

REVIEW

FUNCTIONS OF STEROIDS IN PLANTS*

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(Received 15 August 1974)

Key Word Index—Steroids; saponins; glycoalkaloids; cardiac glycosides; function; hormonal effects; review.

Steroids are sometimes referred to as secondary plant metabolites, because the distribution of certain classes, such as the cardiac aglycones and the alkalamines, is restricted to a few plant families. However, it is becoming increasingly evident that all plants contain steroids of some kind and that they are vitally important cell constituents. In this article I intend to review what is known about the functions of steroids in plants and, extrapolating from this, I would like to advance some further speculations of my own.

STEROLS

Cholesterol (1), is a typical representative of the plant sterols, a class of steroids characterized by a long side chain at C-17. It is perhaps the most widely distributed of all such compounds [1, 2]. This fact was not recognized until 1963 [3], because the amounts present are in most cases very small and the separation and identification of sterols was very difficult until the introduction of GLC and GC-MS and radioactive techniques [4]. Using radioactive precursors such as acetate and mevalonate showed that cholesterol could not only be readily detected but that it is also rapidly synthesized and metabolized. Research over the past decade has shown that cholesterol has a very important function in plants [5] where, as in animals [2], it, or one of its precursors, serves as the

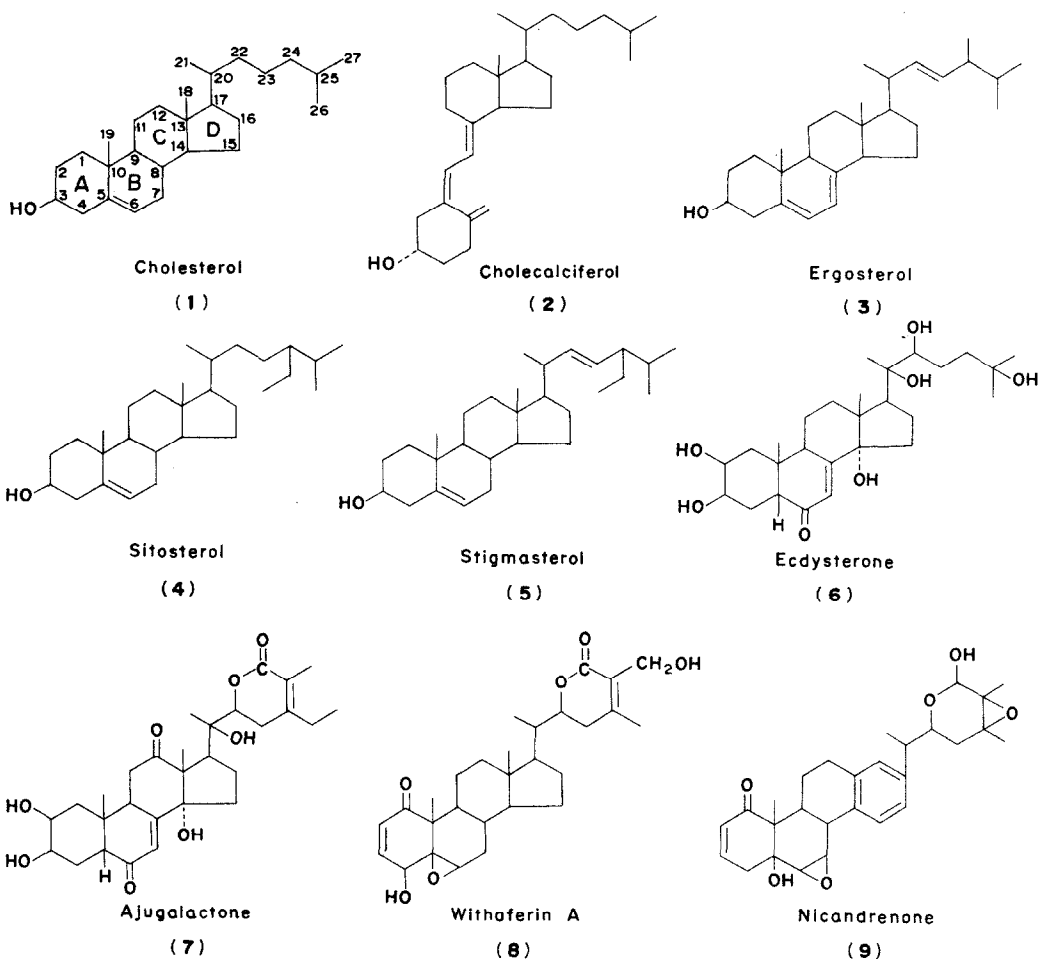
starting material for the biosynthesis of all other steroids.

