6} M estradiol in the germination media greatly reverses the inhibitory effect of heavy metal stress on post-germination events. The cotyledons of estradiol-treated seeds also tended to i) retain higher protease and amylase activities, ii) breakdown more storage compounds (albumin, globulin and starch), and iii) have higher contents

of free amino-acids and glucose than those of untreated controls. Further investigation reveals that heavy metal-induced oxidative damage is reduced by 17β -estradiol application in lentil seedlings [11]. Application of mammalian sex hormones also alleviates salt-stress in wheat seedlings [9]. Similarly, exogenous progesterone application also showed protection of chick pea seedlings against chilling-induced oxidative stress [51]. In another study, Su *et al.* observed that exogenous supply of progesterone alleviates heat and high light stress-induced inactivation of photosystem II in wheat by enhancing antioxidant defense and D1 protein stability [52]. The occurrence and activity of progesterone in the plant kingdom has recently been reviewed [5].

Steroids occur in living organisms as one of the most essential groups of compounds. Continuous research has led to new discoveries and the

revision of existing information concerning the occurrence and the role of steroids, both in animals and plants. Progesterone has been found to moderate abiotic and biotic stresses in *Arabidopsis thaliana* including the infection caused by *Pseudomonas syringae* or *P. fluorescens* [53]. More surprisingly, androstenedione stimulates photosynthesis in drought-stressed soybean [5]. Exogenous progesterone and its cellular binding sites in wheat exposed to drought stress has also been investigated [54]. It is suggested that progesterone involves in drought stress response of wheat. 24-Epibrassinolide and 20-hydroxyecdysone affect photosynthesis differently in maize and spinach [55].

4. *In vitro* bioactivity of mammalian sex hormones and other hormonal substances

Both growth inhibitory and growth promoting effects of steroidal hormones have been observed [56]. But more interestingly, testosterone and estradiol have been found to have sex hormone activity on yeast [57]. An insect-repellent steroid was isolated from the Peruvian weed *Nicandra physalodes* [58]. Antheridiol has been identified as the chemotactic hormone of the water mould, *Achlya bisexualis* (Saprolegniaceae) [20]. The chemistry and biological activities of hormones in sexual reproduction of *Achlya* are discussed later in a separate section of this review. Heftmann *et al.* observed that a structurally related sterol of antheridiol, 5 α -stigmast-22-en-3 β -ol, which is produced by *Dictyostelium discoideum*, triggers the remarkable differentiation this slime mold undergoes [59]. 17 β -estradiol was found to stimulate hyphal growth of endomycorrhizal fungus *Glomus intraradices* [60].

Unlike in plants, Hendrix observed that sterols of fungal, plant and animal origins can regulate sexual reproduction in *Pythium periplocum* and *Phytophthora megasperma* var. *sojae* [61]. In the same study, he found that sterols specifically induced the formation of large zoosporengia in *Phytophthora parasitica*. Some sterols, especially cholesterol, stimulated the growth of peronosporomycetes. However, 17 β -estradiol prevented cholesterol-induced sexual reproduction in *Pythium periplocum*. Inhibition by estradiol was partial at 1×10^{-6} M and complete at 1×10^{-5} M,

the same concentrations at which cholesterol and β -sitosterol were initially and maximally active. Higher concentrations of estradiol were required for growth inhibition than for inhibition of reproduction [16]. The activity of estradiol in inhibiting sexual reproduction of *P. periplocum* at micromolar concentration also suggests that mammalian sex hormones may somehow function on trivial microorganisms. A further study including several other classes of microorganisms such as fungi should elucidate whether this function is general or only limited to specific species.

Steroidal sex hormones can also affect the metabolism, growth, or virulence of pathogenic bacteria [62]. In turn, pathogenic microbiota, and environmental bacteria are able to metabolize and degrade steroid hormones and their related compounds. The role of steroid sex hormones in bacterial-host interactions has recently been reviewed [62].

