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Function of Sterols [and Discussion]

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Function of sterols

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In plants, neither sterol synthesis nor sterol balance is static. While all plant tissues have the ability to synthesize sterols, generally the younger tissue has a higher rate of synthesis. Additionally, the synthesis of the various individual sterols changes with tissue age and environmental factors. For example, the plant apex has a higher level of sitosterol than stigmasterol and as the tissue becomes older the ratio of sitosterol changes in favour of stigmasterol. The level of campesterol is also quite high in the plant apex, especially in the region of cell division. The change in sterols of the various tissues is apparently not due to intercellular sterol transport.

With regard to light, both its intensity and photoperiod are important factors in controlling sterol synthesis, especially the sitosterol to stigmasterol balance. The change in sterol metabolism induced by light does not appear to be due to activation of the 22,23-dehydrogenase enzyme. The change in sterols and their possible relation to the development and physiology of the plant are discussed.

Introduction

During recent years more and more interest in the function of sterols has been generated. Even though the general biosynthetic sequence for selected phytosterols is well defined, their physiological rôle is much less certain. Generally the function of sterols can be placed into two categories: (1) sterols act either directly or indirectly as hormones or as hormone precursors (Geuns 1978), and (2) sterols are integral components of membranes and might control changes in membrane permeability (Grunwald 1975).

Our topic, the function of sterols, can best be approached if we first review briefly the chemistry and biochemistry of sterols, and then show how sterol biosynthesis and physiological function may be interrelated.

Chemistry

Sterols are secondary alcohols with 27–29 carbon atoms, and, unlike common alcohols, are crystalline solids at room temperature. The major plant sterols are sitosterol, stigmasterol, and campesterol (see cholesterol in figure 3 for the steroid numbering system). Cholesterol, which is also shown in the biosynthetic pathway, is the major animal sterol. This sterol, however, is also found in many plants (Johnson, Bennett & Heftmann 1963) and may be an important intermediate in the biosynthesis of various other steroids (Heftmann 1975; Grunwald 1979).

Isoprene (figure 1) can be considered the basic 5-carbon unit, and six isoprenes polymerize to form the sterol molecule. Plant sterols have the perhydro-1,2-cyclopentanophenanthrene ring system with the three 6-carbon rings fused *trans* in a nonlinear arrangement that is attached to a 5-carbon ring (figure 2). Most plant sterols have a hydroxyl group at C-3 which plays an important rôle in the formation of steryl esters and steryl glycosides. Another general characteristic of plant sterols is the methyl group at C-10 and C-13, and a side chain at C-17 with

8–10 carbon atoms. Important sterol intermediates are 4,4-dimethyl and 4-methyl sterols. The former sterol group has two methyl groups at the C-4 position, while the latter only one. The C-3 hydroxy, the C-17 side chain, and the C-10 and C-13 methyl groups have the β-orientation. If a C-4 methyl group occurs, it has the α-orientation. The naturally occurring sterols differ in the number of carbon atoms in the C-17 side chain, and in the degree and position of saturation in the ring system and the side chain.

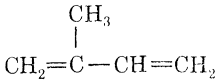

FIGURE 1. Isoprene unit.

BIOSYNTHESIS

Squalene is the most direct sterol precursor (figure 2) and cyclization, to form the sterol ring system, is through 2,3-oxidosqualene (Benveniste & Massy-Westropp 1967; Reid 1968). In higher plants and algae, cycloartenol is the first formed sterol (Goad & Goodwin 1972; Grunwald 1975), while in animals (Frantz & Schroepfer 1967) and fungi (Weete 1973), it is lanosterol. Besides the introduction of the C-3 hydroxyl group, two 1,2-methyl shifts occur from C-14 to C-13 and from C-8 to C-14.

The cyclization reaction is microsomal (Heintz & Benveniste 1970), and anaerobic (Eppenberger, Hirth & Ourisson 1969). Cycloartenol is a 4,4-dimethyl sterol. Experiments with radioactive precursors suggest that cycloartenol is a branch point. To form the major phytosterols (sitosterol, stigmasterol, campesterol), an alkylation at C-24 is required which occurs through transmethylation involving S-adenosylmethionine (Nicholas & Moriarty 1963; Castle, Blondin & Nes 1963). Apparently the double bond at C-24,25 is required for cationic attack at C-24 and it is stabilized by hydrogen migration from C-24 to C-25 (Rees, Mercer & Goodwin 1966).

Before the 9,19β-cyclopropane ring can be opened, a demethylation at C-24 of 24-methylene cycloartenol occurs to form cycloeucalenol (Heintz & Benveniste 1974). Demethylation is through oxidative decarboxylation of the 4α-methyl, and during this process the remaining 4β-methyl is epimerized to the 4α-position (Sharpless *et al.* 1969; Knapp & Nicholas 1970). 24-Methylene lophenol is formed from obtusifoliol through C-14 demethylation. Oxidative decarboxylation of the C-14 methyl group cannot proceed as long as the 9,19β-cyclopropane ring is present. The $\Delta^{8(9)}$ of obtusifoliol appears to be another requirement for C-14 demethylation to proceed.