Cholecalciferol (vitamin D₃) (2) has been isolated from Orchard grass and other plant sources [6]. Vitamin D increases the absorption of calcium and phosphate ions in animals [7], but it is not known whether it has any biological significance for plants. There is good reason to believe that in animals the vitamin acts, like the steroid hormones, at the level of genetic control [7, 8]. Apparently, a metabolite of the vitamin derepresses a gene which directs the synthesis of a component of the calcium transport system, manifested as a calcium-dependent ATPase. It is not impossible that such an activity could take place in plant cells and result in increased calcium and perhaps also phosphate ion absorption by plants.

When fungi or higher plants are analyzed, relatively large quantities of sterols with 28 or 29 carbons are usually isolated—most commonly ergosterol (3), sitosterol (4), and stigmasterol (5). Ergosterol is a precursor of ergocalciferol (vitamin D₂). Irradiated yeast and other irradiated plant products are well-known sources of vitamin D. Sitosterol has been claimed to have estrogenic activity [9], and stigmasterol has been claimed to be a vitamin for guinea pigs, the antistiffness factor [10].

As I have already indicated, one of the functions of sterols in plants is to act as precursors of other plant steroids. Thus, we have observed [11] that *Podocarpus* seedlings convert cholesterol to the insect-molting hormone, ecdysterone (6). In the past few years, dozens of insect-molting hormones have been isolated from a great variety of plants [12].

* Presented at the Symposium on "The Biological Significance of Secondary Metabolites" of the Phytochemical Section of the Botanical Society of America on 18 June 1974, in Tempe, Arizona.



Some are the same C_{27} sterols (e.g. 6) that have also been found in insects, but many of them are C_{28} and C_{29} compounds which are probably produced from precursors such as ergosterol and sitosterol that do not occur in insects. Insect-molting hormones often occur in much larger amounts and greater variety in plants than they do in insects and the plant compounds are often more potent in activity than those found in insects. Like most steroid hormones, the insect-molting hormones contain an α,β -unsaturated carbonyl group. Ajugalactone (7), a hormone analog occurring in *Ajuga decumbens*, also inhibits insects metamorphosis [13]. Withaferin A (8), a structurally related compound which was isolated from *Withania somnifera* and other Solanaceae, is a representative of another large group of plant steroids, called the withanolides [14]. It is a potent antimitotic agent, which inhibits the growth of plant [15] and animal

cells, including tumor cells [16]. Also structurally related is nicandrenone (9), an insect-repellent plant steroid recently isolated from the Peruvian weed *Nicandra physalodes* [17]. Clearly, substances that repel or kill insects are ecologically important to plants [18], but to assert that the *raison d'être* of all secondary plant substances that are unpleasant or toxic to animals is that they are protective agents [19] is, in my opinion, a gross oversimplification.