5. Mammalian sex hormones in the desert weeds of Saudi Arabia and their putative bioactivity

Estrogenic activity of weeds and their effects on grazing animals have been described in many literatures. Reproductive disturbances in animals grazing certain pastures were first observed in Australia in connection with the increased use of subterranean clover (*Trifolium subterranean*) as a sheep pasture [10]. The presence of formononetin and medicarpin in the clover and their effects on the reduction of lambing percentage in ewes in Australia was a striking example of plant-animal (vertebrate) interaction via secondary metabolites. Australian research concerning infertility in sheep associated with estrogens in forage crops has been reviewed by Moule [63]. To see the presence of sex hormones in plants, Janeczko and Skoczowski investigated 128 species from over 50 families. They observed that androsterone and progesterone were present in more than 80% of the investigated species, androgens (testosterone and dihydro-testosterone) in 70% of species and estrogens (estrone and 17 β -estradiol) in 50% of species. The content of total estrogens and 17 β -estradiol in the pollen and in the style of *Ginkgo biloba* L., *Zea mays* L. and *Brassica campestris* L. were estimated by Zhang *et al.* [64]. 17 β -estradiol

contents in the pollen of these plants were in the range of 8-35 pg/g DM. In the style of *Lilium davidi* Duch., 17 β -estradiol was found in the concentration range of 24-40 pg/g DM. Moreover, the above-mentioned authors showed changes in total estrogen and 17 β -estradiol concentrations during flower development. Strain differences in plants must also be considered and may well explain some of the different estrogenic effects of these plants [65].

Recently, Zeitoun and Alsoqeer demonstrated that alfalfa and 17 other natural rangeland native weeds in the desert of central Saudi Arabia possesses significant but highly varying concentration of three steroidal mammalian sex hormones, testosterone, 17 β -estradiol and progesterone [66]. Using enzyme immunoassay kits, they determined the hormone concentration in the oven-dried samples of weeds. Among the tested plants, the highest testosterone concentrations were recorded in *Cakile Arabica* (3.69 ng/g DM) followed by *Cyperus conglomerates* (2.97 ng/g DM). However, six of the other tested species, i.e. *Plantago boissieri*, *Rhanterium epapposum*, *Halexylon salicornicum*, *Heliotropium bacciferum*, *Cenchrus ciliaris* and *Medicago sativa* did not give any detected levels of testosterone. On the other hand, higher levels of 17 β -estradiol were found in *Lactuca serriola* (378.89 pg/g DM), *Eruca sativa* (246.89 pg/g DM) and *H. bacciferum* (229.29 pg/g DM), while no trace of 17 β -estradiol was recorded in *Leptadenia pyrotechnica*, *R. epapposum* and *Stipagrostis pulmosa*. Higher levels of progesterone were found in *L. serriola* (53.96 ng/g DM), *Neurada procumpens* (51.15 ng/g DM), *E. sativa* (51.15 ng/g DM), *Atriplex leucoclada* (46.93 ng/g DM) and *C. arabica* (44.13 ng/g DM). However, weeds, *P. boissieri* (17.43 ng/g DM) and *R. epapposum* (19.82 ng/g DM) gave lower concentrations of progesterone. It was predicted that due to the presence of these mammalian sex hormones in the grasses of deserts, some she camels in Saudi Arabia suffer from cystic ovarian syndrome and delayed pregnancy. In fact, excess external ingestion of estradiol might cause a block on the hypothalamopituitary-ovarian axis, resulting in the impediment for the release of luteinizing hormone in animals with ultimate ovarian follicular cyst [67]. These findings suggest that the presence of mammalian sex hormones in

plants including grasses, and their interactions with herbivores and associated microorganisms seem wide spread.

6. Activities of estrogenic compounds on Peronosporomycete zoospores

One of the remarkable biological activities of isoflavonoidal compounds has been recognized as an estrogenic one. Interestingly, medicarpin (a pterocarpan) isolated from a non-host plant extract exhibited repellent activity toward the zoospores of a damping-off pathogen, *Aphanomyces cochlioides* (a Peronosporomycete). Following this observation, Islam and Tahara tested the effects of a number of xenoestrogens, mammalian estrogens and androgens, and some estrogenic mimics such as bisphenol A, diethylstilbestrol (DES), 17 α -estradiol, 17 β -estradiol, estrone, estriol, dienestrol, pregnenolone, progesterone, testosterone, and mono- and di-*O*-methyl ether of DES on the motility behavior of *A. cochlioides* zoospores [68]. In addition, some flavonoidal estrogenic compounds such as miroestrol, formononetin, naringenin, and three prenylated naringenins were also included in that study [68]. Most of the estrogenic and androgenic substances exhibited a repellent activity against the motility of the zoospores, whereas, progesterone and the natural mimic miroestrol were practically inactive at 1000 μ g/ml. The highest activity was recorded in DES, 17 β -estradiol and estriol (active at 0.5 μ g/ml), followed by 17 α -estradiol, estrone, dienestrol (active at 1.0 μ g/ml), testosterone (active at 50 μ g/ml) and pregnenolone (active at 100 μ g/ml).

