

Mammalian sex hormones in plants

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Abstract: The occurrence of mammalian sex hormones and their physiological role in plants is reviewed. These hormones, such as 17β -estradiol, androsterone, testosterone or progesterone, were present in 60-80% of the plant species investigated. Enzymes responsible for their biosynthesis and conversion were also found in plants. Treatment of the plants with sex hormones or their precursors influenced plant development: cell divisions, root and shoot growth, embryo growth, flowering, pollen tube growth and callus proliferation. The regulatory abilities of mammalian sex hormones in plants makes possible their use in practice, especially in plant *in vitro* culture.

Key words: Mammalian sex hormones - Steroids - Plant growth regulation - Generative development - Enzymatic conversions - Receptors

The discovery and occurrence of mammalian sex hormones in plant tissues

Mammalian sex hormones such as estrogens, androgens and progesterone belong to steroids, a group of compounds which have a basic sterane carbon skeleton. The different steroids in living organisms are determined by the position and types of functional groups attached to the sterane (Fig. 1). In mammals, the steroid sex hormones play a key role in controlling the processes of development and reproduction and they are also engaged in the control of mineral and protein metabolism.

Mammalian sex hormones - estrogens, were first detected in plants in 1926 by Dohrn *et al.* [21] and then in the 1930s, simultaneously by Butenandt and Jacobi [16] and Skarzynski [78]. These results, however, had only an introductory pioneer character because, at that time, the methods of detection were not perfect. Between then and the 1980s, numerous reports concerning quantitative and qualitative analyses of human and animal sex hormones in plants were published (Table 1).

Some analytical methods, like Kober color reaction, which enables the estimation of estrogen-like substances, were critically reviewed by Van Rompuy and Zeveaart in 1979 [83]. These authors suggested that, generally, the detection of estrogens in plants needs more sensitive methods of investigation.

Extensive studies of the occurrence of mammalian steroids in plants (128 species from over 50 families) were carried out using radioimmunoassay in 1989 [77]. These methods allowed detection levels of about one part in 10^9 . Androsterone and progesterone were found in more than 80% of the investigated species, androgens (testosterone and dihydrotestosterone) in 70% of species and estrogens (estrone and 17β -estradiol) in 50% of species. The content of steroids may significantly change during plant development and is dependent on the species, cultivar and plant organ (Table 2).

Zhang *et al.* [91], also using the RIA method, estimated the content of total estrogens and 17β -estradiol in pollen and in the style of *Ginkgo biloba* L., *Zea mays* L. and *Brassica campestris* L. 17β -estradiol was present in the pollen of these plants in the range of 8-35 $\text{pg}\cdot\text{g}^{-1}$ f.w. In the style of *Lilium davidi* Duch., 17β -estradiol was found in the concentration of 24-40 $\text{pg}\cdot\text{g}^{-1}$ f.w. Moreover, the mentioned authors showed changes of total estrogen and 17β -estradiol concentration during flower development. Zhong-han *et al.* [92] proved the presence of testosterone in the pollen of *Pinus bungeana* Zucc. ex

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Abbreviations: BA - benzyl adenine, CC - column chromatography, d.w. - dry weight, ELISA - Enzyme-Linked Immunosorbent Assay, f.w. - fresh weight, GC - gas chromatography, GLC - gas-liquid chromatography, IAA - indole-3-acetic acid, IR - infra-red absorption spectroscopy, KCR - Kober color reaction, MS - mass spectrometry, NAA - naphthaleneacetic acid, NMR - spectroscopy of nuclear magnetic resonance, RIA - radioimmunoassay, TLC - thin-layer chromatography, UV - ultraviolet absorption spectroscopy, VIS - spectrophotometry in visible light