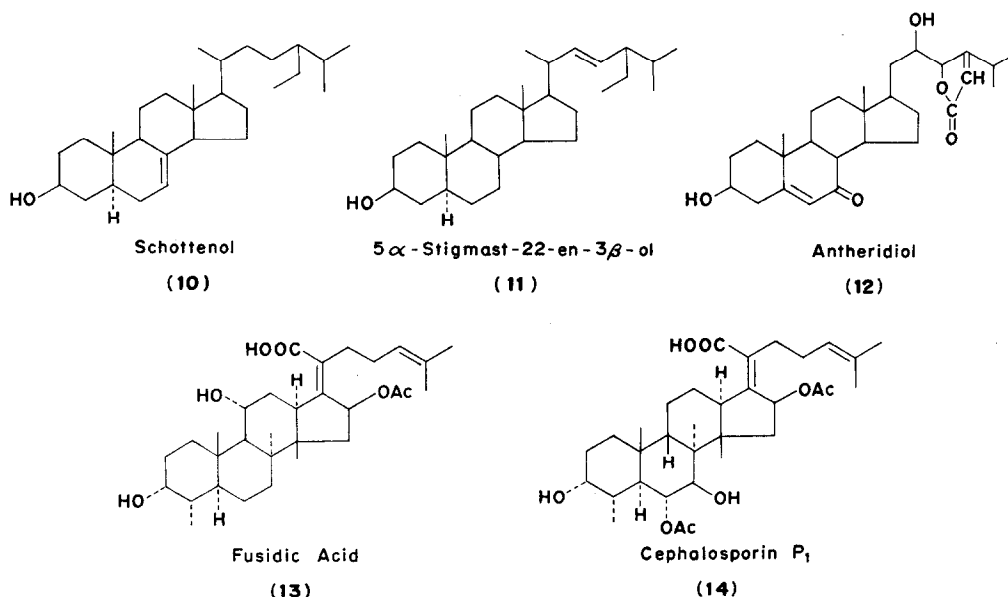
As far as the insect-molting hormones in plants are concerned, there is no evidence that they have any adverse effects on the insects that feed on them [20]. On the contrary, insects, being incapable of synthesizing the steroid nucleus, depend on plants to supply them with suitable sterols. Sterols are by definition, therefore, vitamins for insects. For instance, schottenol (10), which is elaborated by the Senita cactus, *Lophocereus schottii*, is specifi-

cally required by *Drosophila pachea*, which breeds on this cactus, for its larval development. The role of steroid hormones in the metamorphosis of insects is now rather well understood [21]. In essence, the molting hormones (ecdysones) activate specific genes, which produce the ribonucleic acids that direct the synthesis of certain enzymes [22]. This is then manifested in the *de novo* synthesis of certain substances and results in metamorphosis. Other steroid hormones, possessing some structural analogy, apparently act in much the same way [23].

Some years ago, we reported that 5α -stigmast-22-en- 3β -ol (11), which is produced by *Dictyostelium discoideum*, triggers the remarkable differentiation which this slime mold undergoes [24]. Although Bonner's group has since then convincingly demonstrated that 3',5'-cyclic AMP can act as a chemotactic hormone [25], I believe that this is only part of the acrasin story. Cyclic AMP is a second messenger, which mediates the action of a large assortment of hormones [26], and the "real" acrasin may be either a steroid which controls the formation of cyclic AMP from ATP or a steroid the formation of which is controlled by cyclic AMP [27]. Bonner *et al.* [28] have, in fact, suggested that there must be a second chemotactic system, because cyclic AMP attracts only aggregating amoebae but not the vegetative ones. It is conceivable that stigmastenol is a precursor of the

"real" acrasin. A structurally related sterol, antheridiol (12), has been identified as the chemotactic hormone of the water mold, *Achlya bisexualis* [29].

Numerous publications, recently summarized by Hendrix [30], deal with the role of sterols in the growth and reproduction of fungi. Without going into details, it can be stated that small amounts of sterols probably occur in all microorganisms [31] although not all of them are capable of synthesizing them. For instance, many parasitic fungi require an exogenous source of the steroid nucleus. The microbiological transformations of steroids constitute a large body of information, which has been extensively reviewed [32, 33]. The steroids function not only as a source of energy for the microorganisms, but they are also degraded to a number of compounds with hormonal activity in animals. In plant pathology, the sterols play an important role in cell permeability, being localized in the cell membranes of both the host plant [34] and the parasitic fungus [35]. An attack by the fungus on the host's membranes by some fungal metabolite may damage them and permit nutrients to leak out, whereas an attack on the fungal membranes by certain antibiotics may damage the fungus. It is of interest that some steroidal metabolites of fungi may also have antibiotic activity. Examples of steroid antibiotics shown are fusidic acid (13), a product of *Fusidium coccineum* [36], and cephalosporin