Most of the active estrogenic compounds except pregnenolone showed higher repellent activity than that of the androgen, testosterone. The xenoestrogen, bisphenol A showed clear repellent activity at 5 μ g/ml under the same bioassay condition (particle method). Interestingly, the estrogenic and repellent activities of known estrogenic compounds revealed to be correlated. The receptor for estrogenic repellents seemed not to be affected directly by the attractants (e.g. cochliophilin A), because the repellent activity of estrogens was observed in the zoospores suspended in the homogenous solution of host-specific cochliophilin A.

In the zoospore bioassay, the estrogenic and repellent activities of known estrogenic compounds revealed to be correlated. The particle bioassay method is very simple and convenient for testing the motility behavior of fungal zoospores. Thus, this bioassay system seems to be useful for pre-screening the detection of estrogenic activity of naturally occurring compounds. A further investigation to evaluate the usefulness of this repulsion test for bioassay-guided isolation of similar structures of phytoestrogenic compounds from plants and other natural sources is needed. Furthermore, high repellent activity of mammalian sex hormones towards fungal zoospores may be biologically important because such high negative chemotaxis was not yet reported for any zoosporic fungi. It may be important for biorational control of zoosporic peronosporomycetes and/or in studying the molecular basis of chemoresponses of zoosporic phytopathogens.

Phytoestrogen can sometimes be involved in plant growth and development including the sexual expression in plants [27, 28], however, the negative chemotaxis toward Peronosporomycete zoospores has not been described. Chemotaxis of zoospores has been reported in many papers [69, 70, 71, 72, 73, 74, 75] but, reports on negative chemotaxis are very few [76, 77]. Previously, monoacylated phosphatidic acid derivatives containing at least one hydroxyl group at the phosphoryl unit showed repellent activity against *A. cochlioides* zoospores [76]. (\pm)-Medicarpin, an isoflavonoid isolated from *Dalbergia odorifera*, also exhibited repellent activity against the zoospores of *A. cochlioides* [77]. Furthermore, 8-prenylated naringenin showed repellent activity toward zoospores at 1 $\mu\text{g/ml}$. Interestingly, this compound also had estrogenic activity [78]. The mechanism of high repellent activity by mammalian sex hormonal substances on peronosporomycete zoospores is difficult to explain based on the current knowledge. Negative chemotaxis by mammalian sex hormonal substances, most of which are found in plants, raises a question whether the occurrence of this phenomenon is beneficial for peronosporomycete zoospores from a view point of ecology, particularly in host-parasite interaction, and a speculation that minor constituents of phytoestrogens may contribute to defend non-host plants against pathogens.

The structural requirements for repellent activity of steroidal hormones and estrogenic mimics toward trivial zoospores have been investigated using a number of synthetic hormones and their derivatives [68]. Bioassays revealed that the steroidal compounds with an aromatized A ring possess higher bioactivity toward *A. cochlioides* zoospores except 3-*O*-benzoyl-17 β -estradiol. The acetylation and methylation of 17 β -estradiol and acetylation of DES seemed to slightly decrease the repellent activity. Interestingly, mono- and di-*O*-methylated products of DES exhibited a completely opposite biological activity (Table 2). In case of the benzoate group at C-3-OH in 17 β -estradiol, a zero activity was observed at 1000 $\mu\text{g/ml}$ whereas acetylation at the same position showed a little lower repellent activity than the mother compound. Acetylation of the hydroxyl group at C-17 did not affect the repellent activity much indicating that the free hydroxyl group at C-3 is more significant than that at C-17. Testosterone and pregnenolone showed lower activity than the estrogenic compounds having aromatization in the A ring of their steroidal skeleton.

In animals, the sex hormones are formed from progesterone by successive oxidation steps, both at C-17 and at C-19. The oxidative removal of the side chain leads to the C-19 series with androgenic activity, and the oxidative removal of the angular methyl group at C-10 and aromatization of A ring leads to the C-18 series with estrogenic activity [80]. With respect to the structure-activity relationship, it appears that aromatization of A ring together with a free hydroxyl group at C-3 position is necessary for higher repellent activity of estrogenic compounds. Other substituents at the same position (C-3) decreased the bioactivity, which is related to the size of the substituent. Surprisingly, methylation of diethylstilbestrol (DES) exhibited completely different activity *i.e.* both mono- and di-*O*-methyl ethers of DES showed attractant activity. Moreover, the attracted zoospores were found to be encysted and then germinated in the presence of di-*O*-methyl ether of DES. This compound may act through a specific receptor because it was found to be active when host-specific attractants, cochliophilin A and *N-trans*-feruloyl-4-*O*-methyl dopamine, or

Table 2. Bioactivity of some mammalian sex hormonal substances and their derivatives towards motility behavior of *Aphanomyces cochlioides* zoospores (adapted from [79]).