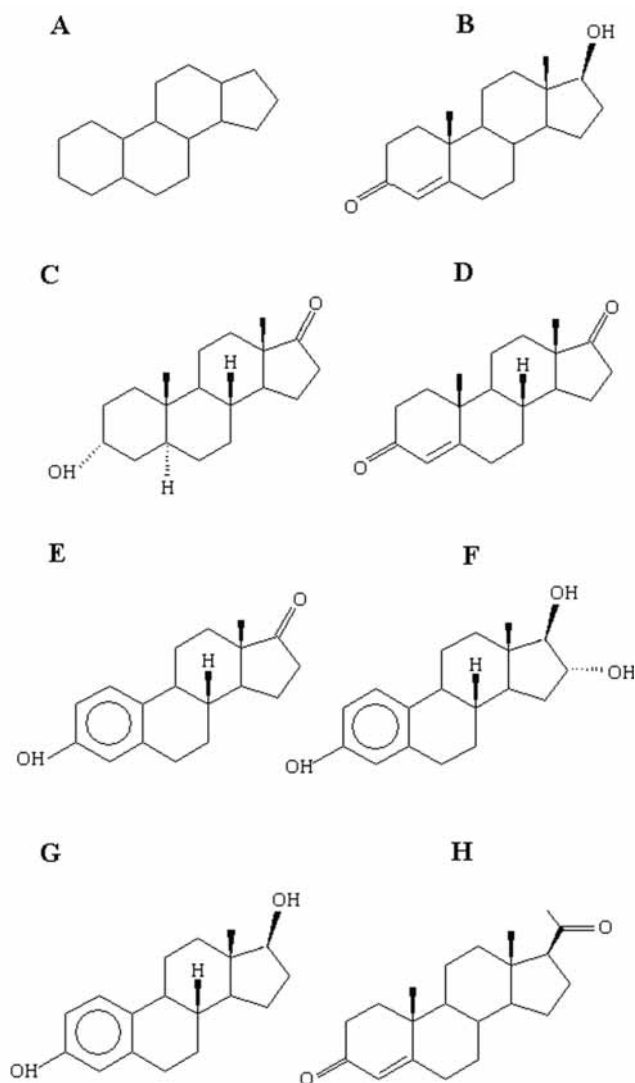


Fig. 1. Skeleton of sterane (A) and selected steroids, including mammalian sex hormones: testosterone (B), androsterone (C), androstenedione (D), estrone (E), estril (F), 17 β -estradiol (G), progesterone (H) (based on [35]).

Endl. (11 ng·g⁻¹ d.w.), *G. biloba* (87 ng·g⁻¹ d.w.) and *Pinus tabulaeformis* Carr. (27 ng·g⁻¹ d.w.) using the ELISA method. Testosterone was not detected in the pollen of *Juglans regia* L. and *B. campestris*. According to the authors, testosterone was also present in the pistil of *L. davidi*.

In 1995, Maier *et al.* [67] used the reconstituted steroid transcription unit in *Saccharomyces cerevisiae* transformed with both a human estrogen receptor expression plasmid (YEPE10) and a reporter plasmid (YR-PE2) to screen plant cell extracts for estrogen compounds. Estrogens, if present, induced the transcription of the reporter gene in transgenic yeast. Estrogenic activity was confirmed in female mulberry (*Morus mic-*

rophylla Buckl.) and osage-orange (*Maclura pomifera* Raf.) plant extracts. The activity caused by the extract from male plants was several times lower than that caused by the extract from female plants. Finally, the authors did not find exactly which substance was present and active in the extracts: 17 β -estradiol, genistein or maybe another phytoestrogen, but they established that the level of transcriptional activity of extracts was changing during plant development and was the highest just before and during flowering.

Steroid hormones have been found in plants as intermediates in different biosynthetic pathways. In *Digitalis*, progesterone is one of the intermediate compounds in the biosynthetic pathway of cardiac glycosides [23, 65, 75]. Moreover, the enzymes responsible for the conversion of progesterone and other pregnane derivatives have been found in *Digitalis* (Fig. 2). Some of these enzymes, such as steroid 5 α -reductase, function in the steroid pathways of animals and man [15, 62, 71].

According to the newest data, 17 β -estradiol and estrone have been detected in lipid fraction of *Solanum glaucophyllum* Desf. organs and calli by electrochemiluminescence immunoassay and radioimmunoassay [68].

The influence of exogenous mammalian sex hormones on plant growth

At the beginning of the 20th century, it was shown that estrone stimulated the growth of an isolated pea embryo *in vitro* [11, 49]. In 1945 Löve and Löve [66] established that meristem activity in the roots of *Melandrium dioecium* ssp. *rubrum* (Weigel) Garcke, *Rumex acetosa* L. and *Anthoxanthum aristatum* Boiss. was accelerated after treatment with estrone and testosterone. The stimulation of pea embryo growth by estrone (1 mg·dm⁻³) was also confirmed by Helmkamp and Bonner [33]. Estrone, at the concentration of 0.1 μ g per plant, also stimulated the growth of *Pisum sativum* L. seedlings by about 40% [50].

