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REVIEW

STEROID HORMONES AND PLANT GROWTH AND DEVELOPMENT

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Key Word Index—Angiosperms; steroid hormones; plant growth; flowering; sex hormones; corticosteroids.

Abstract— The occurrence of steroid hormones in plants is briefly reviewed. Their effects on plant growth, development and flowering are also considered.

INTRODUCTION

Although the number of papers describing the effects of animal steroid hormones on higher plants is still increasing, only a few plant physiologists are convinced that such steroids play a prominent part in plant growth and development. Several steroid hormones have been isolated from plant sources and the literature concerning this topic has been surveyed by Heftmann [1–5], Singh *et al.* [6] and Rees [7]. The physiological aspects of the biosynthesis of the sterols and their role in membranes have been the subject of reviews [8,9] and our previous publications [10–13]. This review will consider the effects of steroids on the growth, development and flowering of higher plants. Studies on the isolation of different groups of steroids* will only be included when the physiological effects of these steroids have also been investigated.

THE ANIMAL STEROID SEX HORMONES

Effects on vegetative development

Fiedler [14] found a stimulative effect (26%) of estrone (**1**) on the growth of isolated corn root tips. Bonner and Axtman [15] observed that folliculine (= estrone, **1**) stimulated the growth of isolated pea embryos grown on a solid culture medium containing salts and 4% sucrose. The root growth was enhanced by 30, 46, 66, and 27% after 1, 2, 3 and 4 weeks respectively. After 4 weeks, shoot growth was stimulated by 50%. Helmkamp and Bonner [16] found that after 4 weeks the shoot growth of isolated pea embryos could be enhanced by 20% by application of estrone. By contrast testosterone propionate (**6**) inhibited the shoot growth by 65% at a concentration of 10 mg/l. Dihydroxyestrin (= estradiol, **2**) and methylandrostenediol (= methandriol, (**8**) were slightly inhibitory.

Kögl and Haagen-Smit [17] noticed that estrone (**1**), added to the culture medium (0.04 mg/l.) of isolated pea embryos, stimulated their shoot growth by some 27%, their fresh weight increased by 18%, and their dry weight increased by 22%. Gioelli [18] observed that estradiol (**2**), when applied in low concentration (3–12 mg/l.) to the culture medium of carrot tissue cultures, stimulated the growth by 100% and favoured the synthesis of chlorophyll. High doses (25–100 mg/l.) were toxic and inhibited the growth of the tissue cultures. Inhibitory effects of 0.01% solutions of hexestrol (**12**), diethylstilbestrol (**13**), estrone (**1**) and testosterone (**5**) have been observed [19] on the development of *Lepidium sativum* seedlings.

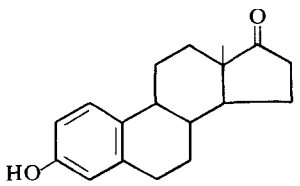
Löve and Löve [20] studied the influence of animal sex hormones on the germination of seeds and pollen. The germination of the seeds of *Melandrium dioecum* was stimulated by estrone (**1**) and 17β-estradiol (**2**) (under certain circumstances an enhanced germination percentage of 200 was observed). The pollen tube growth of *Rumex tenuifolius* was stimulated by estrone (**1**; + 65%), estradiol (**2**; + 71%) and testosterone (**5**; + 34%). The meristem activity in the roots of *Melandrium dioecum* ssp. *rubrum*, *Rumex acetosa* and *Anthoxanthum cristatum* was stimulated by estrone (± 300% more metaphases and anaphases) and by testosterone (75% stimulation). The earlier literature describing the effects of animal steroid hormones on the vegetative and generative development of plants has been reviewed [20].

Kopcewicz [21] observed a stimulative effect of 17 β-estradiol (**2**; 40% stimulation), estrone (**1**; 36%) and sitosterol (**14**; 24%) on the growth of dwarf peas. Cholesterol (**15**) and testosterone (**5**) were inactive. In other experiments with dwarf peas [22] estrone had the same effect as gibberellic acid (40% stimulation of growth). Whereas the growth stimulation of dwarf pea seedlings by estrone (**1**), estradiol (**2**) and estriol (**4**) was about 40%, the growth of pine seedlings was not influenced [23, 24].

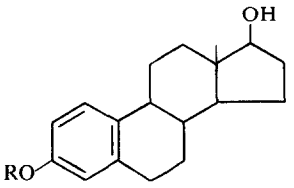
Effects on flowering

Chouard [25] reported flower promoting effects of estradiol (**2**) in *Callistephus sinensis*. Application of estradiol to the culture medium, induced flowering in two *Lemna minor* strains that could not be induced under short or long day conditions, nor by application of sucrose or EDTA [26]. However, flowering could not be induced at any time, which may indicate that additional factors are involved in the flowering process of *Lemna*

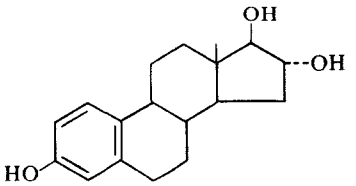
*The numbering of the steroid skeleton is shown on structure **14**. For the conventions of steroid naming see the IUPAC-IUB 1971 Definitive Rules for Steroid Nomenclature [*Rare Appl. Chem.* (1972)**31**, 385].



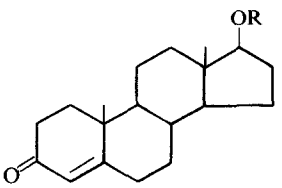
1. Estrone



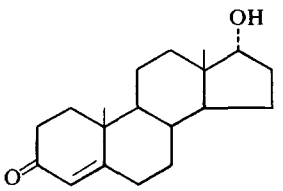
2. R = H; Estradiol
3. R = Bz; Estradiol benzoate



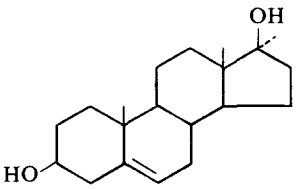
4. Estriol



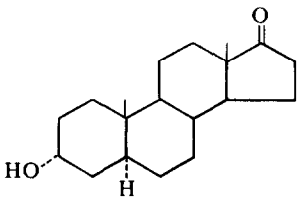
5. R = H: Testosterone
6. R = Pr: Testosterone propionate



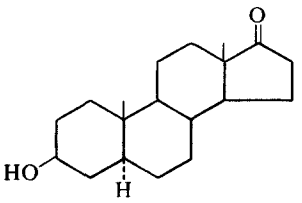
7. Epitestosterone



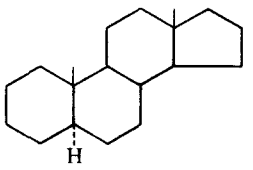
8. Methandriol



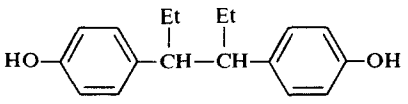
9. Androsterone



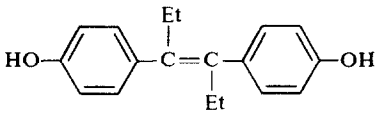
10. Epiandrosterone



11. Androstane



12. Hexestrol



13. Diethylstilbestrol

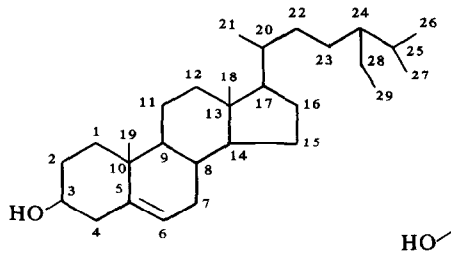
minor. Bonner *et al.* [27] suppressed flower induction of *Xanthium* plants by applying inhibitors of steroid biosynthesis to the leaves shortly before the inductive long night. The inhibitor SKF 7997 had no visible effects on the vegetative growth and it is suggested that the synthesis of a flowering hormone is inhibited by SKF 7997. This hormone could be a steroid (probably estrogen like) or another isoprenoid. However, the inhibition could not be reversed through the use of several steroids and steroid precursors [28]. Leshem [29] found that cholesterol (**15**), 17β-estradiol (**2**) and androsterone (**9**) stimulated the flowering of broccoli curd cuttings grown in culture medium. Root growth, measured as increase in dry weight, was also stimulated by these compounds. The inhibitor of steroid biosynthesis SKF 7997 A3, inhibited flowering as well as root production. Only androsterone could reverse the inhibitory effect of SKF 7997 A3 on flowering.