Name of estrogenic compounds and their derivatives	Observed activity of estrogenic compounds and their derivatives ($\mu\text{g/ml}$)*						
	100	50	10	5	1	0.5	0.1
Diethylstilbestrol (DES) (12)	---	---	--	--	-	-	\pm
17 α -Estradiol (6)	---	---	--	--	-	\pm	na
17 β -Estradiol (7)	---	---	-	--	-	-	\pm
Estrone (8)	---	---	--	--	-	\pm	na
Estriol (9)	---	---	--	--	-	-	\pm
Dienestrol	---	---	--	-	-	\pm	na
Bisphenol A (20)	---	--	--	-	-	na	nt
Testosterone (5)	--	-	na	nt	nt	nt	nt
Pregnenolone (11)	-	\pm	na	nt	nt	nt	nt
Estradiol benzoate	na	nt	nt	nt	nt	nt	nt
Progesterone (11)	na	nt	nt	nt	nt	nt	nt
PRE-4**	na	nt	nt	nt	nt	nt	nt
PRE-5	na	nt	nt	nt	nt	nt	nt
TES-1***	na	nt	nt	nt	nt	nt	nt
Miroestrol	na	nt	nt	nt	nt	nt	nt
Formononetin (13)	++/ss	+/s	na	na	nt	nt	nt
DES diacetate	---	-	\pm	na	na	nt	nt
Estradiol diacetate	--	-	-	\pm	na	na	nt
3-O-Methyl estradiol	-	-	\pm	na	na	nt	nt
DES Dimethyl ether	+++ & e	+++ & e	++ & e	+ & s	+ & s	\pm	na
DES Monomethyl ether	+++/sss	++/ss	+/s	s	\pm	na	na
Estradiol 3-acetate	---	---	--	-	\pm	na	na
Estradiol 17-acetate	---	---	--	-	-	\pm	na

Note: *Particle method; na = non-active; nt = not tested; - = repellent; + = attractant; e = encysting; s = stimulant; \pm = activity is not clear well.

**PRE-4 (70) and PRE-5 (71) are two metabolites of 61 (Farooq and Tahara, 2000).

***TES-1 (69) is a metabolite of 60 (Farooq and Tahara, 2000).

other estrogenic compounds were administered homogeneously into the motile zoospore suspension. Similarly, 8-prenylated naringenin, which is known as a potent phytoestrogen [78], also exhibited equivalent repellent activity (active at 1 $\mu\text{g/ml}$) as shown by 17 β -estradiol, whereas, 6-prenylated naringenin displayed attractant activity

at 10 $\mu\text{g/ml}$. Both naringenin and 3'-prenylated naringenin exhibited repellent activity at 10 $\mu\text{g/ml}$ concentrations, whereas, another natural estrogenic mimic, formononetin had attractant/stimulant activity (50 $\mu\text{g/ml}$). It may be interesting for further investigation to assess how minor modifications in the structure of DES or prenylated-naringenin

completely change the property of biological activity.

7. Steroid hormone-mediated morphogenesis in Peronosporomycete *Achlya* species

Chemical communication is central to evolutionary history. Many types of chemical signals including pheromones/hormones, bioregulators, receptors, transducers, elicitors, effectors and second messengers are almost universally present [81]. It has been known that certain unicellular organisms biosynthesize steroid hormones which are essential for their communication with partners and for sexual reproduction. The role of steroid hormones in sexual morphogenesis of the eukaryotic microorganisms has been subjects of many investigations. Examples include *Achlya ambisexualis*, *Saccharomyces cerevisiae* and *Candida albicans* [20, 82, 83, 84, 85]. Recent studies revealed that these lower eukaryotes also contain steroid receptors as in higher animals.

The filamentous water moulds, *Achlya* species have been known to produce steroidal hormonal substances, which are important for their sexual reproduction. The term 'hormone' can be defined

as a signal transducing molecule which is produced and secreted in one part of an organism and act on another part of the same organism. To unravel biochemical bases of steroid hormones in *Achlya* spp., a considerable amount of research have been carried out by many investigators [20, 21, 22, 23]. Undoubtedly, the role of two diffusible steroids in the sexual differentiation of *Achlya* species was the most classic story of lipid-mediated morphogenesis in peronosporomycetes [20, 86, 87, 23, 88, 89]. In *Achlya*, steroid hormones are derived from fucosterol (**21**). The species-specific and sex-specific hormones, antheridiol (**22**) and oogoniol (**24**), initiate the development of the male and female sex organs, respectively (Fig. 3). Antheridiol is the steroid released by female cells and it induces the development and differentiation of the male sex organs or antheridia. In addition, antheridiol also induces the male to synthesize oogoniol which acts upon the female causing the development of oogonia or egg-bearing structures. Fertilization is accomplished by physical contact of the antheridia with the oogonia and is followed by the production of diploid oospores.