24-Methylene lophenol is another very important branch point in the biosynthesis of phytosterols. The 29 carbon sterols (sitosterol, stigmasterol) need a second alkylation at C-28, but the 28 carbon sterol (campesterol) does not go through this step. As in the first alkylation reaction, methionine is again the methyl donor (Russell, Van Aller & Nes 1967). A cationic site at C-24 is involved which is stabilized through a hydrogen loss from C-28 to form 24-ethylidene lophenol (Goad, Hammam, Dennis & Goodwin 1966). This second alkylation does not appear to be substrate specific (Alcaide *et al.* 1968).

The loss of the second C-4 methyl group of 24-ethylidene lophenol is again through oxidative decarboxylation and gives rise to Δ^7-avenasterol (Knights & Laurie 1967). Δ^7-Avenasterol is a 4,4-demethyl sterol and these sterols are generally referred to simply as sterols. Formation of

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sitosterol and stigmasterol appears to be straightforward from Δ^7-avenasterol but no specific pathway is favoured at present, and a number of pathways are quite possible. Basically, a $\Delta^{7(8)} \rightarrow \Delta^{5,7} \rightarrow \Delta^{5(6)}$ rearrangement and a reduction of the double bond at C-24,28 must occur but the exact sequence of events is ill defined.

The possible conversion of sitosterol to stigmasterol has been debated for a number of years and a 22,23-dehydrogenase has been postulated (Johnson, Heftmann & Houghland 1964). The question of whether stigmasterol is formed from sitosterol, or through an independent pathway

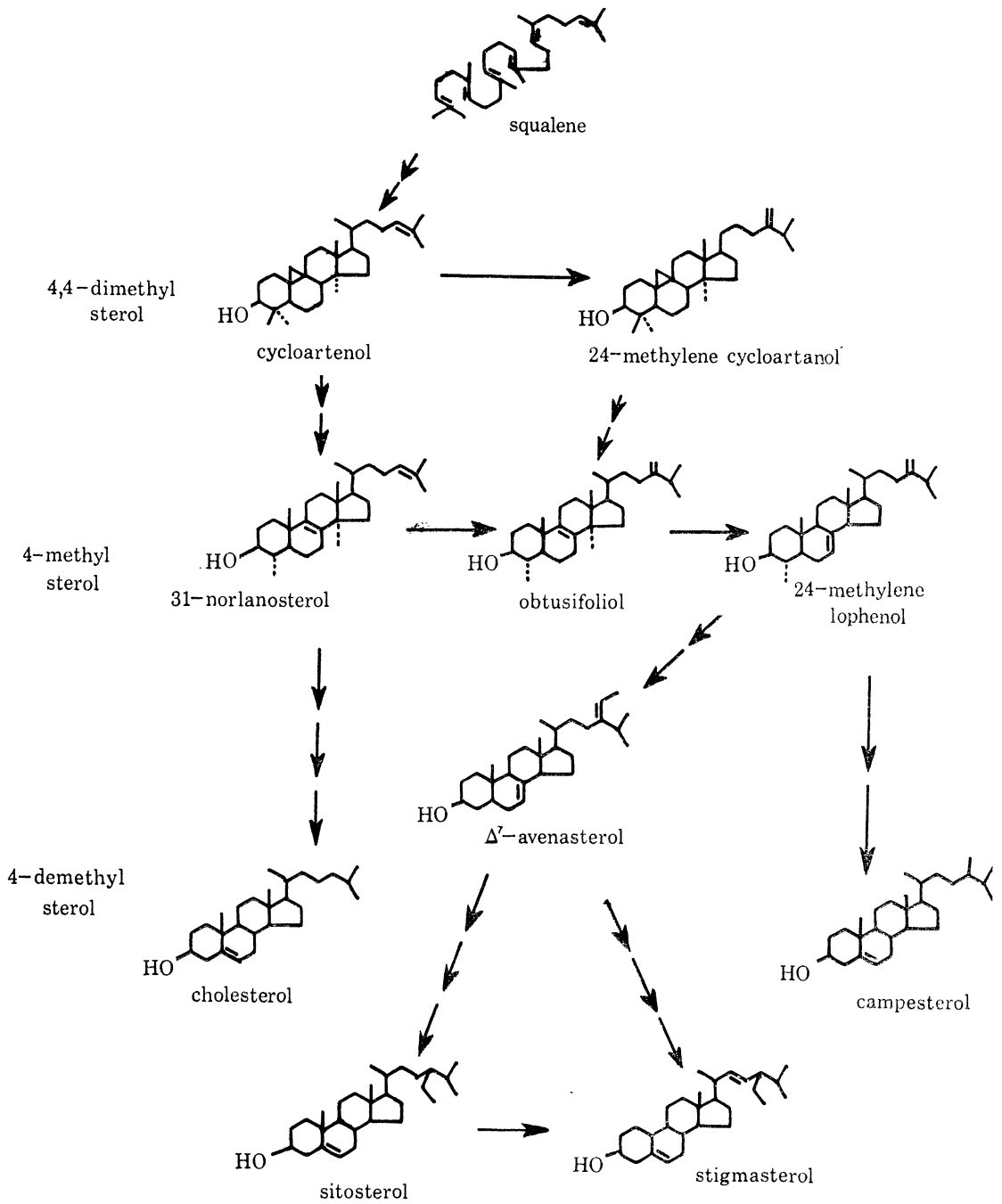

FIGURE 2. Pathway of sterol synthesis in plants.

from Δ^7-avenasterol is of some importance because the sitosterol to stigmasterol ratio changes during seed germination and with plant development and senescence. As will become apparent later, environmental factors (light and temperature) also influence the rate of synthesis of the major phytosterols. Radioactive mevalonic acid is first incorporated into sitosterol and only after longer incubation periods into stigmasterol with a decrease in radioactive sitosterol (Knapp & Nicholas 1970; Bush & Grunwald 1973). However, experiments to prove direct conversion of sitosterol to stigmasterol are unconvincing because of the small amount of radioactivity recovered from stigmasterol (Waters & Johnson 1965; Bennett & Heftmann 1969).