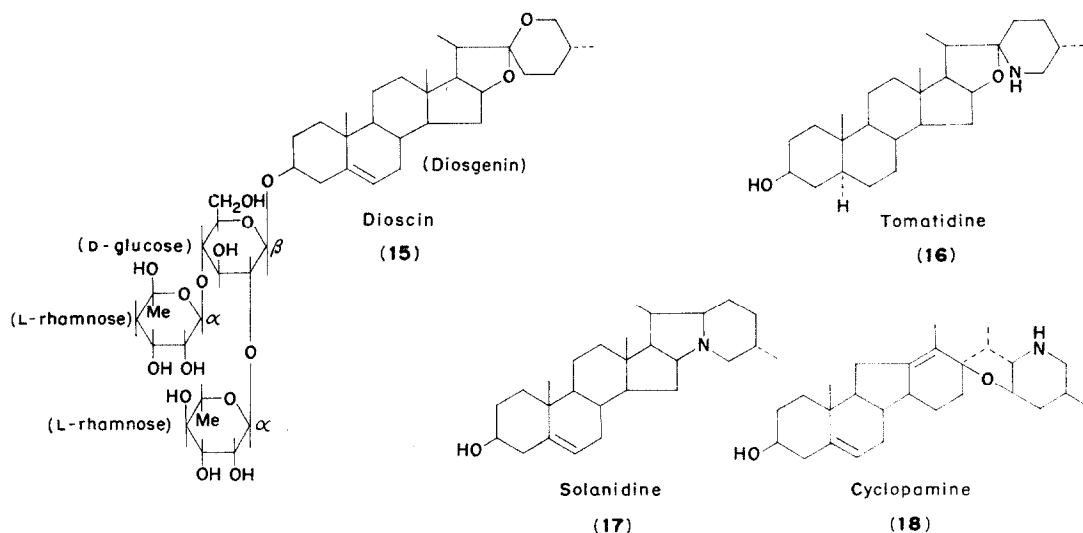


P₁ (14), elaborated by a strain of *Cephalosporium* [37]. The molecular basis for the antibiotic activity of fusidic acid is evidently the inhibition of RNA translation [38].

The role of sterols in the reproduction and differentiation of higher plants is mainly in the realm of speculation. However, even if we are somewhat short of facts, the stakes in understanding and manipulating the flowering process [39, 40] are obviously high enough to outweigh the risk of making the wrong guess. In 1963, we observed that some inhibitors of steroid biosynthesis have the ability to suppress the flowering response of the short-day plants *Xanthium* and *Pharbitis* to dark-treatment [41]. Although these experiments have

the saponins and glycoalkaloids, respectively. The saponins are surface-active agents, which form molecular compounds with many sterols and have hemolytic activity [51]. They are toxic to poikilotherms, but not to homoiothermic animals, although large amounts of saponins, eaten by warm-blooded animals will produce anorexia and bloat due to foaming in the gastro-intestinal tract [52]. It is not generally known that saponins have insecticidal and insect-repellent activity, thus protecting, e.g. wood against termites [53].

The older literature on the effects of steroidal saponins on plants has been reviewed earlier [54, 55]. They are generally toxic to fungi and bacteria [56, 57] and are therefore of some pharmaco-



been confirmed and extended by others [42, 43], we have not come any closer to the identification of a steroid as the flower hormone. Extracts from various plants have been claimed to have florigenic activity [44-47], but experiments of this kind are notoriously difficult to reproduce. The discovery of the phytoecdysones has rekindled the hope of pinning down the elusive flower hormone, but results of direct application of ecdysterone to short-day plants [48, 49] have been negative, and an earlier report ascribing gibberellin activity to ecdysterone could not be confirmed [50].

STEROIDAL SAPOGENINS AND ALKALOIDS

Steroidal sapogenins (15) and alkaloids (16 and 17) occur in plants in the form of their glycosides,

logical interest. Saponins also have some biological activity in higher plants often promoting seed germination and in many cases inhibiting root growth [54, 55]. They are apparently localized in a strategic position in plant cells, being bound to the membrane systems of their organelles, such as mitochondria [58].

The biological effects of the glycoalkaloids (e.g. 16 and 17) are very similar to those of the saponins. They are surface-active, combine with sterols, and cause hemolysis. They also have antibiotic and insecticidal activity, but they are more toxic to warm-blooded animals than the saponins [59, 60]. The physiology of tomatidine (16), which was first isolated from tomato vines, has been studied in some detail [61]. In the tomato plant it apparently

is synthesized in larger quantities wherever physiological changes are about to happen: in the apical buds, root tips, and flowers. Shortly after pollination, the tomatine content of tomato ovaries increases to a level ten times that of the rest of the plant. When the short-day plant, *Lycopersicon glandulosum*, is kept vegetative, its tomatidine content is five times as high as when it is flowering or bearing fruit. As far as the role of tomatine in the protection of plants from pathogenic fungi and insect predators is concerned, there is more evidence against than in favor.