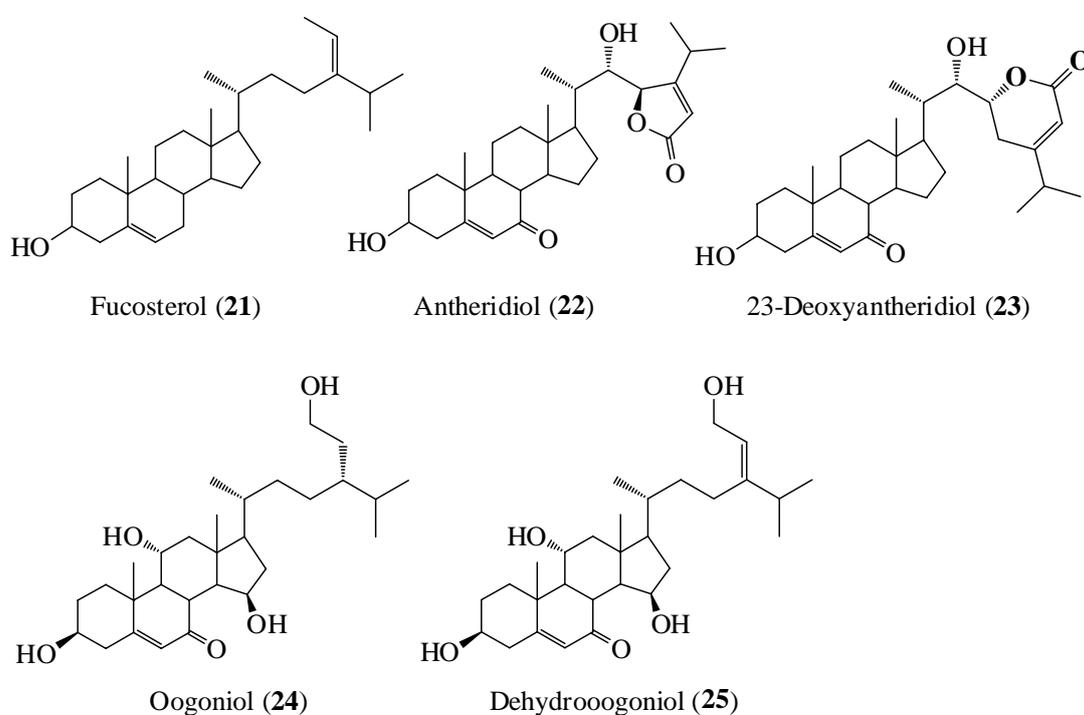


Fig. 3. Structure of fucosterol and *Achlya* hormones.

Several pioneering works have been done on the effect of these diffusible steroids on the growth and development of *Achlya*. In one of the first studies in the late 1930s, John Raper at Harvard University discovered that female hyphae of the water mould *Achlya* release a hormone that induces male hyphae to form short branches bearing male gametes [90]. To understand the detail of the phenomenon, Raper [90, 91] conducted a series of pioneering experiments using heterothallic strain of *Achlya ambisexualis*. He demonstrated that the production of hormone A, a diffusible substance, by the female hyphae induces the production of antheridia in male hyphae. On the other hand, the male hyphae are then induced to produce a second compound, hormone B, which induces oogonial formation. In other early studies, researchers found terpenoid trisporic acids in the Mucorale pin moulds. These suppressed the formation of asexual spores and triggered the production of sexual structures, through interactions between compatible partners by incomplete but complementary biosynthetic pathways.

The structure of *Achlya* hormones A and B were elucidated by several groups [92, 93]. Hormone A was subsequently named antheridiol and hormone B oogoniol. McMorris observed that actively growing female hyphae continuously secrete antheridiol, but male hyphae secrete oogoniols only in response to the presence of antheridiol when stationary phase of growth is reached [94]. Bioassays revealed that oogoniol is at least 3 orders of magnitude less sensitive than that of antheridiol [95]. The response time following exposure to dehydrooogoniol is delayed by 10 to 12 h relative to the biological response to antheridiol. Although the reason for this behavioral response is not fully understood, it is possible that the structural complexity of oogonia, not antheridial branches may be involved in such delayed response.