As already pointed out, the 28 carbon sterol (campesterol) does not require the second alkylation and is therefore somewhat removed from the synthesis of sitosterol and stigmasterol. Conversion of campesterol to sitosterol or stigmasterol has not yet been demonstrated. The final steps of campesterol biosynthesis are similar to those described above for the 29 carbon sterols, a second demethylation at C-4, rearrangement of the double bond in ring B and reduction of $\Delta^{24(28)}$.

Cholesterol (figure 2) has been isolated from a number of higher plants and its possible importance as a precursor in the formation of other steroids is suggested (Heftmann 1975). Formation of cholesterol does not require an alkylation at C-24 and the biosynthetic branch point is therefore quite far removed from the more common phytosterols. Cycloartenol is the most often suggested branch point; however, 31-norlanosterol may be a second point for branching (Heintz & Benveniste 1974). In any case, the steps involved are demethylation at C-4, opening of the 9,19β-cyclopropane ring, rearrangement of the double bond in ring B, and reduction of $\Delta^{24(25)}$.

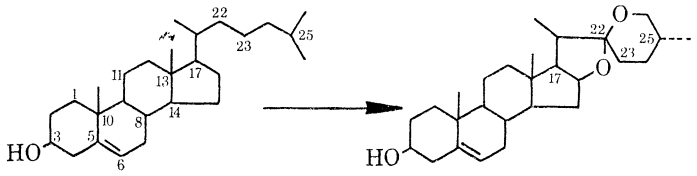

FIGURE 3. Sapogenin formation in plants.

STEROLS AS HORMONES AND HORMONE PRECURSORS

At present not much support can be found for the suggestion that sterols function directly as plant growth hormones, and the few experiments that suggest such action probably need re-examination (Biswas, Paul & Henderson 1967; Kopcewicz 1969). However, the possibility that sterols are precursors to a number of steroids, which in turn might act as hormones, does find support in the literature (for recent reviews see Geuns 1978; Heftmann 1975). Sterols are the precursor to at least four major groups of steroids, all of which have been isolated from plants.

Sapogenin

Sapogenins are 27-carbon steroids (figure 3) widely distributed in a number of monocotyledonous (Liliaceae, Amaryllidaceae, Dioscoreaceae) and dicotyledonous (Solanaceae, Scrophulariaceae) families. In plants both sitosterol (Stohs, Sabatka & Rosenberg 1974) and cholesterol (Bennett & Heftmann 1965) can serve as their precursors. In nature the sapogenins are combined with sugars to form the saponins. While these compounds are used for the

commercial preparation of steroidal hormones, they themselves do not have hormonal activity in plants.

Cardenolides

The cardenolides are steroids with 23 carbon atoms (figure 4). About 50 cardenolides have been isolated from 12 plant families. In plants cardenolides occur as glycosides and the steroid moiety is characterized by a 14β-hydroxyl and an α,β-unsaturated γ-lactone (buteriolide) ring. Cardenolides are found in all types of plant tissues (Singh & Rastagi 1970) and are formed through the condensation of a 21-carbon pregnane derivative with acetate. Many of the cardenolides are poisonous and might have some importance as a defence mechanism but they do not have hormonal properties in plants. Primitive people used these steroids as arrow poison and modern society uses the cardenolides in the treatment of heart diseases.

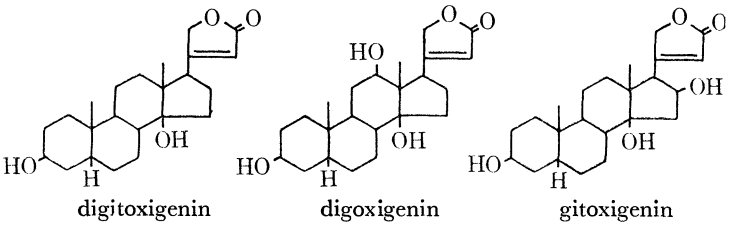

FIGURE 4. Cardenolides isolated from *Digitalis*.