In *Cichorium intybus* the inductive cold period can be replaced by a 17β-estradiol (**2**) or by an estrone (**1**) treatment [30]. Kopcewicz and Porazinski [31] could induce flowering in the long day plant *Salvia splendens*, grown under short day conditions, by 17β-estradiol and also by an unidentified estrogen-like fraction isolated from flowering *Salvia* plants. Flowering in *Chrysanthemum* sp. growing under non-inductive conditions was obtained [32] by application of sitosterol (**14**), lanosterol (**21**), glycocholic acid (**22**) and androstane (**11**).

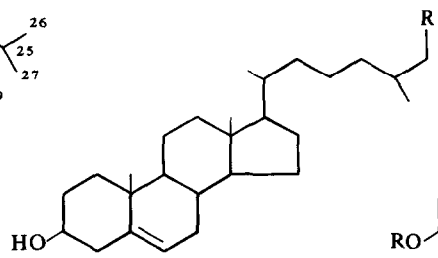
Sex determination of flowers

Besides effects on vegetative development, flower induction and flower development, animal steroid sex hormones effect considerably the sex determination of flowers. Hylmö [33] decapitated *Spinacia* plants above the 3rd to the 5th leaf and treated the decapitated plants with an estrone (**1**) or testosterone (**5**) paste. New lateral shoots developed on the treated plants, but not on the controls. The flowers that arose on the plants treated with estrone developed in a female direction. However, the flowers on plants treated with testosterone developed in a male direction.

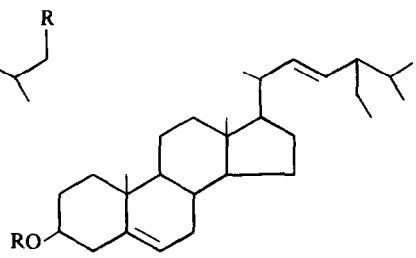
Löve and Löve [20] induced flower buds of *Melandrium dioecum* to develop in a female or male direction by applying estrogens or androgens to the shoots before the flowering. The hormones used were estrone (**1**), estradiol (**2**), estradiol benzoate (**3**), testosterone (**5**) and testosterone propionate (**6**). They were administered in a lanoline paste to the cut end of decapitated shoots. The effects of the hormones were observed when new axillary shoots grew through the lanolin, and produced flowers. Estradiol and



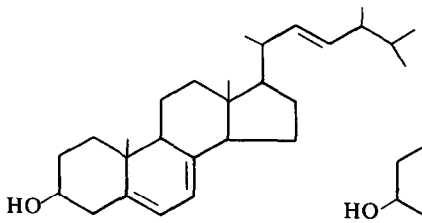
14. Sitosterol



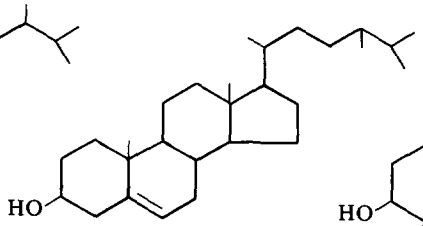
15. R = H; Cholesterol
16. R = OH; 26-Hydroxycholesterol



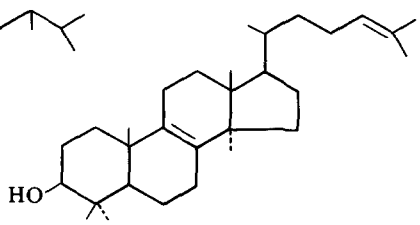
17. R = H; Stigmasterol
18. R = $C_6H_{11}O_5$; Stigmasterylglucoside



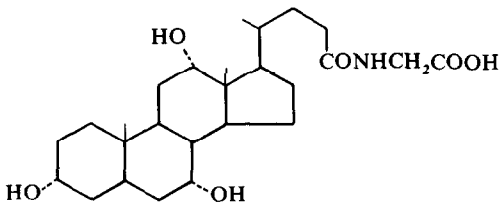
19. Ergosterol



20. Campesterol



21. Lanosterol



22. Glycocholic acid

estradiol benzoate produced similar effects to estrone; testosterone propionate was similar to testosterone. As to their sex expression the plants belonged to five categories as follows: (1) Normal female flowers. Estrone was inactive, testosterone caused flowers to develop rudimentary anthers. (2) Female flowers with rudimentary anthers. By estrone treatment the anthers disappeared in a high percentage of treated plants, flowers becoming fully female. With testosterone treatment a high percentage of flowers developed with enlarged anthers. (3) Androhermaphroditic individuals belonging to different generations of a family with an interchange between the X- and Y-chromosomes. When treated with estrone, all flowers developed like normal female flowers, though with small rudimentary anthers. By applying testosterone the flowers became like normal male flowers. (4) Male flowers with a hairlike rudiment of gynaecium in the centre of all flowers. Estrone caused the flowers of the only surviving plant to develop normal gynaeciums with small rudimentary anthers. When treated with testosterone the hairlike gynaecium disappeared completely. (5) Normal male individuals. Testosterone was inactive. After treatment with estrone a hairlike gynaecium developed. In the case of the higher estrone concentration (0.1%) even a few flowers were found which contained a large gynaecium with 2 stigmas but with low fertility. Kopcewicz [34] found similar results when studying the sex determination of *Ecballium elaterium*. Applied estrogens (estrone **1**, estriol **4**, or 17β-estradiol **2**) enhanced the number of flowers by 18–35% and increased the percentage of female flowers by *ca* 66%. Androgens (androsterone **9**, androstenedione **28** and testosterone **5**) did not influence the number of flowers, but decreased the percentage of female flowers 61% in favour of male flowers. The flowers of *Cucumis sativus* could be influenced to develop in a male or female direction through the use of testosterone (**5**) or estradiol (**2**) [35].

Although ferns and horse tails do not belong to the flowering plants, a few results obtained with steroid sex hormones deserve to be mentioned. Laroche *et al.* [36] influenced the sex expression of *Equisetum arvense* prothallia in a female direction by adding testosterone (**5**) to the culture medium. The prothallia were more ramified and had a higher chlorophyll content, which are female characteristics. Archegonia developed much earlier after 20 days instead of 50 days. These results may appear to be contradictory. However, in animals testosterone can be converted into estrogens [37]. It is possible that the applied testosterone was converted by the *Equisetum* prothallia into estrogens, which then stimulated the prothallia to become female. Montardy-Pausader [38] observed that estradiol (**2**) increased the germination of *Gymnogramme sulphurea* spores and also increased cell divisions. Higher concentrations of estradiol disturbed the sexualisation of the prothallia and retarded the formation of archegonia.

Prothallia of *Lygodium japonicum*, grown on a solid

culture medium, formed many hairlike outgrowths on both sides but afterwards the hairs died. These prothallia were grown on medium supplemented with estradiol (**2**) (10μg/30 ml), testosterone (**5**, 10 μg/30 ml) or both (10 μg of each/30 ml) [39]. With estradiol treatment the number of hairlike outgrowths decreased, but at the lower side of the prothallia archegonium like outgrowths were formed possessing a central canal. Testosterone treatment resulted in a decrease of the hairlike outgrowths and the appearance of antheridiumlike organs at the upper side of the prothallia, in which small cells were formed, although without cilia. When estradiol and testosterone were applied together, the number of hairs decreased, and at the upper side antheridiumlike organs were formed while at the lower side archegonium like ones appeared. In the controls only hairlike outgrowths were formed. With lower concentrations of estradiol (5 μg/30 ml) and testosterone (5 μg/30 ml) normal archegonia and antheridia developed. However, spermatozoïds had no cilia and were immature.

Isolation of steroid sex hormones from plants

Early investigators [cf. 20] isolated substances from plants possessing the same properties as the animal sex hormones when tested on animals. In this respect the importance of the so-called phytoestrogens (chiefly isoflavones) should not be underrated. Phytoestrogens mainly occur in the Leguminosae and possess estrogenic activity when given to animals, e.g. coumestrol (**23**) [40], daidzein (**24**), genistein (**25**) and biochanin A (**26**) [41, 42] for recent reviews, see [43, 44]. Their function in higher plants remains obscure. Newsome and Kitts [45] have reported an easy technique for separating estrogens from phytoestrogens.

Recent studies unequivocally prove the natural occurrence of steroid sex hormones in plants. Bennett *et al.* [46] isolated estrone (**1**) from the seeds and pollen of the date palm (*Phoenix dactylifera*) Heftmann *et al.* [47] isolated estrone from pomegranate seeds, the isolated compound showed physiological activity in the mouse uterus test [48]. Heftmann *et al.* consider pomegranate seeds to be a rich source of plant estrogens. However, Dean *et al.* [49] using pomegranate seeds from the same source, could not confirm the high yield of estrone (17 mg/kg) reported. They used a more specific identification technique (competitive protein binding) and could find estrone but no 17β-estradiol. The oestrone was at a concentration 4000 times less than that cited by Heftmann's group (*ca* 4 μg/kg against 17 mg/kg). Dean *et al.* explain the difference in yield as a result of the more specific identification technique. Possibly also different physiological conditions of the seeds at the time of extraction may account for differences in yield.