There is a good deal of information about the distribution of the solanidine glycosides (17) in various parts of solanaceous plants, not only during their life cycle [62] but also variations during the diurnal cycle [55]. They are formed in the meristem and are localized, for certain periods of time, in the growing, young parts of the plant [63]. The glycoalkaloid concentration in the lobed nightshade, *Solanum laciniatum*, increases during the night and decreases during the day, mainly in response to the temperature changes [64]. Various investigators have postulated a connection between the glycoalkaloid content and resistance to fungal infection. *Alternaria solani*, which causes early blight in potatoes, is inhibited by the high glycoalkaloid levels in young potato leaves [65]. However, no correlation was found between the resistance to late blight, caused by *Phytophthora infestans*, and the glycoalkaloid content of various clones of potato plants [66].

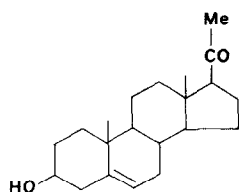
Two genera of the lily family, *Veratrum* and *Fritillaria*, contain C₂₇ alkaloids of the C-nor-D-homosteroid series. In contrast to the *Solanum* alkaloids, they occur either in the free state or in the form of esters. The alkaloids in this group are highly toxic to animals, having effects on the heart which are somewhat similar to those of the cardiac glycosides. Various effects on animal tissues can be interpreted in terms of an increased sodium influx into the cells [67]. Cyclopamine (11-deoxojervine) (18), which has been isolated from *Veratrum californicum* [68], is of special interest, because ewes browsing on this weed produce lambs with a cyclopiian malformation. There has been some concern about a possible link between human birth defects and presumed teratogenic substances in potato tubers [69]. The significance of these potent alkaloids for *Veratrum* plants is puzzling, because it is

difficult to see how they could fail to affect ionic transport and cell division in them. Treatment of oat and rye seedlings with *Veratrum* alkaloids inhibits their growth, apparently through a specific effect on DNA stability [70].

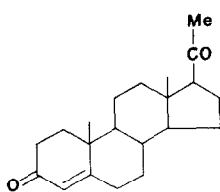
PREGNANE DERIVATIVES

The key reaction in the biosynthesis of steroid hormones in animals is the degradation of cholesterol (1) to pregnenolone (19) [2]. Because the same reaction has been observed in a variety of plants [71], and because cholesterol is now known to be widely distributed in the Plant Kingdom, I believe that progesterone (20) must occur in many plants besides those from which it has been isolated [72]. Moreover, we have observed that the most common plant sterol, sitosterol [4], can also be degraded to progesterone by *Digitalis* plants [73]. Analogous compounds are produced by the degradation of other plant steroids. Thus, we have found that tomatoes carry out the same degradation of tomatidine (16) to allopregnenolone (21) as the basic step in the partial synthesis of steroid hormones [74]. I believe it is safe to assume that steroidal sapogenins are also metabolized in this way by higher plants. We already know that certain microorganisms can convert sapogenins to analogous pregnane derivatives [75]. The degradation of ecdysterone to poststerone (22) which was isolated from a plant, *Cyathula capitata* [76], has so far not been demonstrated in plants but does occur in the blowfly [77].

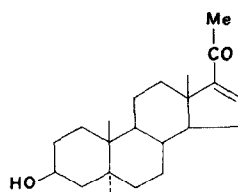
Just as in the adrenal glands of animals [2], the hormone deoxycorticosterone (23) is synthesized from progesterone in *Digitalis lanata* [78]. This undoubtedly happens in many plants; deoxycorticosterone was recently isolated from rice bran oil [79]. We have found that *Mallotus paniculatus* can carry out the conversion of cortisol (24) to cortisone (25) [80], and several other experiments of this kind are also reported in the literature [72]. Microorganisms—the filamentous fungi in particular—are remarkably adept in degrading steroids of various types to progesterone (20), in hydroxylating this compound and degrading it to androgens and further to estrogens [32, 33]. Many higher plants contain neutral pregnane derivatives. One of these, holadysone (26), which was found in