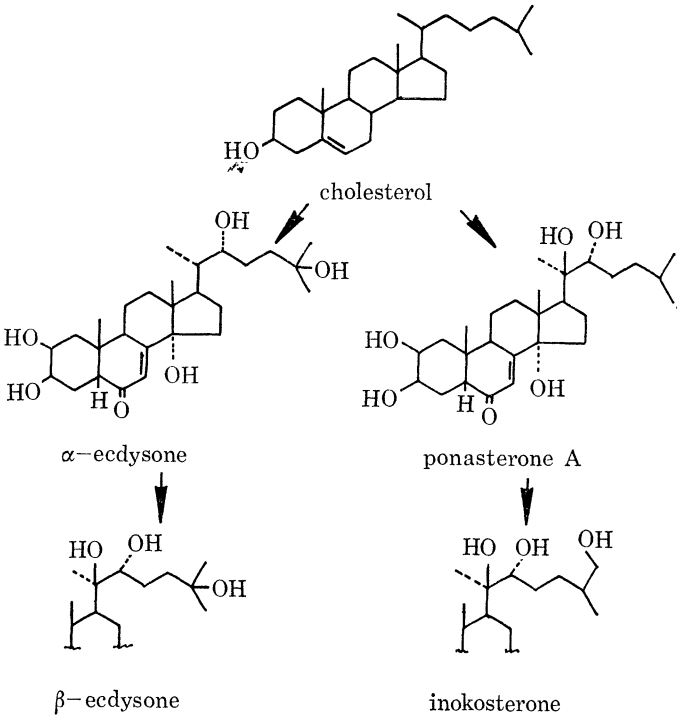

FIGURE 5. Ecdysone synthesis in plants.

Ecdysteroids

Ecdysteroids are polyhydroxylated steroids with 27–29 carbon atoms (figure 5) and the major differences in the various ecdysteroids are in the C-17 side chain (Hikino & Takemoto 1974). Cholesterol can serve as a precursor in the formation of ecdysones; however, experiments to

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establish that sitosterol can also be a precursor have been unsuccessful (Boid, Rees & Goodwin 1975). Phytoecdysteroids are of recent discovery and at the present time over 40 ecdysteroids have been isolated from about 80 plant families, but no taxonomic relation has been established (Hikino & Takemoto 1974).

The function of ecdysteroids in plants is ill defined but they probably do not have hormonal activity. In insects, ecdysones induce ecdysis or moulting and, therefore, are often called moulting hormones. Unfortunately, the term moulting hormone is also often used in the plant literature; this term does not, however, describe their function in plants. The action of ecdysteroids in insects is probably at the gene level, by acting on specific sites and thereby regulating metamorphosis (Karlson 1974). The question has been raised whether or not ecdysteroids are also involved in the metamorphosis of plants (Heftmann 1975), but no qualitative or quantitative effects on flowering or vegetative development of shoot tips and intact plant culture of *Xanthium pennsylvanicum* could be demonstrated (Jacobs & Suthers 1971). Ecdysterone was also inactive in the following gibberellin bioassay tests: α-amylase induction, plant growth of dwarf pea, leaf growth with dwarf corn, and germination of *Anemia* spores (Hendrix & Jones 1972).

A second suggestion for the function of ecdysteroids is that these triterpenes might act as potent anti-feeding agents (Williams 1970). Ingestion of ecdysones by insects has little or no effect on the insect (Robbins *et al.* 1968), but the analogue ajugalactone, which is found in *Ajuga decumbens*, inhibits insect metamorphosis (Koreeda, Nakanishi & Goto 1970).

Progestogens

This group of steroids has 21 carbon atoms and includes pregnenolone, progesterone, and deoxycorticosterone (figure 6). All these steroids have been isolated from plants (Tschesche 1966; Gawienowski & Gibbs 1968; Bahadur & Srivastava 1971); however, deoxycorticosterone is the only corticosteroid so far isolated from plants. Biosynthetically the progestogens are derived from sterols by oxidative cleavage of the C-17 side chain, and both cholesterol and sitosterol can serve as substrates (Bennett & Heftmann 1965; Bennett, Heftmann & Winter 1969). The progestogens are important intermediates in the biosynthesis of other steroids, e.g. cardenolides, oestrogens and androgens.

A number of corticosteroids (cortisone, cortisol, cortisol acetate) are active in growth tests (Ratsimamanga, Nigeon-Dureuil & Boiteau 1958; Ratsimamanga & Diot 1959; Donnet, Chevalier & Pruneyre 1960), but deoxycorticosterone, the only presently known corticosteroid to occur in plants was inactive and even inhibited growth (Geuns 1977). Of the active corticosteroids, those with an 11β-hydroxy group (cortisol, Δ^1-cortisol, corticosterone) were more active than those with an 11-oxo function (cortisone, Δ^1-cortisone). The number of flowers could also be stimulated with corticosteroids (Kopcewicz 1971). Since most of the tested corticosteroids have not yet been isolated from plants, it is difficult to assess the significance of these results. It appears quite unlikely that deoxycorticosterone is converted to one of the 11β-hydroxy or 11-oxo compounds.

Oestrogens and androgens

Oestrogens and androgens are steroids with 18 or 19 carbon atoms (figure 7). These steroids are mammalian hormones and their possible importance as plant hormones has been debated for many years. Even today few plant physiologists are convinced that these steroids play a meaningful rôle as plant growth regulators. The biosynthesis of oestrogens has been demonstrated in plants (Young, Knights & Hillman 1977) and it has been suggested that the

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pathway is similar to the one found in animals and microorganisms (Heftmann 1975). The proposed pathway would require pregnenolone and progesterone, which have been isolated from plants, to be intermediates.