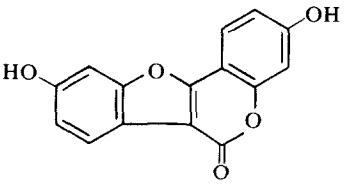
Kvanta [50] isolated estrone (**1**) from an acetone extract of a standardized pollen mixture from six plant species. Amin *et al.* [51] isolated estrone from the roots of *Clossostemum bruguieri* (moghat) and from the pollen of *Phoenix dactylifera.* Gawienowski and Gibbs [52] found estrone in apple seeds. Amin and Paleologou [53] isolated estrone from the kernels and the pollen grains of the Doum palm (*Hyphaene thebaica*). Awad [54] isolated estrone and estradiol (**2**) from *Prunus armeniaca* seeds. The seeds and also the vegetative and flowering plants of *Phaseolus vulgaris* contained estradiol (2–10 μg/kg fresh wt in seeds and leaves) and estradiol was biosynthesized from applied mevalonic acid, estrone and potassium estrone sulphate [54a].

Androstanetriol (= 5α-androstane-3β, 16α, 17α-triol, **27**) has been isolated from *Haplopappus heterophyllus* [55]. Bennett and Heftmann [56] could not find this substance in the same species but this may be due to taxonomic confusion, since there are at least two plant species that are called 'rayless goldenrod'. Saden-Krehula *et al.* [57] isolated testosterone (**5**), epitestosterone (**7**) and androst-4-ene-3, 17-dione (**28**) from the pollen of *Pinus sylvestris.*

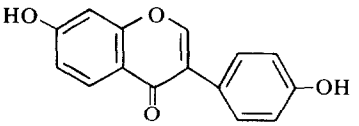
Biotransformation of applied estrogens and androgens

Further indirect evidence for the occurrence of steroid sex hormones in plants comes from the fact that plants are able to metabolize applied steroids. This implies the possession of the enzymes necessary for steroid interconversions, or that these enzyme systems are quickly induced by the applied substrates. This fact shows that plants apparently possess the genetic information required for steroid specific enzyme synthesis.

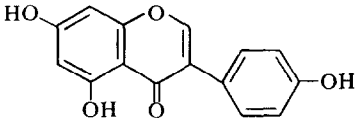
Stohs and El-Olemy [58] showed that androstenedione (**28**) was converted into 3β-hydroxy-5α-androstane-17-one (**29**) and 5α-androstane-3β,17β-diol (**30**) by cell suspension cultures of *Dioscorea deltoidea.* Hirotani and Furuya [59] studied the metabolism of testosterone and other androgens in the cell cultures of tobacco. Applied testosterone (**5**) was converted into androst-4-ene-3, 17-dione (**28**), 17β-hydroxy-5α-androstan-3-one (**31**), 5α-androstane-3β, 17β-diol (**30**) and its dipalmitate ester and 3- and 17-monoglucosides, epiandrosterone (**10**) and and its palmitate and glucoside, and to testosterone



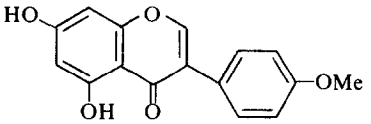
23. Coumestrol



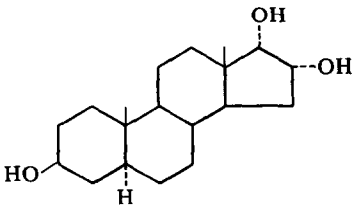
24. Daidzein



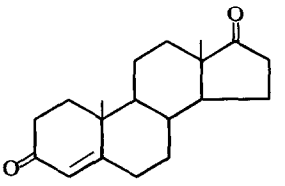
25. Genistein



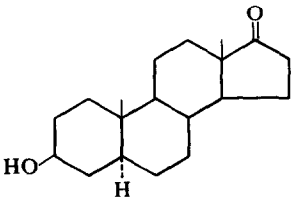
26. Biochanin A



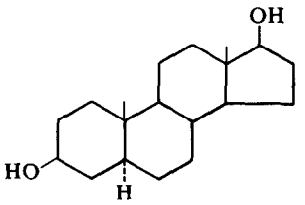
27. Androstanetriol (5α, 3β, 16α, 17α)



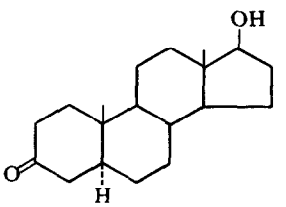
28. Androst-4-ene-3, 17-dione



29. 3β-Hydroxy-5α-androstan-17-one



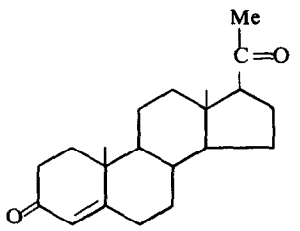
30. 5α-Androstane-3β, 17β-diol



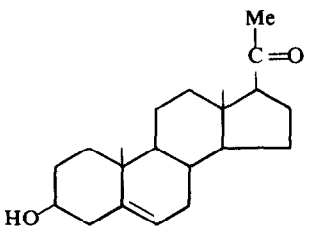
31. 17β-Hydroxy-5α-androstan-3-one

glucoside. Also androst-4-ene-3, 17-dione (**28**) and epiandrosterone (**10**) were actively metabolized by the tobacco cell cultures. *Phaseolus vulgaris* plants converted estrone and potassium estrone sulphate into estradiol [54a]. Heftmann [3–5] cites authors who have studied the biotransformations of animal steroids in plants.

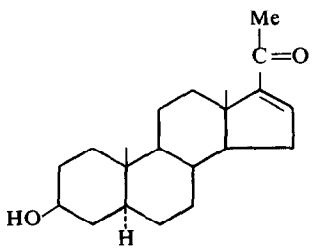
The biosynthetic pathway for estrogen and androgen production from sterols has not yet been studied in higher plants. It is likely that the biosynthesis of these hormones will occur via progesterone (**32**) and C_{19}-steroids as in animals [37], The intermediates progesterone (**32**) and pregnenolone (**33**) have been isolated from higher plants. Leboeuf *et al.* [60] found progesterone (**32**) in *Holarrhena floribunda* and Gawienowski and Gibbs [61] isolated this substance from apple seeds. Pregnenolone (**33**) has been isolated from *Xysmalobium undulatum* [62] and from *Trachycalymna fimbriatum* [63], in the former as a mixture with 3β-hydroxy-pregnan-20-one (**35**). Yamauchi *et al.* [64] isolated four pregnenolone glucosides from *Nerium odorum* root and trunk bark and other pregnane derivatives were found in the root bark of *Nerium odorum* [65]. Kaneko *et al.* [66] isolated 3β-hydroxy-pregna-5,16-diene-20-one (**36**) from *Veratrum grandiflorum*.