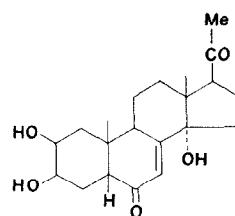
Pregnenolone
(19)



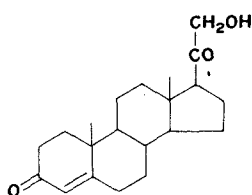
Progesterone
(20)



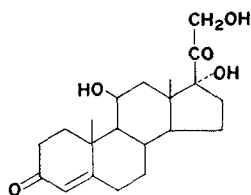
Allopregnenolone
(21)



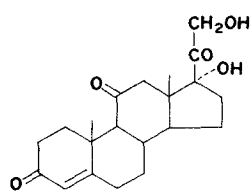
Poststerone
(22)



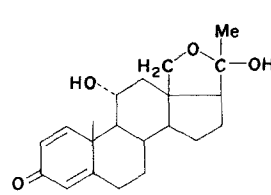
Deoxycorticosterone
(23)



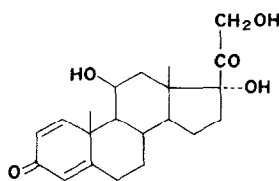
Cortisol
(24)



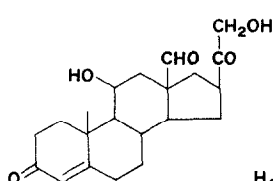
Cortisone
(25)



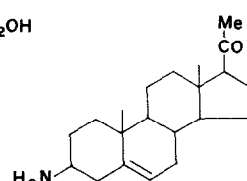
Holadysone
(26)



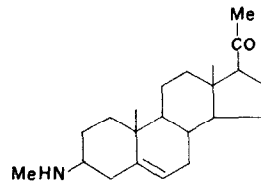
Prednisolone
(27)



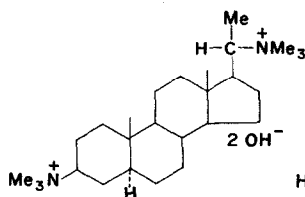
Aldosterone
(28)



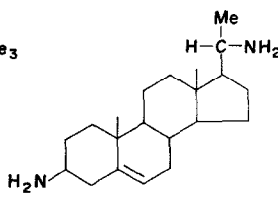
Holaphyllamine
(29)



Holaphylline
(30)



Malouetine
(31)



Irehdiamine A
(32)

Holarrhena antidysenterica, shows structural features similar to those of the potent adrenocortical hormones prednisolone (27) and aldosterone (28).

The literature contains only sparse references to the biological effects of progesterone (20) on plants. In mammals, progesterone produces a number of changes that prepare the uterus for the implantation and maintenance of the fertilized ovum. What effects, if any, could it exert on plant organs? A possible answer to this question may lie in the fact that progesterone can do so many things in the animal organism. The hypothesis that pro-

gesterone, like other steroid hormones, releases some of the genetic information stored in the chromosomes not only fits the observations best but also permits us to propose that progesterone could act on plant chromosomes the same way it does on animal chromosomes [81-83].

The adrenocortical hormones perform vital functions in animals by regulating the water and mineral balance and the carbohydrate metabolism [2]. The mineralocorticoids, such as aldosterone (28), are believed to act on transport systems involving the ATPase in the renal tubules [84, 85].

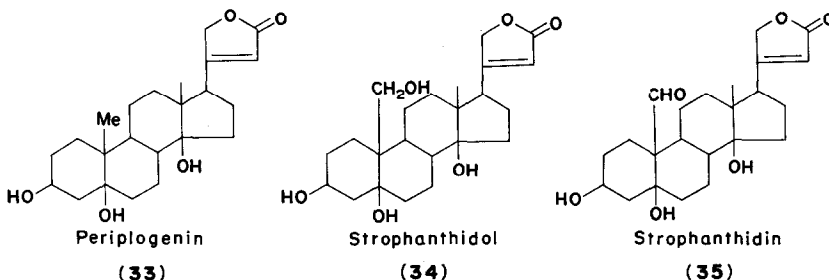
Glucocorticoids, such as cortisol (24), apparently increase glycogen production by stimulating transaminase activity [86]. They also stabilize lysosomal membranes against leakage [87]. I see no reason why these hormones could not regulate the electrolyte and carbohydrate metabolism in plant cells by stimulating the synthesis of the appropriate enzymes at the transcriptional level. I am even convinced that plants contain lysosomes [88], but no effect on them by corticosteroids has been demonstrated so far [89]. Such effects of adrenal steroids on higher plants as have been reported are difficult to interpret and probably difficult to repeat [90]. They generally inhibit the growth of microorganisms [91–93] and antagonize the antibacterial activity of fusidic acid [94].