Most of the debate does not concern itself whether or not oestrogens and androgens influence vegetative growth, flower formation or sex expression (Geuns 1977), but whether or not these terpenes are naturally occurring products of plants (Grunwald 1979). In general, oestrogen treatment brings about an increase in flower formation and the sex ratio is usually in favour of

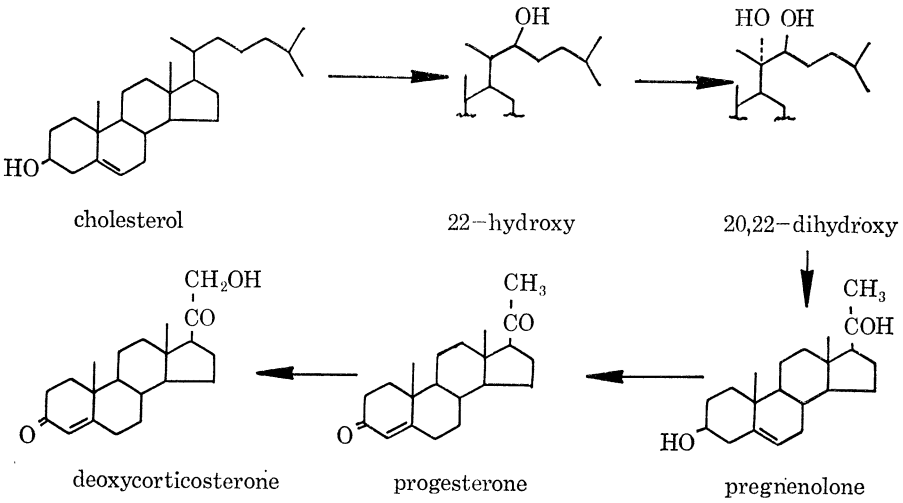

FIGURE 6. Postulated progestogen synthesis in plants.

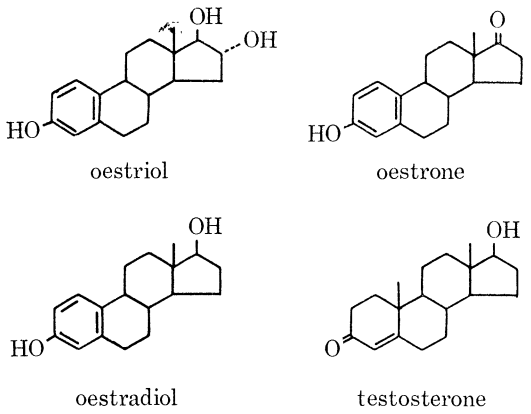

FIGURE 7. Oestrogens and androgens.

femaleness. Androgens give only a slight increase in flower number but the sex ratio generally shifts to maleness. Early experiments relied on bioassay techniques to identify oestrogens (Butenandt & Jacobi 1933; Skarzyński 1933) and some of the estimates were as high as 2 mg of oestrogen per kilogram of tissue. Only lately have these procedures been challenged (Jacobsohn, Frey & Hochberg 1965). But even today many of the oestrogen characterizations are based on colour reactions, mobilities in chromatographic systems, melting points, bioassays, and ultraviolet and infrared spectroscopy. With the use of the extremely sensitive competitive protein binding techniques, it was estimated that even the supposedly richest source of oestrogen does not contain more than 3–9 μg/kg (Dean, Exley & Goodwin 1971). Of course, these levels might

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be high enough to control plant growth and development. The report of androgen isolation from plants is rare but testosterone (figure 7) and androstenedione have supposedly been found in Scotch pine (Săden-Krehula, Tajić & Kolbah 1971). The possibility does exist that oestrogens and androgens act indirectly on plant growth and flowering by influencing gibberellin and auxin biosynthesis (Grunwald 1975).

Sterols as membrane components

The bulk of the sterols of plant cells is localized in the intracellular organelles (Kemp & Mercer 1968*b*; Grunwald 1970) and the plasma membrane (Hartmann, Normand & Benveniste 1975). Cellular fractions contain not only the free sterols but also steryl esters and steryl glycosides, but the function of the latter two groups is unknown. Generally, mitochondria and microsomes contain the largest quantity of sterol, and the individual sterol composition of the various cellular fractions is not always the same (Brandt & Benveniste 1972) indicating a relation between membrane composition and membrane function.

The question 'What is the function of sterol in membranes?' may be raised, and one suggestion is that the various phytosterols control membrane permeability (Grunwald 1971). It has been suggested that sterols in higher plants play an important rôle in the structure of membranes through their stabilizing properties (Grunwald 1971). Based on permeability studies, for sterols to be active as membrane stabilizers, they must have (1) a free C-3 hydroxyl group to allow for interaction with membrane phospholipids, and this is a very specific requirement; (2) a relatively flat molecular configuration, similar to cholesterol, to fit into phospholipid cavities – any increase in the bulkiness of the C-17 side chain (campesterol, sitosterol, stigmasterol) would decrease membrane stabilization; and (3) the perhydrocyclopentanophenanthrene ring system must have at least one double bond. Experiments suggest that cholesterol is more effective in stabilizing a membrane than campesterol, which is more effective than sitosterol or stigmasterol. However, the reported slight changes in the ratio of stigmasterol to sitosterol are difficult to explain purely on the above criteria.