Chemical studies on several *Achlya* species revealed that fucosterol is quantitatively a major sterol in *Achlya* [96, 97]. The *Achlya* spp. also have cholesterol and 24-methylene cholesterol [98]. Antheridiol and structurally related 23-deoxyantheridiol (**23**) are oxygenative derivatives of fucosterol (Fig. 3). The possible biosynthetic

pathways of the steroidal compounds in *Achlya* species have been presented [99]. Chemical structures of all *Achlya* hormones and related compounds were confirmed by synthesis [100, 101, 102, 103]. Fucosterol-derived several oogoniols and dehydrooogoniols were also isolated from *Achlya heterosexuality* and their structures were also confirmed by synthesis [104]. Dehydrooogoniol induced oogonial initiation from 1.3 μM to 13 μM concentrations, which was much lower than those required for oogoniol [105]. Data on bioassays and analytical chemical techniques of these natural and synthetic hormones have been compiled [105]. Indeed, oogonium development in female strains of *Achlya* is initiated in nature by a mixture of oogoniol, dehydrooogoniol and their C-3 esters [95]. McMorris and co-workers tested the biological response of the female strain *Achlya ambisexualis* 734 to synthetic and natural dehydrooogoniol and its analogues. When tested individually, the synthetic steroid dehydrooogoniol exhibited similar biological activity as that of natural steroids. Surprisingly, synthetic oogoniol alone did not show any biological activity. However, oogoniol had a synergistic effect on the activity of dehydrooogoniol at low concentrations but inhibitory effect at high concentrations. The 7-deoxo analogue of dehydrooogoniol, a putative biosynthetic intermediate, is an inhibitor of the hormone. These *in vitro* bioassays suggest the possibility of a complex interplay of steroids in the sexual development of *Achlya* [95]. Further studies revealed that oogonial initials produce more antheridiol than do vegetative hyphae. Thus, a proper concentration gradient is created in the medium to provide the chemotropic stimulus for the antheridial hyphae to grow toward the oogonial initials, leading to further development of the antheridia and oogonia and ultimately to fertilization.

Cooper-Palomar and Powell investigated the changes in cellular calcium concentration during sexual reproduction of the heterothallic water mold, *Achlya ambisexualis* [106]. They demonstrated that an increase in Ca^{2+} concentration in the male strain was associated with antheridial branch formation, vectorial growth toward the oogonial initial and fertilization. In the female strain, they observed that development of the oogonial cytoplasm

after septal delimitation, oosphere formation, and oospore maturation correlated with calcium increase. Hynes *et al.* found that during antheridiol-mediated morphogenesis, a protein with molecular weight of 69 kDA was induced in the male strain of *A. ambisexualis* [107, 108, 109]. The induction of the male sexual organ primordia (antheridial hyphae) by the steroid hormone antheridiol in the water mold *A. ambisexualis* requires both transcription and translation [110]. Inhibition of either of these processes eliminates the expected increase in the production and release of the enzyme cellulase, which accompanies the formation of the antheridial hyphae. The steroid-binding protein from a primitive eukaryotic microbe, *Achlya* has *in vitro* biochemical properties that are similar to those of other known steroid receptors in higher organisms [111].

In the case of antheridiol, specific binding of the hormone to a protein receptor in *Achlya* cytosol has been demonstrated. The receptor is saturable and of low capacity, and partial characterization has shown it to be remarkably similar to steroid receptors in higher organisms. Antheridiol has also been shown to induce biosynthesis of certain proteins, and thus it behaves in much the same way as mammalian steroid hormones.

8. Discovery of mating hormone in *Phytophthora*

The genus *Phytophthora* also includes heterothallic species that consists of two mating types, A1 and A2 [112, 113]. Each individual can produce both male (antheridia) and female (oogonia) organs. Fertilized oogonia develop into sexual spores called oospores, which have a thick-walled survival structure. Due to sexual reproduction, the notorious phytopathogens under *Phytophthora* genus are difficult to control in the practical field. Hormonal regulation of sexual reproduction in *Phytophthora* was first proposed in 1929 [114]. However, chemical basis of that claim has not been unambiguously established until 2005. Nearly hundred years later, Qi *et al.* first characterized the mating hormone $\alpha 1$ from more than 1,830 l of culture broth of an A1 mating type *P. nicotianae* [24]. Ethyl acetate extracts of the culture broth were successively chromatographed on silica gel followed by reverse phase and high

performance liquid chromatographies to give 1.2 mg of pure hormone. The chemical structure of the hormone was elucidated as $\alpha 1$ or (MH- α) by rigorous spectroscopic analyses including one- and two-dimensional NMR. The hormone $\alpha 1$ is a diterpene, which consist of an array of 1,5-stereogenic centers.