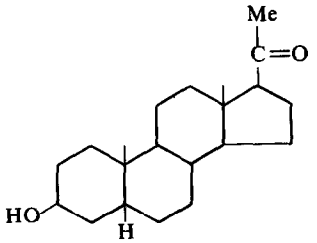
32. Progesterone



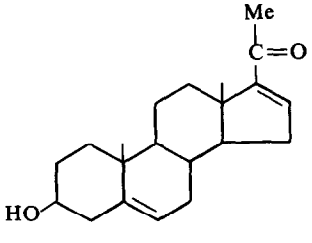
33. Pregnenolone



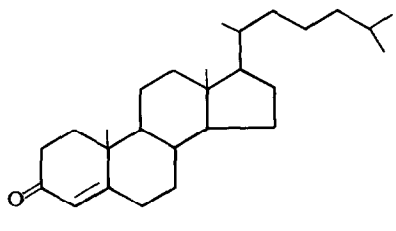
34. Allopregnenolone



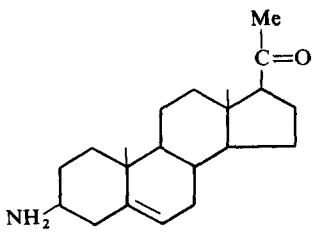
35. Pregnan-3β-ol-20-one



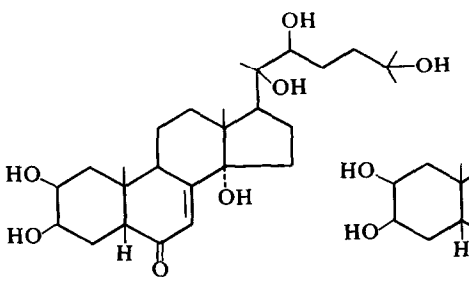
36. 3β-Hydroxy-pregna-5, 16-dien-20-one



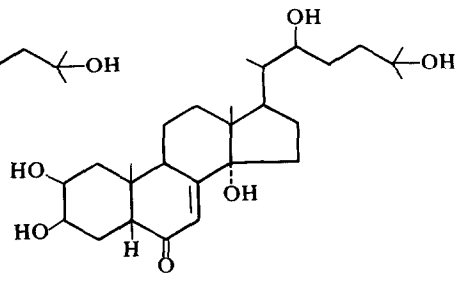
37. Cholest-4-en-3-one



38. Holaphyllamine



39. Ecdysterone



40. β-Ecdysone

Bennett and Heftmann [56] found that *Holarrhena floribunda* plants were able to convert applied pregnenolone (**33**) into progesterone (**32**) and this substance was rapidly metabolized by the plants. Pregnenolone was converted into progesterone by sterile cell suspension cultures of *Digitalis purpurea*, *D. lutea* and *Nicotiana tabacum* [67] and *Digitalis lanata* plants converted pregnenolone into progesterone [68, 69]. Stohs and El-Olemy [70] obtained progesterone from pregnenolone in leaf homogenates of *Cheiranthus cheiri*, *Digitalis purpurea* and *Strophanthus kombé*. Cholesterol (**15**) was converted into pregnenolone (**33**) by *Digitalis purpurea* [71] by *Haplopappus heterophyllus* [56] and by the leaves of *Punica granatum* [72]. The first metabolites of cholesterol in potato plants, were identified as 26-hydroxycholesterol (**16**) and cholest-4-en-3-one [73]. Sitosterol (**14**) was converted into progesterone (**32**) by *Digitalis lanata* plants [74] and pregnenolone was formed from holaphyllamine (**38**) in *Holarrhena floribunda* [75].

Pregnenolone and progesterone can be converted into a great number of other steroids [67, 76–78] including a corticosteroid (11-desoxycorticosterone (**52**) [79]. Related pregnane-derivatives were also metabolized by several higher plant species [78, 80, 81].

Occurrence of steroid sex hormones and plant flowering

The occurrence and the concentration of estrogens in higher plants is often related to flowering or to other physiological states of the plant. Kopcewicz and Chromiński [82] found a higher estrogen content (identification only by the Kober colour reaction) in *Cucurbita pepo* plants which were induced to develop as females through treatment with 2-chloro-ethylphosphonic acid. Bennett *et al.* [28] found phenolic isoprenoids (estrogenlike ?), formed from mevalonic acid [2-^{14}C] only in flowering *Haplopappus heterophyllus*. The labelled material was not identical with any of the common steroidal estrogens, but the possibility that it was a steroid can not be dismissed. Kopcewicz [83] showed that during the flowering period of *Phaseolus vulgaris* the estrogen content increased (measured by the Kober colour reaction). No estrogens were detected in ripe seeds and young seedlings. Estrogens were found during the formation of the flower buds and in the course of their development to flowers. The highest estrogen concentration was found in the leaves of the flowering plants. In ripening pods the estrogen content decreased.

In *Perilla ocimoïdes* and *Chenopodium rubrum*, estrogen biosynthesis occurred only when the plants were held under inductive light conditions [84]. Estrogenlike substances were also found [85] in *Hyacinthus orientalis* bulbs (Kober colour reaction and spectrophotometric assay). Cold-treatment of the bulbs, necessary for the normal growth of the inflorescence and the leaves, caused an increase in the estrogen content, which was highest in the leaves and the inflorescences.

Interactions of animal steroid sex hormones with the plant hormones

Not much is known about the way in which animal steroid sex hormones realize the effects discussed above. A few studies have been undertaken to examine the influence of animal steroid sex hormones on the content of the accepted plant hormones and vice versa. Kopcewicz and Chromiński [82] found a higher estrogen content in *Cucurbita pepo* plants treated with 2-chloro-ethylphosphonic acid (source of ethylene production). Plants which had been treated in this way mainly formed female flowers. The growth of dwarf peas was stimulated by estrone (**1**), probably through a stimulated biosynthesis of gibberellins (quantitated in lettuce hypocotyl biotest) [21, 22, 86]. Estrone (**1**), 17β-estradiol (**2**) and estriol (**4**) caused an increase in the auxin content (concentration determined in the Avena cylinder test) of dwarf pea and young pine seedlings. At the same time the growth of the pea seedlings was stimulated by *ca* 40%. The growth of the pine seedlings, however, was not influenced [23, 24].

Kopcewicz [87] demonstrated that applied kinetin increased the estrogen content of bean seedlings, whereas applied abscisic acid decreased the estrogen content. Auxin and gibberellins were inactive. The increased estrogen biosynthesis after kinetin treatment may be involved in the flower promoting effect of kinetin in *Cichorium intybus* grown under non-inductive conditions [88]. Kopcewicz [30] obtained evidence that in this long day plant, requiring a cold period, the inductive cold period could be replaced by a gibberellic acid treatment, just as by a 17β-estradiol (the effect equals 85% of the GA_3-treatment) or by an estrone treatment (55% of the GA_3-effect). However, the plants treated with gibberellic acid were taller and flowered earlier than plants treated with estrogens. Possibly the effect of applied estrogens can be explained through a stimulated gibberellic acid biosynthesis. Kopcewicz and Porazinski [31] induced flowering in the long-day plant *Salvia splendens*, grown under short-day conditions, by application of a mixture of GA_4 and GA_7, estradiol, N_6benzyladenine and also by an unidentified estrogen-like fraction isolated from flowering *Salvia splendens* plants. This latter extract was more active than estradiol itself. Biswas *et al.* [32] obtained flowering in *Chrysanthemum* spp. growing under non-inductive conditions; sitosterol (**14**), lanosterol (**21**), glycocholic acid (**22**) and androstane (**11**) were active. However, one should be critical of the interpretation of these results, because the authors used only a very limited number of plants (2×5) per treatment.

The results cited above suggest that animal steroid sex hormones can play a prominent part in growth, differentiation and flowering of certain higher plants. Undoubtedly estrogens and androgens have been isolated from different plant sources and through the use of new sophisticated analytical methods they will certainly be detected in many other plant species. Many physiological effects of applied animal steroid sex hormones have been observed. The effects on the sex expression of reproductive organs are extremely interesting. A study of the interactions of animal steroid sex hormones with the known plant hormones should be a promising approach to the process of sex determination which, for most plant species, is far from understood.

The few reports on flower formation under non-inductive conditions by applied animal steroid sex hormones may be an indication that steroid hormones are synthesized during normal flower induction. A generally known inhibitor of steroid biosynthesis, SKF 7997, also inhibits flower formation. This supports the suggestion that steroid hormones are indeed involved in flower formation. However, a detailed biochemical study of the steroid hormone production under well-defined

physiological conditions, using sensitive radioisotope techniques under aseptic conditions, is still required to prove the involvement of steroid sex hormones in flower formation. As animal steroid sex hormones influence the vegetative development of certain plants it is possible that certain effects of applied steroid hormones on flower formation are indirect.

ECDYSONES

Several authors describe the isolation of insect moulting hormones from plants [7, 89–93] and it has been shown that plants are able to convert cholesterol (**15**) into ecdysterone (**39**) [7, 94–96].

The function of ecdysones in plants remains unknown [7, 89].

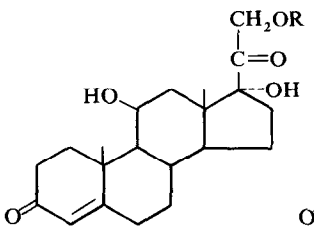
There seems to be no direct evidence that the plant ecdysones play an important part in plant protection by interference with the metamorphosis of phytophagous insects. Carlisle *et al.* [97] found that a locust extract which contained ecdysone (**40**) activity when tested on insect larvae, could stimulate the growth of dwarf peas (only 10% of the gibberellin activity), while gibberellin accelerated the moulting of insect larvae. Hendrix and Jones [98] tested β-ecdysone (**40**) in gibberellin bio-assays (α-amylase induction, leaf growth test with dwarf maize, growth of dwarf peas and the germination of *Anemia* spores) and found it to be completely inactive. Consequently, one should perhaps be cautious when interpreting results obtained with crude extracts of unknown hormone composition, like those of Carlisle *et al.* [97].