A number of physiological observations relating to membrane behaviour and sterol concentration have been reported. The polyene antibiotic filipin increases cellular permeability to solutes by complexing with the sterol molecules. Addition of free sterol to the medium reverses cellular leakage (Mudd & Kleinschmidt 1970). The amount of leakage induced with filipin is directly related to the free sterol content of the tissue (Carbonero, Torres & Garcia-Olmedo 1975). These latter observations were made with tissues differing in phenotype as to sterol content, and not through application of exogenous sterols. The uptake of K^+ and NO_3^- is also reduced in filipin-treated tissue and the ion uptake capabilities can be restored with sterol application (Hendrix & Higinbotham 1973). The photochemical activity of chloroplasts is also reduced with filipin treatment, probably through interaction with membrane sterols (Bishop 1973). The steroid digitonin, a sapogenin, forms equimolar insoluble sterol complexes and treatment of chloroplasts with digitonin reduces photosynthesis as measured by oxygen evolution (Robinson & Wiskich 1975).

Plants treated with ozone generally show an increase in membrane permeability and the same treatment decreases the free sterol content of the tissue (Tomlinson & Rich 1971). A pretreatment with sterols inhibits the ozone effect (Spotts, Lukezic & Lacasse 1975). Similarly, kinins protect plants against ozone damage and also prevent the decrease in free sterols

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(Tomlinson & Rich 1973). The induction of anthocyanin in red cabbage is phytochrome mediated and it is assumed to influence membrane behaviour. Cholesterol is capable of reversing phytochrome-induced anthocyanin production (Bassim & Pecket 1975). While the above observations do not constitute direct proof that sterols are an absolute requirement for proper membrane function, they certainly make a strong case.

Sterol distribution

The importance of sterols in higher plants cannot be over emphasized because these triterpenoids have been isolated from all angiosperms and gymnosperms examined so far (Bean 1973) and the most often encountered 4-demethyl sterols are sitosterol, stigmasterol, and campesterol (Stoll & Jucker 1955; Goad & Goodwin 1972). Examination of liverworts (Matsuo, Nakayama, Maeda & Hayashi 1973), mosses (Marsili & Morelli 1970), horsetail (Ludwiczak & Stachowiak 1963), and ferns (Berti & Bottari 1968) have shown that sitosterol is also a major sterol in these plants. Sterols have also been found in amoebae (Smith & Korn 1968), fungi (Weete 1973), and lichens (Lenton, Goad & Goodwin 1973), but the major sterol in these organisms is ergosterol. The algae also contain sterols but the specific sterols found depends upon the algal group under investigation. The green algae probably show the largest variation and a number of Δ^5, Δ^7 and $\Delta^{5,7}$ sterols have been reported (Patterson 1974). Brown algae have fucosterol and 24-methylene cholesterol (Patterson 1968) while the red algae have desmosterol, cholesterol, and 22-dehydrocholesterol (Chardon-Loriaux, Morisaki & Ikekawa 1976). Poriferasterol is a major sterol of the golden brown algae and diatoms (Rubinstein & Goad 1974). The blue-green algae may contain sitosterol and cholesterol (Reitz & Hamilton 1968). For many years it was believed that bacteria did not contain sterols but more recently it has been demonstrated that even these organisms may contain sterols such as cholesterol and sitosterol (Schubert, Rose, Tümmler & Ikekawa 1964; Schubert *et al.* 1968).

In higher plants sterols have been isolated from all types of tissue such as leaves, roots, and stems (Kemp & Mercer 1968*a*), bark (Rowe 1965), flowers (Sliwowski & Kasprzyk 1974), pollen (Hügel, Barbier & Lederer 1964), fruit (Bennett, Heftmann, Purcell & Bonner 1961), and seeds (Knights & Laurie 1967).

From what is known at the present time, all plant tissues are capable of synthesizing sterols (Evans 1974; Atallah, Aexel, Ramsey & Nicholas 1975*a*), but the rate of synthesis depends upon the type of tissue and the age of the tissue (Geuns 1973, 1975). The microsomes are involved in sterol synthesis, and at the cellular level most of the sterols are found in the intracellular organelles and the microsomes are richest in sterols (Kemp & Mercer 1968*b*; Grunwald 1970).

Sterol changes during seed germination and plant development

During the first 2 or 3 days of seed germination, sterol synthesis is essentially nil (Baisted 1971), and the slight increase sometimes observed is probably due to a weight loss of the germinating seeds. As is evident from table 1, sterol accumulation is very slow during the first few days but starts to increase as germination progresses. After 3 or 4 days tobacco seeds are germinated, and it is at this point that sterol accumulation occurs. The sterols mostly responsible for this increase are stigmasterol and campesterol. Sitosterol, which accounts for almost 60 %

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of the sterols in seed, does not increase during germination, and after 6 days of germination accounts for only 35–40 % of the total sterols. It has been suggested that the general increase in sterols is probably due to membrane biogenesis during germination (Dupéron 1971). The vigour of the seedling also influences the sterol level and plants grown with proper nutrients show a higher sterol content than those grown below optimum conditions (Dupéron 1971).