Pure $\alpha 1$ induces oospore formation on mycelia of A2 mating type at a dose of 3 ng and the number of oospores increases dose-dependently (Fig. 4) [24] but it did not induce any oospore formation on the A1 mating type at a dose of 300 ng. This hormone equally induces oospore formation in other *Phytophthora* species (*P. capsici*, *P. cambivora* and *P. infestans*) in a similar manner and suppresses the formation of sporangia and sexual spores (asexual reproduction), indicating that $\alpha 1$ is a universal mating hormone in the heterothallic species of *Phytophthora*. The *Phytophthora* mating hormone $\alpha 1$ has an array of 1,5-stereogenic centers. Three years after the discovery of the natural hormone $\alpha 1$, Harutyunyan *et al.* synthesized 2 of the 16 possible stereoisomers of $\alpha 1$ by using a unique methodology of catalytic enantioselective conjugate addition of Grignard reagents [115]. They assigned relative and absolute configurations as well as optical rotations of two synthetic isomers. In fact, the four stereocenters in MH- $\alpha 1$ can lead to 16 possible stereoisomers. The synthetic isomers also induce the formation of viable oospores in three heterothallic *Phytophthora* species, *P. infestans*, *P. capsici*, and *P. nicotianae* (Fig. 4). The vegetative growth of *Phytophthora* was also accelerated by the hormone. However, the doses required for oospore formation was much (27-fold) higher than that of natural hormone $\alpha 1$. It is important to test the biological activities of other remaining 14 stereoisomers of the MH- $\alpha 1$ for better understanding the structure-activity relationships.

MH- $\alpha 1$ is the first mating hormone identified in *Phytophthora*. The availability of synthetic compounds mimicking the activity of *Phytophthora* mating hormone will be instrumental for further understanding on sexual reproduction in this important group of plant pathogens. The structure of mating hormone produced by A2 mating type strains and the type of receptors involved in perceiving the mating hormone are still unknown.

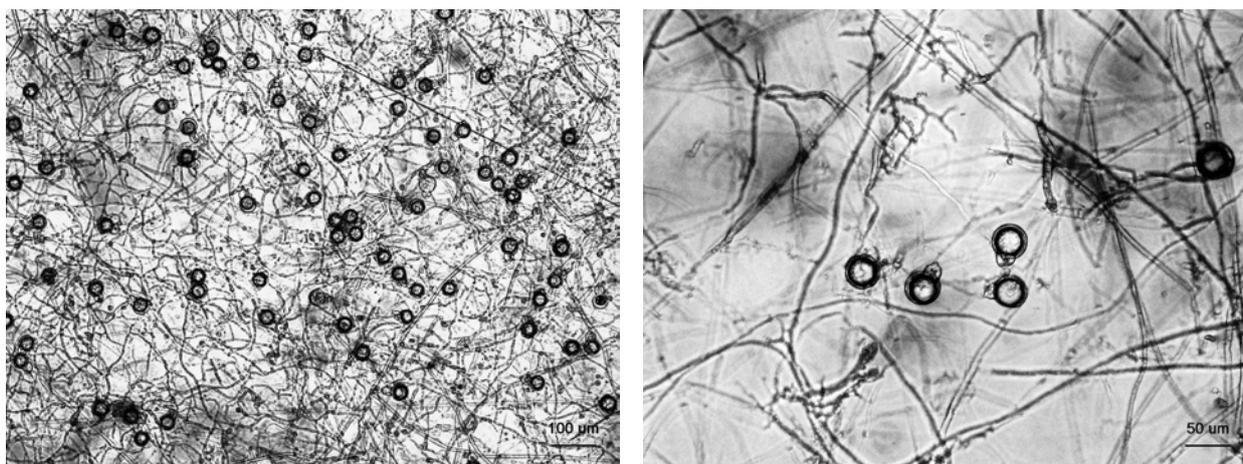


Fig. 4. Induction of oospore production in A2 mating type of *Phytophthora nicotianae* (right and left micrographs) by hormone α -1 from A1 mating type. The photomicrographs are generously provided by Prof. Makoto Ojika of Nagoya University, Japan.

However, in *P. infestans* the mating type locus has been mapped [116, 117], and it is anticipated that comparative genomic analysis and the forthcoming annotation of the *P. infestans* genome will help in identifying the genes that determine the A1 and A2 mating type [118, 119]. The second mating hormone of *Phytophthora*, α 2 was discovered by Ojika *et al.* [25]. They described the absolute stereostructure of the second mating hormone α 2 by the aid of spectroscopic analysis and total synthesis. Through a series of study, they uncovered not only the interspecies universality of α hormones but also the pathway by which α 2 is biosynthesized from phytol by A2-mating type strains and metabolized to α 1 by A1 strains.