In sunflower seedlings, 17 β -estradiol and progesterone (0.25 μ g per plant) increased shoot growth but inhibited root growth, however, root elongation was promoted by progesterone at the concentration 0.1 μ g per plant. Testosterone promoted cotyledone axillary bud formation at the concentrations of 0.1 and 0.25 μ g per plant [7].

In tomato seedlings, estrone and 17 β -estradiol (as sulphate derivatives, at the concentration of 1 μ M in nutrient solution), reduced root growth as well as root number in shoot cuttings [30].

Watering *Medicago sativa* L. with nutrient solutions containing estrone and 17 β -estradiol at the concentrations of 0.005-0.5 μ g·dm⁻³ favoured the growth and increased in the dry weight of shoots and roots of plants, but at the concentration of 50-500 μ g·dm⁻³ inhibited plant growth [76]. The effect of estrone was stronger than that of 17 β -estradiol. The authors pointed out that

Table 1. Selected results of quantitative and qualitative analysis of steroids - including mammalian sex hormones - in plant material (1965-1983)

Species	Steroids (amount, if estimated)	Methods	References
<i>Phoenix dactylifera</i> L. (seeds)	estrone	TLC, CC	[32]
<i>Punica granatum</i> L. var. <i>nana</i> Pers. (seeds, root, flower)	estrone ($4 \mu\text{g}\cdot\text{kg}^{-1}$) estrone ($4.5 \mu\text{g}\cdot\text{kg}^{-1}$) estrone ($2.5 \mu\text{g}\cdot\text{kg}^{-1}$)	TLC, RIA	[20]
<i>Pinus silvestris</i> L. (pollen)	testosterone ($0.8 \mu\text{g}\cdot 10 \text{ g}^{-1}$) epitestosterone ($1.1 \mu\text{g}\cdot 10 \text{ g}^{-1}$) androstenedione ($5.9 \mu\text{g}\cdot 10 \text{ g}^{-1}$)	VIS	[73]
<i>Phaseolus vulgaris</i> L. (whole plant)	estrogen-like substances (in estrone equivalent $0.8 \mu\text{g}\cdot 100 \text{ g f.w.}^{-1}$, changes during development)	KCR, TLC, CC	[53]
<i>Salvia splendens</i> Sell. (whole plant)	estrogen-like substances (in estrone equivalent $0.50 \mu\text{g}\cdot 100 \text{ g d.w.}^{-1}$, changes during development)	KCR, TLC	[54]
<i>Hyoscyamus niger</i> L. (whole plant)	estrogen-like substances (in estrone equivalent $0.35 \mu\text{g}\cdot 100 \text{ g d.w.}^{-1}$, changes during development)	KCR, TLC	[54]
<i>Perilla ocimoides</i> L. (whole plant)	estrogen-like substances (in estrone equivalent $0.35 \mu\text{g}\cdot 100 \text{ g d.w.}^{-1}$, changes during development)	KCR, TLC	[55]
<i>Chenopodium rubrum</i> L. (whole plant)	estrogen-like substances (in estrone equivalent $0.20 \mu\text{g}\cdot 100 \text{ g d.w.}^{-1}$, changes during development)	KCR, TLC	[55]
<i>Hyacinthus orientalis</i> L. (bulbs)	estrogen-like substances (in estrone equivalent $0.62 \mu\text{g}\cdot 100 \text{ g f.w.}^{-1}$)	KCR, TLC	[57]
<i>Hyphaene thebaica</i> L. (kernel)	estrone ($5.13\text{-}5.25 \text{ mg}\cdot\text{kg}^{-1}$)	KCR, UV, IR, NMR	[2]
<i>Prunus armeniaca</i> L. (seeds)	estrogens	TLC	[3]
<i>Phaseolus vulgaris</i> L. (seeds and leaves)	17β -estradiol ($2\text{-}10 \mu\text{g}\cdot\text{kg f.w.}^{-1}$) (preliminary data)	TLC, RIA, LC, GC-MS	[89]
<i>Pastinaca sativa</i> L. (root)	androstenon ($5.5\text{-}11.4 \text{ ng}\cdot\text{g}^{-1}$)	RIA, GLC-MS	[18]
<i>Zea mays</i> L. (oil) <i>Olea europea</i> L. (oil) <i>Olea europea</i> L. (kernel)	estrone ($4 \mu\text{g}\cdot 100 \text{ ml}^{-1}$) estrone ester ($9 \mu\text{g}\cdot 100 \text{ ml}^{-1}$) estrone ($8.1 \text{ mg}\cdot 240 \text{ g}^{-1}$)	KCR, TLC, UV, IR, NMR	[1]
<i>Pinus nigra</i> Ar. (pollen)	testosterone ($0.7 \mu\text{g}\cdot 10 \text{ g}^{-1}$) androstenedione ($0.8 \mu\text{g}\cdot 10 \text{ g}^{-1}$) progesterone ($0.8 \mu\text{g}\cdot 10 \text{ g}^{-1}$)	RIA	[74]
<i>Pinus nigra</i> Ar. (pollen)	androsterone ($0.22 \mu\text{g}\cdot 10 \text{ g}^{-1}$) androstenedione ($0.90 \mu\text{g}\cdot 10 \text{ g}^{-1}$) dehydroepiandrosterone ($1.5 \mu\text{g}\cdot 10 \text{ g}^{-1}$)	TLC, GLC, RIA	[72]