CORTICOSTEROIDS

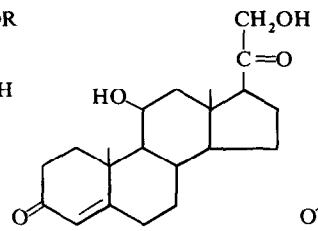
Physiological effects

Corticosteroids are biosynthesized in the animal adrenal cortex. This section will consider those C_{21}-corticosteroids that possess 'mineralocorticoid' and 'glucocorticoid' activity. Corticosteroids are pregnane derivatives and the basic structural requirements for biological activity in animals are demonstrated by 11-desoxycorticosterone (**52**). The particular features are [37] (*a*) a double bond between C-4 and C-5, (*b*) an oxo group at C-3, (*c*) an oxo group at C-20, (*d*) a hydroxyl group at C-21 which is required for any effect on carbohydrate and protein metabolism ('glucocorticoid activity') but it also enhances the capacity of sodium retention and water metabolism ('mineralocorticoid activity').

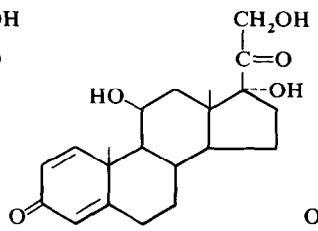
Moreover, for glucocorticoid activity, an oxygen is necessary at C-11 (11-oxo or 11β-hydroxyl group) but this 11-oxygen function decreases the mineralocorticoid activity. Aldosterone (**57**) is an exception because it is the most potent natural mineralocorticoid, although it possesses an 11β-OH group.



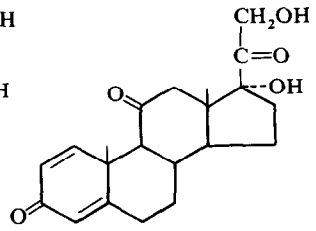
41. R = H; Cortisol
42. R = Ac; Cortisol acetate



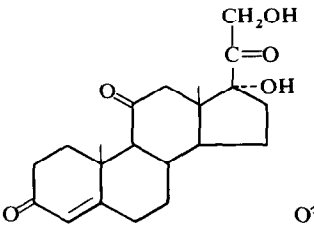
43. Corticosterone



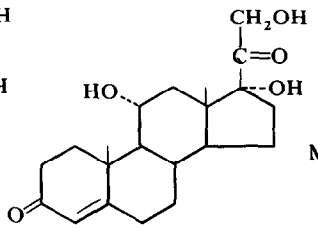
44. Δ^1-Cortisol



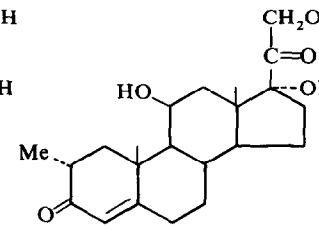
45. Δ^1-Cortisone



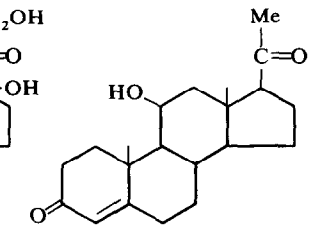
46. Cortisone



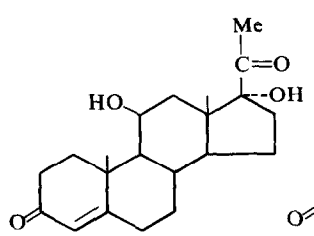
47. Epicortisol



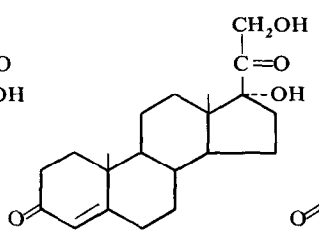
48. 2α-Methylcortisol



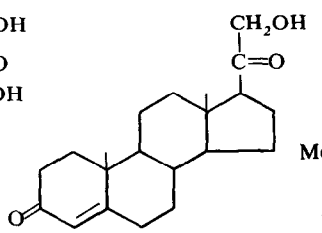
49. 11β-Hydroxyprogesterone



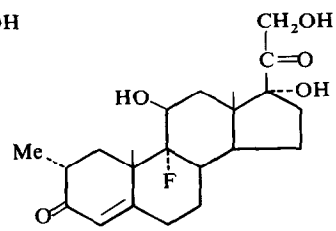
50. 21-Desoxycortisol



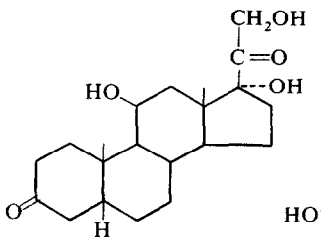
51. 11-Desoxycortisol



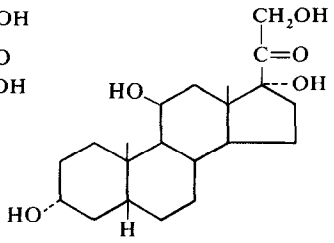
52. 11-Desoxycorticosterone



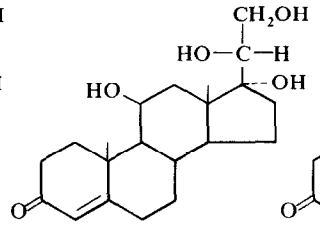
53. 2α-Methyl, 9α-fluorocortisol



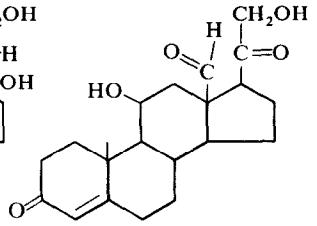
54. 5β-Dihydrocortisol



55. Tetrahydrocortisol (THF)



56. Reichstein's substance E



57. Aldosterone

Helmkamp and Bonner [16] observed an inhibitory effect of cortisone (**46**) when applied to the culture medium of isolated pea embryos. In concentrations of 1 and 10 mg/l. cortisone inhibited the shoot growth by 22 and 85% respectively, whereas the root growth was only inhibited (75%) by the higher cortisone concentration.

Boiteau and Ratsimamanga [99] reported a minor influence (8% stimulation) on fresh weight increase and on the germination of *Ervum lens* after applying cortisone (**46**) for 25 days. Cortisone influenced the fresh weight increase of *Ervum lens* seedlings (10% stimulation after 11 days), the root length (43% stimulation after 20 days) and the shoot length (34% stimulation after 20 days) [100]. Hydrocortisone (= cortisol, **41**) was active in *Ervum lens* [101] but the results are difficult to interpret because the percent differences are given not the absolute values of the measurements. When hydrocortisone was applied, the fresh weight increased by some 33% after 5 days, the shoot length by some 24% after 8 days. Donnet *et al.* [102] reported a 52% stimulative effect of hydrocortisone acetate (**42**) on *Ervum lens*, *Triticum sativum* and *Phaseolus vulgaris* plants after 3 weeks, but these authors did not specify what exactly they had measured. Kopcewicz [34] found that the number of flowers on *Ecballium elaterium* could be enhanced (15% stimulation) by applied cortisone (**46**). The sex expression of the flowers was not influenced. Kallistratos [103] observed that hydrocortisone (= cortisol, **41**) and prednisolone (= Δ^1-cortisol, **44**) applied together with cytostatica to *Vicia faba* roots caused abnormal mitotic divisions. When both thiocolchicin (**58**) and cortisol were applied many cells were observed with two or more nuclei, probably because the formation of the phragmoplast was blocked. Convallamarin (**59**) (1.5 g/l.) and cortisol (0.75 mg/l.) when applied together, induced the formation of tripolar anaphases. Cortisol (0.6 mg/l.) or prednisolone (0.6 mg/l.), when applied together with thiocolchicin (**58**) (40 mg/l.) synergistically induced polyploidy.

The effect of applied corticosteroids on the growth and development of mung bean seedlings has been examined [104–106]. The optimum concentration for applied cortisol (**41**) was about 15 mg/l. ($=4.1 \times 10^{-5}$ M). After a 48–72 hr growth period in cortisol solution (15 mg/l.) the lateral root number and the root length were doubled, whereas the growth of the hypocotyl was stimulated by 20–50%. Neither the germination of the mung bean seeds, nor the fresh and dry weight increases of the roots and the hypocotyls were influenced by the cortisol treatment, which suggested that, due to cortisol treatment, the growth became oriented along the longitudinal axis of the organs. Measurements of cell lengths made clear that the observed length increase in the roots was due to a stimulated cell elongation growth and not to a stimulated activity of the apical meristem. The increased lateral root production which had been observed was due to a stimulation of lateral root initials as had been demonstrated by decapitation experiments and also by counting the lateral root initials after the application of a clearing technique. Even in 36 hr old treated seedlings an enhanced number of lateral root meristems could be observed [104]. Cortisol, very active when tested on the intact seedlings, was not active in mung bean hypocotyl section tests nor in *Avena* cylinder tests (J. Geuns, unpublished).