TABLE 1. FREE STEROL CHANGES DURING GERMINATION OF TOBACCO PLANTS (ADAPTED FROM BUSH & GRUNWALD 1972)

(Concentrations in milligrams per gram dry mass.)

germination time/h	total sterols	campesterol	cholesterol	stigmasterol	sitosterol	ratio, stig.:sito.
1	1.36	0.20	0.05	0.30	0.81	0.37
2	1.39	0.21	0.05	0.31	0.82	0.38
3	1.41	0.22	0.08	0.32	0.79	0.41
4	1.50	0.25	0.09	0.36	0.80	0.45
5	1.63	0.30	0.10	0.42	0.81	0.52
6	2.04	0.40	0.13	0.70	0.81	0.86
7	2.11	0.45	0.15	0.72	0.79	0.91

TABLE 2. MEVALONIC ACID INCORPORATION INTO THE STEROLS OF MUNG BEAN HYPOCOTYL SECTIONS (ADAPTED FROM GEUNS 1975)

hypocotyl zone cm	sterol fraction	control count/min	NAA count/min	increase (%)
1st	4,4-dimethyl	940	1060	13
	4-demethyl	1380	3270	137
5th	4,4-dimethyl	930	1180	27
	4-demethyl	480	930	93

Medium: 3 ml containing 0.06 μCi [2-^{14}C]MVA. Incubation: 25 hypocotyl sections for 20 h in the dark at 23 °C.

The rate of sterol biosynthesis depends upon the age of the tissue. Generally, young tissue has a higher rate of sterol synthesis than old tissue, and experiments by Geuns (1975) probably best demonstrate this point (table 2). Synthesis of 4-demethyl sterols in the first centimetre of mung bean hypocotyl, as measured by mevalonic acid incorporation, is almost three times that of its synthesis in its fifth 1 cm section. However, if one examines the 4,4-dimethyl sterols no decrease in synthesis with age can be detected, suggesting that the rate-limiting step lies past cycloartenol. Furthermore, it is interesting to note that the auxin naphthalene acetic acid does not stimulate the biosynthesis of 4,4-dimethyl sterol but does stimulate the conversion of 4,4-dimethyl sterol to 4-demethyl sterol. The auxin effect is larger with young tissue, but even the fifth 1 cm of mung bean hypocotyl shows a doubling of mevalonic acid incorporation into 4-demethyl sterols. Again the point of control must be somewhere in the later stages of sterol synthesis and it is suggested that the enzyme S-adenosylmethionine-Δ^{24} sterol methyltransferase might regulate sterol synthesis (Baisted 1971; Hartmann & Benveniste 1974).

As the newly germinating seedling continues to grow, the accumulation of sterols ceases and a plateau region is reached. This plateau remains essentially until the tissue starts to age, when another increase in sterol is registered (Grunwald, Bush & Keller 1971). The accumulation of stigmasterol accounts for most of the sterol increase during early and late stages of plant development. However, at the present time nothing is known about the biological reason for

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the increase in stigmasterol. It appears that the absolute changes in individual sterols is probably not as important as the shift in sterol ratio, especially the change in the stigmasterol to sitosterol ratio. The magnitude of change can best be demonstrated with a few examples and one of these is the already cited germinating seed (table 1). The hypocotyl of mung bean is another good example (Geuns 1973). The hypocotyl curve contains only 10% stigmasterol and 62% sitosterol, but moving down the hypocotyl the percentage of stigmasterol increases while that of sitosterol decreases; 6 cm down from the curve, the percentage of these two sterols is about equal, 41 and 40% respectively. Changes in sterol composition with tissue age do not only occur in seedlings and hypocotyls but also in the whole plant (table 3). In a recent study (Grunwald

TABLE 3. STEROL CONTENT OF TOBACCO BY LEAF POSITION (ADAPTED FROM GRUNWALD 1978)

leaf position	free sterol mg/g dry mass	campesterol (%)	cholesterol (%)	stigmasterol (%)	sitosterol (%)	ratio, stig.:sito.
top	1.31	18	10	33	39	0.85
middle	1.40	15	11	47	27	1.74
bottom	1.59	15	10	49	26	1.88

TABLE 4. EFFECT OF GA ON STEROLS IN PEA

treatment	pea variety	plant height/cm	free sterol mg/g dry mass	stigmasterol (%)	sitosterol (%)	ratio, stig.:sito.
control	Alaska	14.9	2.46	27	59	0.46
0.5 mM GA_3	Alaska	14.0	2.40	27	61	0.44
control	Progress	3.2	2.00	38	53	0.72
0.5 mM GA_3	Progress	12.6	2.09	34	54	0.63

Plants were treated 7 days after germination and harvested 7 days later.

1978) it was shown that the top two leaves of field-grown tobacco contained 1.31 mg sterol/g dry mass and that the stigmasterol:sitosterol ratio was 0.85, while lower leaves, leaves 19 and 20, contained 1.59 mg of sterol, and the sterol ratio increased to 1.88. Leaves from middle stalk positions had intermediate sterol compositions. A similar change in sterol composition has also been reported for single leaves (Davis 1972). The leaf tip had 1.56 mg sterol/g dry mass, and the stigmasterol:sitosterol ratio was 0.87. The part of the lamina closer to the petiole had 2.24 mg of sterol, and the sterol ratio was 1.80. Storage tissue is also high in sitosterol and low in stigmasterol and as the tissue 'ages' the ratio of stigmasterol to sitosterol increases (Hartmann & Benveniste 1974).