estrogens found in sewage water ($0.3 \mu\text{g}\cdot\text{dm}^{-3}$) could affect the vegetative growth of alfalfa plants.

According to relatively new data, estrogens, and also progesterone ($1 \mu\text{M}$), stimulated the winter wheat seedling roots and leaves growing *in vitro*. The same steroids at the concentration tenfold higher inhibited the seedlings growth by a few percent [37].

The growth of callus. Already in the 1940s, Gioelli [cit. in 27], reported that 17β -estradiol ($3\text{-}12 \text{ mg}\cdot\text{dm}^{-3}$) stimulated the growth of *Daucus carota* L. callus in tissue culture by 100% and also favoured chlorophyll synthesis. The same hormone promoted the induction and proliferation of *Polygonatum verticillatum* L. callus [44, 81]. Androstenedione stimulated the proliferation of *Arabidopsis thaliana* L. callus tissue [37, 40]. Androste-

rone and androstenedione ($1 \mu\text{M}$) promoted the germination and growth of immature embryos of winter wheat (*Triticum aestivum* L.) accompanied by the induction and proliferation of callus tissue on the scutellum [37, 39]. Estrogens, especially estrone limited the germination of immature embryos, but they did not counteract the induction of callus tissue.

Other effects. Morphological abnormalities, such as epinasty and leaf rolling, were observed in tomato and mung bean seedlings treated with estrogens [30, 31]. 17β -estradiol treatment ($10^{-12}\text{M}\text{-}10^{-7}\text{M}$) increased the chlorophyll and carotenoid content in *Chlorella vulgaris* [4]. This hormone, at the concentration of $10^{-13}\text{M}\text{-}10^{-8}\text{M}$, stimulated the growth of *Chlorella vulgaris* and increased sugar and protein content in algal cells [5].

Table 2. Occurrence of mammalian sex hormones and related mammalian steroids in selected plants, estimated by radioimmunoassay in 1989 by Simons and Grinwich [77] (modified).

Species and organs	Date (collected)	Androgens (ng·g d.w. ⁻¹)	Androstenone (ng·g d.w. ⁻¹)	Estrogens (ng·g d.w. ⁻¹)	Progesterone (ng·g d.w. ⁻¹)
<i>Triticum aestivum</i> L. cv. Glenlea (leaf tissue)	7 July	0	220	0	0
cv. Benito (leaf tissue)		0	140	0	0
<i>Hordeum vulgare</i> L. (leaf tissue)	14 May	41	0	78	31
<i>Monstera deliciosa</i> Liebm. (leaf tissue)	27 April	140	8400	420	84
<i>Zea mays</i> L. (leaf tissue)	12 May	170	0	86	280
<i>Prunus virginiana</i> L. (leaf tissue)	20 May	140	1300	34	13
<i>Brassica campestris</i> L. cv. Torch (leaf tissue)	7 July	0	250	0	6
<i>Crassula arborescens</i> Willd. (leaf tissue)	27 April	3200	1300	320	130
<i>Daucus carota</i> L. (stem tissue)	5 June	0	310	0	15
<i>Urtica dioica</i> L. (shoot tissue)	30 April	99	0	500	79
<i>Thlaspi arvense</i> L. (shoot tissue)	29 April	95	190	240	76
<i>Acer negundo</i> L. (female inflorescence tissue, male inflorescence tissue)	30 April	49	0	98	10
		82	41	82	8
<i>Syringa vulgaris</i> L. (inflorescence tissue - early bud, inflorescence tissue - full bloom)	24 May 24 June	44 0	180 270	44 0	18 0
<i>Bromus inermis</i> Leyss. (mature seeds)	-	44	0	0	18
<i>Brassica napus</i> L. cv. Oro (mature seeds)	-	11	0	0	9