Cortisol also stimulated the adventitious root formation on the hypocotyls of intact mung bean seedlings grown in cortisol solution (730% stimulation). However, it was practically inactive on adventitious root formation on hypocotyl cuttings [105]. However with hypocotyl cuttings the number of adventitious root initials was greatly enhanced by cortisol treatment (J. Geuns, unpublished results). It is not clear why the numerous initials do not develop to form adventitious roots.

When measuring the cell lengths in the root zones between 10 and 12 mm from the root apex, it was also observed that the nucleoli in the segments of roots treated with cortisol were much larger (Table 1). The nucleolus volume in the treated roots was 1.88 times higher than that of the control roots. This may be an indication of an enhanced *r*RNA (ribosome subunits)

Table 1. Effect of cortisol (15 mg/l.) on the nucleolus volume in 48 hr old roots of etiolated mung bean seedlings grown at 30°. Each value is the mean of 50 nucleoli (J. Geuns unpublished results)

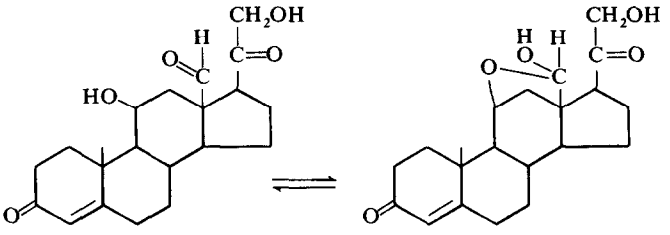
Exp	Control radius (μm)	Control volume (μm³)	Cortisol radius (μm)	Cortisol volume (μm³)	vol cortisol / vol control
1	1.072 ± 0.024	5 160	1.439 ± 0.040	12 482	2.42
2	0.962 ± 0.020	3 728	1.244 ± 0.038	8 064	2.16
3	1.183 ± 0.037	6 935	1.309 ± 0.052	9 395	1.35
4	1.205 ± 0.028	7 329	1.409 ± 0.048	11 717	1.60
Mean	1.105	5 788	1.350	10 414	1.88

synthesis stimulated by cortisol (also suggesting its glucocorticoid activity in plants, see below).

The measurements of the root cell lengths suggested [105] that cortisol interferes with one or more compounds (IAA or ABA?) limiting the growth of control roots since although cells of the root bases (formed during the first 24 hr after germination) had a length of some 100 μm in both control and treated roots, cells in the zone between 10 and 12 mm from the apex of 48 hr control roots were only about 50 μm long, against 100 μm for cells from the corresponding zone of roots treated with cortisol. Interaction studies of cortisol with IAA constituted a first approach to elucidate this problem. In these studies it was proved that the growth inhibition obtained by the higher IAA concentrations (10^{-4}, 5×10^{-5}. 10^{-5} M) could be counteracted by adding cortisol [107].

Structure requirements for activity in higher plants

Cortisol is not the only active corticosteroid. Structure–activity relationship studies [106] with sixteen corticosteroids revealed that in plants a distinction also has to be made between 'glucocorticoids' (regulating the organic metabolism of animal cells) and 'mineralocorticoids' (influence on the salt and water metabolism of animal cells). As the 11-oxygen function is very important for the type of effect of the hormone, the corticosteroids may be grouped into three categories: (i) The corticosteroids with an oxygen at C-11 have a pronounced glucocorticoid activity and little effect on water and electrolyte metabolism. A 17α-OH group enhances the glucocorticoid activity. (ii) The corticosteroids without an oxygen at C-11 have a pronounced mineralocorticoid activity without much glucocorticoid activity. (iii) A special case is aldosterone (**57**) with an aldehyde group at C-18 and an 11β-OH group. This hormone is the most potent mineralocorticoid. In solution the hormone exists in hemiacetal and aldehyde forms.



Aldehyde Hemiacetal

(**57**) Aldosterone

It has been demonstrated [106] that glucocorticoids are very active in mung bean seedlings. Most of them double the elongation growth of the root as well as the lateral root number. After a 72 hr growth period, the hypocotyl growth was stimulated up to *ca* 50%. The compounds tested were cortisol (**41**), corticosterone (**43**), Δ^1-cortisol (**44**), cortisone (**46**) and Δ^1-cortisone (**45**). In animals 2α-methylcortisol (**48**) has the same effects as cortisol (**41**). In mung beans, however, it inhibited the growth and lateral root formation. 11α-hydroxycortisol (**47**) was completely inactive.

The glucocorticoids with an 11-oxo-function (cortisone **46**, Δ^1-cortisone **45**) were less active than their corresponding 11β-hydroxy-forms (cortisol **41**, Δ^1-cortisol **44**), possibly because the conversion of the 11-oxo group into an 11β-hydroxy is very slow in mung beans [104]. In animals the 11β-hydroxyl form of the steroid is the active substance [108–109]. The reduction of 11-oxo-steroids into the 11β-hydroxyl forms has not been studied in higher plants but Bennett and Heftmann [110] studied the reverse reaction and showed the conversion of cortisol (**41**) into cortisone (**46**) in *Mallotus paniculatus*.

Precursors of corticosteroids with an 11β-hydroxyl group (11β-hydroxyprogesterone, **49**; 21-desoxycortisol **50**) were very active [106]. Steroids without the 11β-hydroxyl group (11-desoxycortisol **51**; 11-desoxycorticosterone **52**) inhibited the growth or were inactive.

11-Desoxycorticosteroids (11-desoxycortisol **51**, 11-desoxycorticosterone **52**) are probably not converted into their corresponding 11β-hydroxy forms [106]. In animals these steroids have a mineralocorticoid activity like aldosterone (**57**) and 2α-methyl-9α-fluoro-cortisol (**53**). These mineralocorticoids inhibited the growth or were inactive [106].

The reduced products of cortisol (excretion compounds in animals) were very active in mung bean seedlings, although they are inactive in animals [106]. To this group belong 5β-dihydrocortisol (**54**), tetrahydrocortisol (= THF **55**) and Reichstein's compound E (**56**). It is not yet known whether these reduced corticosteroids are active as such or after conversion into the more oxidized cortisol.

Metyrapone (**60**), an inhibitor of steroid hormone biosynthesis in animals, inhibited the elongation and the fresh and dry weight increase of roots, hypocotyls and leaves of etiolated mung bean seedlings. The lateral root formation was also inhibited. In addition chlorophyll, carotenoid and anthocyanin biosynthesis were inhibited by the metyrapone treatment [111]. In animals metyrapone inhibits steroid hydroxylation reactions, mainly the 11β-hydroxylation of corticosteroids [112]. It exerts its inhibitory activity by binding to cytochrome P450, which has to be considered as an active constituent for hydroxylation reactions. Cytochrome P450 has been isolated from many plant species [111] and it is involved in several hydroxylation reactions in plants. The above results with corticosteroids support the suggestion that the inhibitions obtained by metyrapone are due to an inhibition of the steroid hormone (corticosteroids) production in plants. However, the correctness of this hypothesis has to be verified by detailed biochemical work.

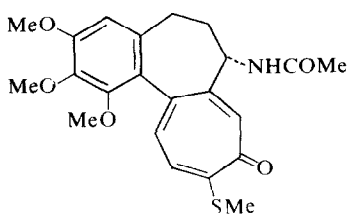
Isolation of corticosteroids

So far only one corticosteroid has been isolated from a

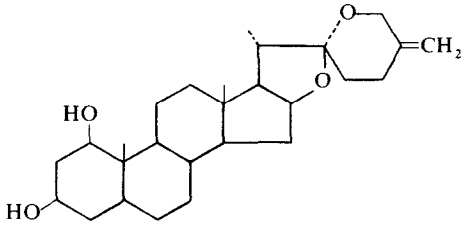
higher plant. Bahadur and Srivastava [79] isolated 11-desoxycorticosterone (**52**) from the unsaponifiable matter of *Oryza sativa* husk oil. The conversion of applied progesterone into 11-desoxycorticosterone (**52**) by *Digitalis lanata* plants has been observed by Caspi *et al.* [113]. Pregnenolone (**33**) and progesterone (**32**), the precursors of corticosteroids and other steroid hormones (in animals) have been isolated from several plant species. Corticosteroids are rather labile compounds that usually occur in very low concentrations in animals. If present in plants, they probably also occur at low concentrations, as estimated from incorporation experiments with cortisol [^{3}H] (J. Geuns, unpublished results). This may be the reason why so little is known about the natural occurrence of corticosteroids or similar compounds in plants. Nevertheless many plant species possess steroids oxygenated at C-11. This is interesting because of the importance of the 11-oxygen function for the activity of corticosteroids in plants [106]. It also proves that many plants possess the enzyme system required for the 11-oxygenation which, in animals only takes place in the mitochondria of the adrenal cortex [37, 114]. Many 11-oxo-steroids, 11β-hydroxy or 11α-hydroxy-steroids have been isolated from plants [115, 116]. Applied pregnenolone (**33**) was converted into an 11-oxo-steroid (digifologenin **68**) by *Digitalis lanata* [69, 117]. Glycyrrhetinic acid (**61**), a pentacyclic triterpene with an 11-oxo function, has been isolated from the root of *Glycyrrhiza glabra* [118]. This compound is used in medicine because of its corticosteroid activity in man (cortisonelike anti-inflammatory acitivity and desoxycorticosterone acetate like effects on sodium and potassium metabolism) [119–124] and the 11-oxo group is required for its physiological activity [125]. It is interesting that plants possess compounds with corticosteroid activity in animals, whereas the animal corticosteroids stimulate the elongation growth and root formation in plants. Perhaps compounds, such as glycyrrhetinic acid, play a normal role as naturally occurring plant corticosteroids.