The mating hormones α 1 and α 2 induce sexual reproduction of the phytopathogenic genus *Phytophthora*. To demonstrate the structural elements responsible for the hormonal activity, Molli *et al.* [120] synthesized 17 derivatives of α 1 and α 2 and evaluated their hormonal activity (oospore-inducing activity). The terminal ester derivatives of α 1 (diacetate and dibenzoate) retained the hormonal activity, whereas a dicarbamate derivative completely suppressed the activity. Even monocarbamates showed weak activities; among them the 1-*O*-carbamate was less active than 16-*O*-carbamate, suggesting that the 1-OH group is a little more significant than 16-OH. Dihydro, dehydro, and demethyl derivatives

exhibited the minimum level of activity. Surviving activity of 15-*epi*- α 1 suggested a less significance of this stereochemistry. Contrary to α 1, not only the terminal diacetate derivative but also monoacetates of α 2 exhibited no or little activity. Among the monoacetates, 1-*O*-acetyl- α 2 exhibited little yet relatively better activity than the others. No activity was observed for mono- and dicarbamoyl derivatives of α 2. Dihydro α 2 with the saturated double bond lost most of the activity. These findings suggest that both the mating hormones α 1 and α 2 require most of the functional (hydroxyl, keto and olefinic) groups they possess in their natural form for inducing the sexual reproduction of *Phytophthora*.

9. Role of sterols in Pythiaceae and its importance in plant-pathogen interactions

Although most of the organisms belonging to peronosporomycetes synthesize sterols, some plant pathogenic Peronosporomycete species such as *Pythium* and *Phytophthora* are sterol auxotrophs. A considerable amount of work has been reported on sterol-mediated morphogenesis in these plant parasites [17, 18, 19]. Hendrix observed that sterols of fungal, plant, and animal origin can regulate sexual reproduction in *Pythium periplocum* and *Phytophthora megasperma* var. *sojiae* [61]. He also found that sterols specifically induced the formation of large zoosporangia in *Phytophthora parasitica* and some sterols, especially cholesterol,

also stimulated the growth of some peronosporomycetes. Similarly, a related sterol auxotrophic species, *Lagenidium giganteum*, which is a facultative parasite of mosquito larvae, has also been found to be useful for comparable studies on sterol chemistry [121, 122, 123].

10. Presence of a steroidal secondary metabolite, terresterol in *Saprolegnia terrestris*

The peronosporomycetes are ubiquitous eukaryotic microorganisms. Reports on secondary metabolite production by this class of microorganisms are rare. Recently, Kikuchi and co-workers discovered a rare polyoxygenated lanostanoid namely terresterol from *Saprolegnia terrestris* [26]. This compound showed immunomodulating activity against bacterial infection. Compounds having a 21, 22, 24, 25-tetraoxygenated side chain similar to terresterol have only been rarely reported. Therefore, isolation of such a compound from *S. terrestris* indicates that peronosporomycetes are also a promising source of natural product chemistry.

11. Conclusion and future perspectives

Occurrence of major mammalian sex hormones such as testosterone, 17 β -estradiol, estrone and progesterone in the plant kingdom is wide spread and their biosynthetic pathways have partially been known. They show diverse effects on growth promotion and reproduction of plants *in vitro*. Some of them enhance stress tolerance in plants and also affect the growth and reproduction of other organisms including the environmental microorganisms. However, the significance of these steroidal secondary metabolites in plants is still illusive. The mammalian estrogens and estrogenic compounds display repellent activities toward zoospores of the phytopathogenic peronosporomycete, *Aphanomyces cochlioides* at micromolar concentrations. Interestingly, the estrogenic and repellent activities of known estrogenic compounds revealed to be correlated. However, the molecular mechanism of this interesting biological activity is unknown. Some peronosporomycetes such as *Phytophthora* and *Achlya* also produce steroidal and non-steroidal hormonal substances for their mating for sexual reproduction. Understanding the underlying molecular mechanism of their functions in sexual reproduction

would lead to the development of an effective management strategy against the pathogenic species of the peronosporomycetes. Recent advances of techniques in genomic and post-genomic research in both plants and peronosporomycetes might lead to better understanding of plant-peronosporomycete interactions via mammalian sex hormones.

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CONFLICT OF INTEREST STATEMENT

The author declares no conflict of interest.

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