In experiments conducted in 2000, Bhardwaj and Thukral [6] established that the treatment by steroids (estrone, testosterone and pregnenolone acetate), at the concentrations of 10^{-8} M and 10^{-6} M, of maize plants enhanced their growth and evoked earlier anthesis and better yield.

The influence of mammalian sex hormones on plant generative development

Since estrogens and androgens act as sex hormones in animals and man, the related functions of these substances, accompanied with generative development, were investigated in plants.

Induction of flowering. The first indication that the steroids influence the generative development of plants is the paper of Chouard from 1937 [cit. in 27]. The author found that 17β -estradiol stimulated the generative development of *Callistephus sinensis* L. Later, it was shown that 17β -estradiol also stimulated the flowering of *Lemna minor* L. [19].

Bonner *et al.* [12] used a steroid biosynthesis inhibitor (SK&F 7997) to block the flowering process in a short day plant *Xanthium pensylvanicum* Wallr. However, this inhibitor upsets the biosynthesis of all steroids including membrane sterols. Nevertheless, Leshem [61] in

experiments on broccolis showed that the inhibition of steroid biosynthesis by SK&F 7997, could be reversed by androsterone but not by cholesterol or 17β -estradiol.

The generative development of *Salvia splendens* Sell. growing in non-inductive conditions was induced by 17β -estradiol applied during 15 days in the quantity of 5-15 μ g per plant [56]. In non-vernalized plants of *Cichorium intybus* L., the flowering was stimulated by estrone and 17β -estradiol [51]. These estrogens evoked flowering in 55% and 85% of plants, respectively, whereas the control plants remained vegetative.

The observation that the selected estrogens stimulate flowering in plants which require photoperiodic induction or vernalization was confirmed in *in vitro* cultures of *Arabidopsis thaliana* L. and in winter wheat (*Triticum aestivum* L.) [38, 41]. Several other sex hormones; female hormone - progesterone, male hormones - androsterone and androstenedione (testosterone precursor), applied to these two plants were also active. According to other reports, androgens such as testosterone were unable to induce the generative development in *Salvia splendens* Sell. [56]. In the experiment of Biswas *et al.* [10] androstane and androsterone did not stimulate flowering in *Chrysanthemum sp.* Nevertheless, in *A. thaliana in vitro* culture androsterone and androstenedione at the concentration of 0.1 μ M, stimulated over 90% of

plants to the generative stage [41]. Treatment of suboptimally vernalized winter wheat with these two chemicals at the concentration of 10 μ M caused a significant increase in the percentage of plants reaching the stage of heading. Even 100% of heading plants was observed in group of plants previously cultured *in vitro* on a medium containing androsterone and androstenedione. The control plants showed 0-8% heading. Moreover, all tested steroids, such as androsterone, androstenedione, progesterone, estriol, estrone and 17 β -estradiol, were able to accelerate the onset of heading in comparison to control by 14 to even 30 days - depending on the hormone applied and its concentration [38].

Unlike in plants, in the fungus *Pythium periplocum* Drechs., 17 β -estradiol prevented sexual reproduction - it reduced it at the concentration of 10⁻⁶M and eliminated it at the concentration of 3.3·10⁻⁶M [34].

Sex expression in plants. According to some reports, mammalian sex hormones can modulate sex expression in flowers of dioecious plants (which have masculine and feminine flowers on the same plant). For example, in *Ecballium elaterium* L., the application of estrogens considerably affected the total number of flowers as well as increased the ratio of female to male flowers. On the other hand, androgens increased the ratio of male flowers [52]. In cucumber, the number of female flowers increased after treating the plants with 17 β -estradiol as well as with testosterone [26]. Moreover, cucumber plants treated with these hormones produced the first flower on the first node while control plants produced it on the fourth node. Other authors, however, do not confirm the results cited above [46]. They tested the influence of 17 β -estradiol, estrone and testosterone on sex expression in cucumber, pumpkin and spinach. No significant modification of sex expression tendency in the flowers of these plants was observed under the influence of the mentioned hormones.