The apocyanaceous *Holarrhena floribunda* converts cholesterol (1) via pregnenolone (19) to progesterone (20) and to various C_{21} alkaloids [71]. Whereas holaphyllamine (29) and holaphylline (30) are not very toxic, malouetine (31), which occurs in another plant belonging to the Apocynaceae, is a potent curare-like poison. Irehdiamine A (32), which is found in *Funtumia* species, and malouetine inhibit the bacteriophage-directed DNA synthesis in infected *Escherichia coli* [95]. These diamines may bind to the closed circular replicative form of $\phi\chi$ -174 DNA [96] and cause uncoiling and reversal of the DNA supercoils [97]. What these alkalamines do in the higher plants that produce them is a mystery, but it is already clear that they undergo seasonal changes in composition [98], which one day may give us a clue.

The concentration, location, and composition of the cardiac glycosides changes not only with the season [99], but also with the time of day [100] and environmental conditions [101]. This lends support to the claims of herbalists but not to those of teleologists. There are quite a few insects that can live on plants containing cardiac glycosides [102]. Moreover, these insects are still eaten by birds [103], lizards, and mice [104].

To find an indication of the possible significance of cardiac glycosides for plants, I believe we have to examine their mode of action in animals [105–107]. Although some questions remain, most investigators agree that cardiac glycosides act on cell membranes by inhibiting ATPase. This inhibits cation transport and results in a cellular accumulation of sodium and loss of potassium ions. What happens in the heart muscle can also happen in plant tissues. Thus, a strophanthin-sensitive ATPase has been discovered in corn [108], and the inhibition of sodium extrusion by ouabain has been demonstrated in carrot [109] and barley [110] roots. The cytotoxic activity reported for many cardiac glycosides [111–113] parallels the inhibition of transport ATPase [114], but it is not easy to understand how plants can contain such substances without being adversely affected by them.

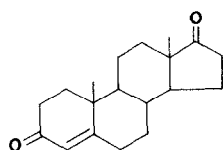
Periplogenin (33), strophanthidol (34), and strophanthidin (35) always seem to occur together [1], forming a triad with an increasing state of oxidation at C-19. Evidently, stepwise oxidation occurs at this carbon atom, because when we



CARDIAC GLYCOSIDES

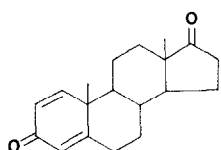
A limited number of genera include plants with the ability to synthesize cardiac aglycones with either 23 or 24 carbon atoms from a C_{21} steroid, such as progesterone (20) or pregnenolone (19) and a 2- or 3-carbon fragment, respectively [71, 72].

administered radioactive progesterone to *Strophanthus kombé* plants, all three genins could be isolated in radioactive form [115]. Several such triads have been identified [1]. Oxidation of steroids at C-19 is frequently observed in microbiological transformations [116] and may have a rather general significance.



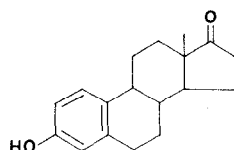
Androstenedione

(36)



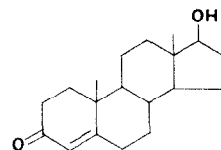
Androstadienedione

(37)



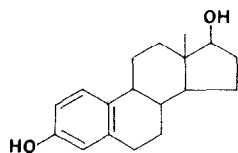
Estrone

(38)



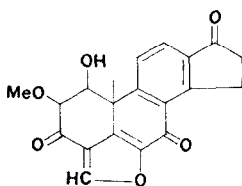
Testosterone

(39)



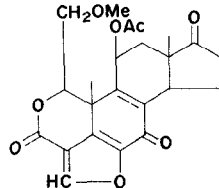
Estradiol

(40)