The cucurbitacins constitute another group of 11-oxygenated triterpenoids [126] and many of them possess an 11-oxo or 11β-hydroxyl function, a 3-oxo-function and a Δ^5-double bond. They chiefly occur in the Cucurbitaceae, Cruciferae and Scrophulariaceae. Their function in plants remains practically unknown. Guha and Sen [127] studied the effect of five cucurbitacins on rice seedling growth. Cucurbitacin B (**62**; 9 µg per plant) alone had only a slight inhibitory effect, but when applied together with GA_3 (5 µg per plant), it inhibited the GA_3 effect by 50%. Also cucurbitacins E (**63**), I (**64**), J (**65**) and K (**66**) (5–10 µg/l.) behaved as strong antigibberellins when applied together with GA_3 (5 µg/plant).

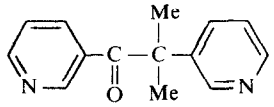
Although lateral root formation, adventitious root formation and also the elongation of roots and hypocotyls are stimulated by glucocorticoids, this does not necessarily mean that corticosteroids influence these phenomena directly. Glucocorticoids are known to regulate organic metabolism in the animal cell and they may well have a similar role in the plant cell. Beside their 'glucocorticoid activity', glucocorticoids play a so-called 'permissive role' in animal cells, i.e. for the response of many hormones to take place, a minimum concentration of glucocorticoids has to be present in the cells [37, 114, 128]. In this respect the interaction of corticosteroids with the known plant hormones should certainly



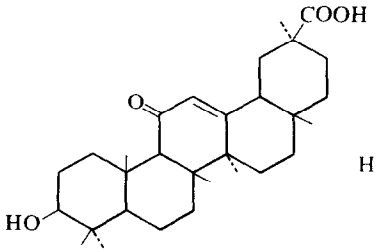
58. Thiocolchicin



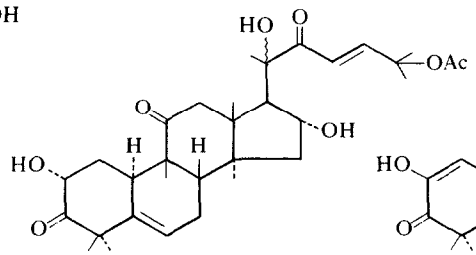
59. Convallamarogenin



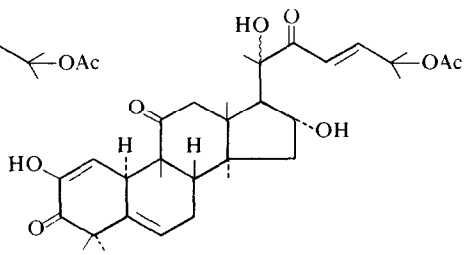
60. Metyrapone



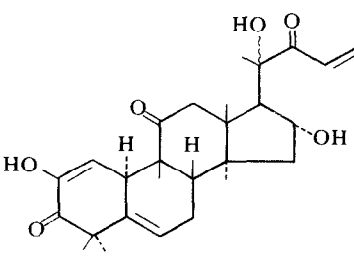
61. Glycyrrhetinic acid



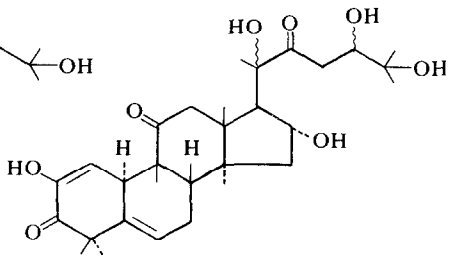
62. Cucurbitacin B



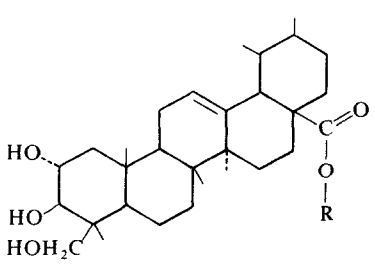
63. Cucurbitacin E



64. Cucurbitacin I



65. and **66.** Cucurbitacin J and K, epimeric at C-24



67. Asiaticoside

provide interesting results. Although direct proof is lacking, it may be suggested that the mineralocorticoids have a similar function as in animals, i.e. the regulation of water and electrolyte metabolism.

OTHER GROUPS OF STEROIDS

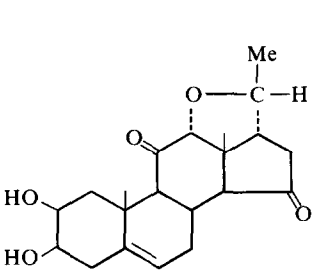
Asiaticoside

Oxyasiaticoside, derived from the pentacyclic triterpene asiaticoside (**67**), isolated by Boiteau and Ratsimamanga [129], stimulated growth and chlorophyll biosynthesis in radish, *Linum grandiflorum*, pea and *Lupinus* × *polyphyllus*. Also anthocyanin biosynthesis was stimulated in radish [99, 130]. Whereas high concentrations of oxyasiaticoside inhibited growth, more dilute solutions stimulated the pea root growth by 23% and the shoot growth by 68% as measured 15 days after the application of the substance. In radish lateral roots, chlorophylls and anthocyanins were observed 24 hr earlier than in the controls.

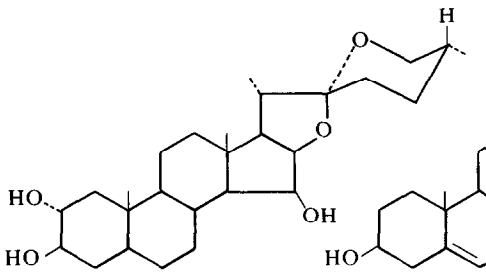
Saponins, digitonin and tomatine

In a series of papers Balansard *et al.* [131–138] reported that saponins may elicit plant growth responses They used various concentrations (1 to 1000 mg/l.) of quillaja, polygala, saponaria and sapindus saponins. Whereas higher concentrations were almost poisonous, lower concentrations stimulated growth. The growth rate of isolated wheat embryos was approximately doubled by low concentrations of saponin. Applied saponins also increased the rate of development of the shoots and roots on *Begonia* leaves. Treatment of the seeds of cereals and tomato resulted in an increased growth rate. Treated pea embryos imbibed more water and the imbibition process itself was also accelerated. The imbibition by *Zea mays* grains was faster and enhanced with a subsequent increased growth rate of the seedlings.

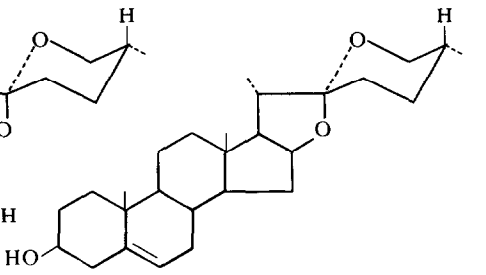
Von Euler [19] germinated seeds of *Lepidium sativum* and *Hordeum vulgare* in solutions of digitonin (**69**) (0.02%) and quillaja saponin. Root growth of *L. sativum* was inhibited by *ca* 75% after 12 days in the digitonin solution. Also the root growth of *H. vulgare* was inhibited by the digitonin treatment. Quillaja saponin (0.03 and 0.01%) inhibited the shoot and root growth of *Lepidium* by *ca* 40% and *ca* 90%. The mitotic activity in the root tips of *H. vulgare* was strongly inhibited. The few mitoses were normal. Olah [139] reported abnormal mitoses in *Allium sativum* root tips after digitonin treatment.