The effect of mammalian sex hormones on plant pollination and fertilization. Estrogens and testosterone may play a role in the process of pollination and fertilization in plants. According to Zhang *et al.* [91], the level of total estrogens in the style of *Lilium davidii* Duch. decreased after self-pollination of the open flower. After self-pollination at the bud stage, which partly overcame self-incompatibility, the levels of both 17 β -estradiol and total estrogen increased in style, in comparison with unpollinated control.

In the anthers of *L. davidii*, the testosterone level increased during their development reaching a maximum at anthesis. After pollen shedding, testosterone rapidly decreased [92].

Pollen germination and pollen tube growth. Estrogens and testosterone stimulated pollen tube growth of *Rumex tenuifolius* Waller. [66]. The most efficient con-

centration was 0.1%. 17 β -estradiol at this concentration enhanced pollen tube growth up to 171% and testosterone up to 134% in comparison with the control. In *Nicotiana tabacum* L., the same hormones as well as progesterone at the concentration 10 μ M stimulated pollen tube growth [88]. Androstenedione, the testosterone precursor, did not influence the growth of male gametophytes.

Enzymatic conversions of mammalian sex hormones in plant tissues

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 authors, but the question remains how these steroids are biosynthesised, transported and eventually converted in plant organisms.

Conversion of estrogens. Biosynthesis of 17 β -estradiol from 2-¹⁴C mevalonic acid was observed in *Phaseolus vulgaris* seedlings [89]. The same authors demonstrated in *P. vulgaris* interconversion between estrone and 17 β -estradiol and the ability to hydrolyze estrone sulphate to free estrone [90]. According to Young *et al.* [89, 90], enzymes capable of converting estrone to estradiol are present or inducible in leaf tissue. Bioconversion of 17 β -estradiol can also be carried out by less specific enzymes, such as phenoloxidase, which is capable of *ortho*-hydroxylating a range of phenolic substrates. After complexation with β -cyclodextrin, 17 β -estradiol could be *ortho*-hydroxylated by phenoloxidase (EC 1.14.18.1) mainly into 4-hydroxyestradiol in the cells of *Mucuna pruriens* Linn. grown *in vitro* [87].

Conversion of androgens. In *Pisum sativum* seedlings, radioactive 4-androstene-3,17-dione [4-¹⁴C] added to leaves was reduced to testosterone [64]. 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 [36]. *Cucumis sativus* L. plant transformed 4-androstene-3,17-dione [4-¹⁴C] to testosterone and to other androstane derivatives [63]. 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.

Conversions of progesterone. The conversions of female hormone, progesterone, are relatively well known. Leaf homogenate of *Cheiranthus cheiri* L., however, converted 20 α -hydroxycholesterol-7-³H into pregnenolone and progesterone [79]. Progesterone was modified to different derivatives of pregnane by suspension cultures of *Dioscorea deltoidea* Wall. and *Digitalis purpurea* L. [80, 25]. Some algae were capable of transforming progesterone. *Chlorella emersoni* C211-8H trans-

formed this compound to hydroxyprogesterones and dihydroxyprogesterones [28]. The described conversions of steroids might be driven by unspecific enzymatic systems, but not necessarily. In 1973 Furuya *et al.* [25] proposed the scheme of biotransformation of progesterone in *Digitalis purpurea* L. callus culture and suggested that 20α -hydroxysteroid dehydrogenase may be involved in this process. It is well known now 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. (Fig. 2). The enzymes participating in the conversions of compounds along this pathway were described by Seitz and Gärtner [75], Lindemann and Luckner [65] and Finsterbusch *et al.* [23]. In the cardenolide pathway, Δ^5 - 3β -hydroxysteroid dehydrogenase/ Δ^5 - Δ^4 -ketosteroid isomerase (Δ^5 - 3β -HSD) transforms pregnenolone to progesterone. Then, progesterone- 5β -reductase transforms progesterone to 5β -pregnane-3,20-dione or progesterone- 5α -reductase transforms progesterone to 5α -pregnane-3,20-dione (Fig. 2). In mammals, hydroxysteroid dehydrogenase- and steroid 5α -reductase-type enzymes participate in the biosynthesis and control of steroid hormone activity, including activity of sex hormones. Hence, similar enzymes were searched in plants. It seems interesting that in brassinosteroid-deficient *Arabidopsis* plants lacking steroid 5α -reductase activity, the introduction of the human type of that enzyme restored normal plant growth without exogenous brassinosteroid application [62].