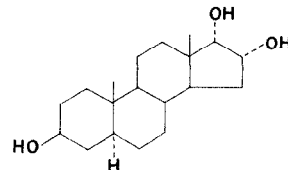
Viridin

(41)



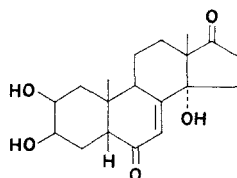
Wortmannin

(42)



Androstanetriol

(43)



Rubrosterone

(44)

SEX HORMONES

In animals, the sex hormones are formed from progesterone (20) by successive oxidation steps, both at C-17 and at C-19. The oxidative removal of the side chain leads to the C_{19} series with androgenic activity, and the oxidative removal of the angular methyl group at C-10 and aromatization of ring A leads to the C_{18} series with estrogenic activity [2]. The ability to manipulate steroids is rather common among microorganisms, and many of them are exploited for the production of pharmaceutically useful hormones belonging to the corticosteroid, androgen, and estrogen series. For instance, *Arthrobacter simplex* [117], *Mycobacterium phlei* [118], *Streptomyces olivaceus* [119], and *Nocardia asteroides* [120] degrade cholesterol (1) to androstenedione (36) or androstadienedione (37). *M. phlei* can also produce these two steroids from diosgenin (15) [121]. The conversion of 19-hydroxycholesterol to estrone (38) was first observed in *N. restrictus* [122], but by now many *Nocardia* and *Mycobacterium* species are known to be capable of this transformation [123]. The degra-

dation of progesterone (20) to testosterone (39) can be accomplished by a number of microorganisms [124] and even by cell-free preparations of *Penicillium* [125] and *Cylindrocarpum* [126]. The aromatization of androstenedione (36) to estradiol (40) was observed in the presence of human intestinal bacteria [127].

Gliocladium virens produces an antibiotic, viridin (41), which is related to the 17-ketosteroids [128], and a structurally related steroidal antibiotic, wortmannin (42), is elaborated by *P. wortmanni* [129]. Whereas the biological significance of antibiotic steroids is clear, that of the rather commonly produced steroid hormones is somewhat puzzling. Information on the effects of steroid hormones on microorganisms is very sparse [72]. Both growth-promoting [130] and -inhibiting [131] effects have been observed, but more interestingly, testosterone and estradiol have been found to have sex hormone activity on yeast [132].

Higher plants contain compounds belonging to the androgen and estrogen series [72, 81]. We assume that they are made by the same processes

as have been observed in animals and micro-organisms, but so far we have not been able to demonstrate this [48]. Testosterone (39) and androstenedione (36) have been identified in the pollen of Scotch pine, *Pinus silvestris* (133), and 5 α -androstane-3 β , 16 α , 17 α -triol (43) was found in Rayless goldenrod, *Haplopappus heterophyllus* [134]. Rubrosterone (44) is undoubtedly a metabolite of insect-molting hormones occurring in *Achyrantes rubrofusca* [135], although we were unable to show this [48]. It has no molting-hormone activity, but it stimulates protein synthesis in the isolated mouse liver [136]. As far as estrone (40) is concerned, it is now established that it occurs in palm kernels and pollen and in pomegranate and apple seeds, among other plant sources [72].

If there is some reluctance to admit that steroid hormones do occur in higher plants [137, 138], most people will just reject the idea that these agents could have any effects on them. This is probably because they associate the effects of steroid hormones with the endocrinology of higher animals. Yet, on the molecular level there must be some interactions between plant chromosomes and steroid hormones that are analogous to those between animal chromosomes and steroid hormones [139–141]. Earlier research on the effects of hormones on higher plants has been reviewed previously [54, 81, 142–144]. In summary, there are numerous reports in the literature of effects on the germination, growth, flowering, and sex expression of plants, produced by the application of steroid hormones. More recently, the stimulating effect of steroidal estrogens on seed germination and growth has been confirmed [72]. Both Kopcewicz [145] and Leshem [146] were able to promote floral development by estrogen treatment. Administration of male and female sex hormones to plants in the cucumber family has changed their sex expression [147, 148], and treatment of *Equisetum arvense* with testosterone (39) has increased the number of female prothalli [149]. Experiments of this kind are notoriously difficult to repeat, but in view of the great theoretical and practical significance of the potential chemical regulation of plant reproduction these leads are, I believe, worth following.

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