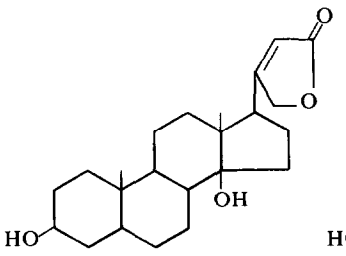
68. Digifologenin



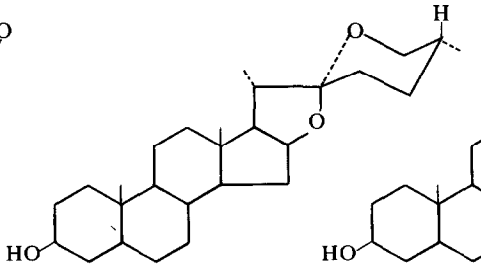
69. Digitogenin



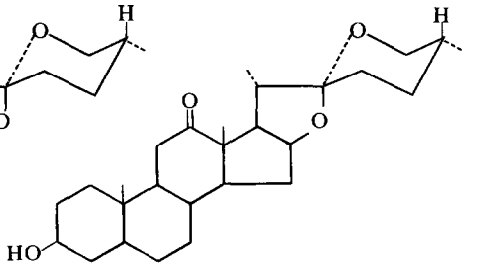
70. Diosgenin



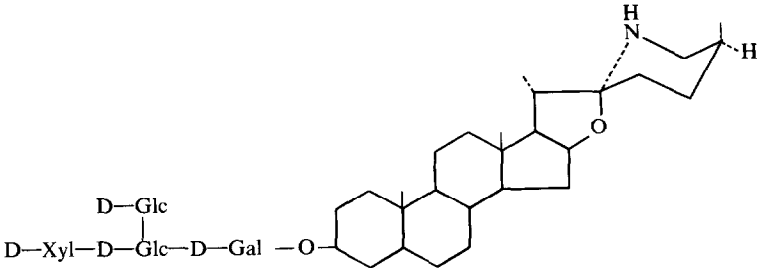
71. Digitoxigenin



72. Tigogenin



73. Hecogenin



74. α-Tomatine

Helmkamp and Bonner [16] applied saponins to the culture medium of isolated pea embryos. At an optimum concentration of 10 mg/l. three relatively crude saponin preparations as well as one pure compound, diosgenin saponin (**70**), stimulated the stem growth by 17 to 43% and the root growth by 32–47%. Jonas [140] observed that the aqueous extracts of *Digitalis purpurea* stimulated the adventitious root formation on tomato cuttings, although they were toxic to a number of plant species. The application of pure digitoxin (**71**) emphasized these symptoms.

Vendrig [141] found a stimulative effect of digitonin (**69**), tigogenin (**72**), hecogenin (**73**) and tomatine (**74**) in the *Avena* section test. This stimulation of the cell elongation growth by digitonin and tomatine could not be reproduced by other authors [142, 143]. Roddick [143] did not find auxin activity of tomatine (**74**). In oat celeoptile sections it was growth inhibitory at 10^{-3} M, in wheat coleoptile tests at 10^{-4} M. In interaction studies with oat coleoptiles, antagonistic effects were observed at higher alkaloid concentrations. 10^{-4} M tomatine reduced the IAA-enhanced growth by 65%, although, alone, it was inactive. Tomatine possibly acts via an alteration of the membrane structure or function. It is known that tomatine forms 1:1 complexes with sterols [144]. Its inhibitory effect may be due to an alteration of the membrane structure or to a membrane permeability change, for it is known that sterols regulate membrane permeability [145]. Roddick [146] also studied the interactions of tomatine and IAA in the elongation growth of wheat coleoptile sections. Whereas 10^{-5} M tomatine alone did not influence the growth, it reduced the IAA-enhanced extension growth by *ca* 50%. The results are discussed in relation to the ability of tomatine to alter the membrane function, and to the hypotheses implicating membranes in the primary action of (applied) IAA.

Roddick [147] also studied the effect of α-tomatine on the permeability of plant membranes. The leakage of betacyanin from beetroot disks was measured, together with the efflux of UV (260 nm) absorbing substances from potato, apple and carrot disks. Tomatine induced the efflux from all tissues, though there was a difference in sensitivity towards the alkaloid. The addition of $CaCl_2$ or $MgCl_2$ reduced the tomatine induced efflux of cell material. In potato disks, applied cholesterol (**15**) caused a small reduction in cell efflux. High concentrations of applied tomatine stimulated the K^+ efflux from the fruit disks of tomato [148], but there was no influence of the developmental stage of the fruits. The growth of tomato seedlings in 1 mM tomatine solution was not affected, whereas the growth of the hypocotyls and roots of lettuce seedlings was inhibited by 34 and 73% at 0.1 mM, and by 73 and 88% at 1 mM tomatine. A review on tomatine has appeared [149].

An interesting feature of the steroid alkaloids is that steroid hormones can be formed from them. Bennett *et al.* [75] found that holaphyllamine (**38**) is metabolized to pregnenolone (**33**) by *Holarrhena floribunda*. Tomatine (**74**) applied to ripe tomatoes is converted into allopregnenolone (**34**) [150]. This substance could function as an intermediate for further steroid hormone production in plants. However, it is known that many plants possess a more direct route for the biosynthesis of steroid hormones, i.e. the conversion of cholesterol (**15**) or sitosterol (**14**) into pregnenolone (**33**) and progesterone (**32**) [2, 48, 151, 152].

Sterols

Stimulatory effects of applied sterols have been reported. Sitosterol (**14**) and lanosterol (**21**) induce flowering in *Chrysanthemum* spp. growing under non-inductive conditions. The applied sterols (cholesterol **15**, its acetate, sitosterol **14**, stigmasterol **17** and lanosterol **21**) did not influence the stem elongation growth [32]. Some doubt may exist about the stimulating effects because the authors only tested 2 × 5 plants per treatment. Cholesterol (**15**) (25 mg/l.) promoted the flowering (+171%) and flower peduncle length (+300%) of broccoli curd cuttings [29]. The root formation, as measured by the dry weight production per curd, was doubled by cholesterol treatment (50 mg/l.). Helmkamp and Bonner [16] found no effect of applied sitosterol (**14**) and stigmasterol (**17**) on the growth of excised pea embryos. Applied cholesterol (**15**; 0.1 mg/l.) and ergosterol (**19**; 10 mg/l.) inhibited the root growth by 20 and 28% respectively, the stem growth being unaffected. Kopcewicz [21] reported a stimulative effect of sitosterol (**14**) on the growth of dwarf peas but cholesterol (**15**) was inactive.

The growth inhibition obtained by Amo 1618, CCC and Phosphon D in *Nicotiana tabacum* seedlings could be counteracted by gibberellic acid [153]. In Amo 1618 and CCC-treated plants the addition of sitosterol (**14**), stigmasterol (**17**) or cholesterol (**15**) could also restore the normal growth. In Phosphon D treated plants, however, applied sterols were unable to restore the normal growth.

Kimura *et al.* [154] reported a synergistic effect of stigmasteryl-β-D-glucoside (**18**) with IAA in the *Avena* coleoptile segment test. In a later paper, however, the same authors could not reproduce the synergistic action of various steryl glucosides with IAA [155]. Although these authors report stimulative effects with the glucosides of stigmasterol (**17**), sitosterol (**14**) + campesterol (**20**) and cholesterol (**15**) in the *Avena* cylinder test, the growth of *Lepidium sativum* seedlings and the H^+-efflux from *Vicia faba* leaf protoplasts, one should perhaps be critical since the authors failed to demonstrate the reproducibility of their results.

The polyene antibiotic filipin (100 μg/ml), applied on the apical and the next five axillary buds of *Xanthium*, just before each of the three 16 hr photoperiods that are necessary to induce flowering, inhibited the development of the buds into flowers (inhibitions of 25, 54, 56 and 80% of respectively the apical buds and the first, second and third axillary ones: In the fourth and fifth axillaries no development to flowering took place) [156]. The effect of filipin was probably due to its interaction with the membrane sterols, as a pretreatment of the buds with cholesterol (50 μg/ml) reversed the filipin action.

CONCLUSIONS

It is now certain that steroid hormones (estrogens, androgens, progestogens, ecdysones and a corticosteroid) have been isolated from plant sources. In addition, many physiological responses have been obtained by applying steroid hormones to plant bio-assays. It is true that various authors were unable to reproduce the stimulative effects of applied steroid hormones. The poor solubility of most steroid hormones in water, often necessitating an appropriate co-solvent, may have been one of the causes for their failure. In view of the present-day knowledge of the similarities between animal and plant cells, especially

in their biosynthetic pathways to steroids, the study of steroid hormone biosynthesis and metabolism in plants deserves more attention than is the case at present

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