Receptors of mammalian sex hormones in plant cells

The molecular mechanism of mammalian sex hormones activity in plants is not explained and still requires investigations. There are two papers reporting in plant cells the presence of estrogen receptors which may be involved in steroid actions. The endogenous receptor for 17β -estradiol was found in the ovules of *Gladiolus primulinus* Bak. in 1984 by Janik and Adler [45]. According to the authors, the receptor passed from cytoplasm to the nucleus upon activation by 17β -estradiol, estriol and diethylstilbestrol. Recently, Milanesi *et al.* [68] proved the presence of estrogen receptors in callus of *Solanum glaucophyllum* Desf. The authors found the proteins which were tested positive as estrogen binders in ligand blot experiments with the use of 17β -estradiol derivatives as ligands.

Brassinosteroids and phytoestrogens

Currently, the interest in the occurrence and action of mammalian sex hormones in plants declined and the research is focused on steroid-related compounds, brassinosteroids and phytoestrogens. Brassinosteroids disco-

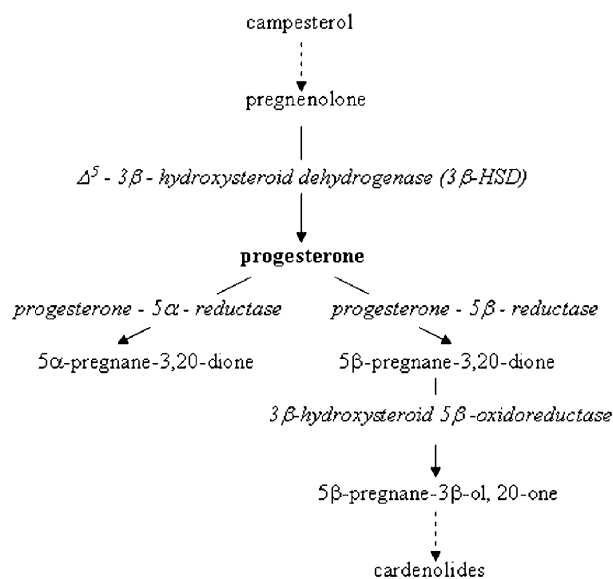


Fig. 2. Progesterone in cardenolide biosynthesis pathway of *Digitalis lanata* Ehrh. (according to [65], modified).

vered in 1979 [29], such as brassinolide or 24-*epibrassinolide* are considered to be a new class of plant hormones - the first steroid hormones confirmed in the plant kingdom [47]. Brassinosteroid receptor has been discovered in the plasma membrane and the signal transduction pathways in cell are partly explained [9, 24, 48, 86]. Brassinosteroids exhibit a multitude of physiological activities, influence plant growth and crop [13, 70, 84] and increase resistance to some stresses [8, 42, 59, 85]. They also stimulate photosynthesis and photosynthetic pigment production in leaves [13, 22, 60]. The physiological activity of brassinosteroids is still a field of intensive investigations.

Apart from typical mammalian sex hormones, in plants naturally occur phytoestrogens (genistein, daidzein, formononetin, enterodiol), secondary metabolites of plant origin which exhibit structural and functional resemblance to the main female sex hormone 17β -estradiol. Phytoestrogens under certain circumstances can have actions like human estrogens by binding estrogen receptors in some tissues and activating or down-regulating cellular responses [69, 82]. They are present mainly in *Leguminosae* plants (soy, clover, bean) [43, 82]. Phytoestrogens are applied in medicine: they show some anti-cancer properties and can be useful in heart disease prevention [14, 17]. They are also used in menopausal disturbances in woman [58].

Conclusions

The involvement of mammalian sex hormones in the physiology of plants has been proved in many experiments during the last few decades. Although the available knowledge does not allow us to consider these

compounds as plant hormones, it has been established that they appear in many plants and frequently their metabolic pathways have been partly revealed.

The regulatory abilities of mammalian sex hormones in plants can be used in practice, especially in *in vitro* culture for callus proliferation, embryo growth stimulation and flowering promotion of micropropagated plants.

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