Translocation of sterols from one part of the plant to another could be one explanation for the change in sterol composition, except that exposure of one leaf to radioactive mevalonic acid does not show a redistribution of sterols. Even 14 days after a single leaf is treated, essentially no radioactive sterol can be found in other parts of the plant (unpublished data). However, if sterols are applied to the leaf surface, translocation of some sterols may occur (Atallah *et al.* 1975*b*). Likewise, sterols introduced into the stem are translocated (Tso & Cheng 1971). It appears that higher levels of sitosterol are required during cell division and early stages of cell enlargement, and that the requirement for stigmasterol increases as cells pass into a more 'inactive' stage of development. Experiments with abscisic acid, a plant hormone which induces dormancy in many plants, did not, however, confirm this generalization (Mercer &

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Pughe 1969). Additionally, dwarf pea seedlings which were treated with gibberellic acid showed an increase in plant growth; however, there was no corresponding increase in sterol content or change in sterol composition (table 4). The report that gibberellic acid stimulates sterol biosynthesis in hazel seeds (*Corylus avellana*) might be more an induction requirement for germination rather than an induction in sterol metabolism (Shewry & Stobart 1974).

Environmental effects on sterols

Physiological factors which have the largest influence on plant growth and development are temperature, water, and light. However, very little is known about the effects of temperature and water stress on sterol metabolism, even though their importance in membrane function and chemistry is well documented.

Only one report deals with the influence of temperature on sterol metabolism (Davis & Finkner 1972). In this study, it was found that the shoots of wheat plants grown below optimum temperatures had lower sterol levels than those grown at or near optimum temperatures. In shoots sitosterol was highest when the plants were grown near optimum growing temperatures but for roots the reverse was true.

When it comes to water stress and its effect on sterols, no published information is available. In some of our preliminary studies, water stress for 48 h, even at -16 bar,† did not influence the sterol composition of soybean leaves (table 5). The plants used in these studies were 21 days old and had only three trifoliate leaves. The water potential of the plants was determined at time of harvest and, therefore, may not have been the same over the 48 h test period.

Table 5. The influence of water stress for 48 h on the free sterol composition (%) of soybean leaves

water potential/bar	2.1	8.1	16.0
sterol			
sitosterol	49.3	49.0	51.3
stigmasterol	39.5	38.8	39.6
campesterol	11.2	12.2	9.1

Light influences a number of biochemical, physiological, and morphological plant characteristics. One important process that light influences, besides photosynthesis, is senescence (Woolhouse 1967). A decrease in photoperiod or light intensity will generally accelerate plant ageing, and it is therefore only natural in the study of sterol metabolism to use light to manipulate senescence.

Light-grown plants generally have a lower sterol content than dark-grown plants (Dupéron 1968; Bush, Davis & Grunwald 1971), and the increase in sterol content is mainly due to an increase in stigmasterol. The suggestion has been made that phytochrome might be involved in the control of sterols (Hartmann, Benveniste & Durst 1972). In an experiment to test this hypothesis, tobacco plants were grown and at the end of the photoperiod either 5 min of red or far-red light was applied. After 60 days the plants did not show a change in sterol composition (unpublished data). On the other hand, plants grown under a 12 h photoperiod had a stigmasterol:sitosterol ratio of 1.11 and if transferred to the dark the ratio changed to 1.41 within 6 days. Similar changes in sterol ratio were also found in tobacco plants transferred

† 1 bar = 10^5 Pa.

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to red or far-red light conditions (unpublished). The stigmasterol:sitosterol ratio was also changed if the light energy to which the plant was exposed was reduced (table 6). The incorporation of radioactive acetate and mevalonate into sterol was not stimulated by increased light intensity, but if CO_2 was used a definite increase was observed (Kasprzyk, Wojciechowski & Jerzmanowski 1971). It has been postulated that compartmentation and changes in permeability can be responsible for the above described results (Grunwald 1975).

TABLE 6. EFFECT OF SHADE ON STEROL COMPOSITION IN TOBACCO PLANTS (ADAPTED FROM GRUNWALD 1978)

percentage shade	free sterol mg/g dry mass	campesterol (%)	cholesterol (%)	stigmasterol (%)	sitosterol (%)	ratio, stig.: sito.
0	1.43	18	10	33	39	0.84
26	1.55	18	11	36	35	1.03
67	1.41	18	11	38	33	1.15
90	1.42	16	12	42	30	1.40

TABLE 7. EFFECT OF PHOTOPERIOD ON STEROL COMPOSITION (%) OF SOYBEAN

			trifoliate leaf			
	sterol	apex	1	2	3	4
control	campesterol (%)	22	14	15	13	10
	stigmasterol (%)	20	29	35	39	42
	sitosterol (%)	58	57	59	48	48
	ratio, stig.:sito.	0.34	0.51	0.59	0.81	0.88
test	campesterol (%)	20	15	15	14	12
	stigmasterol (%)	23	35	43	45	46
	sitosterol (%)	57	50	42	41	42
	ratio, stig.:sito.	0.40	0.70	1.02	1.10	1.10

Soybean plants were grown under an 18 h photoperiod for 21 days and then transferred to an 8 h photoperiod for an additional 4 days. Control plants were kept at an 18 h photoperiod for 25 days.

As already indicated, photoperiod has also an influence on the sterol ratio (table 7). Plants grown with an 18 h photoperiod and then transferred to an 8 h light régime show a definite increase in stigmasterol and a decrease in sitosterol. The ratio of stigmasterol to sitosterol of the first trifoliate leaf under an 18 h photoperiod is 0.51 but after only 4 days under an 8 h photoperiod the ratio increases to 0.70. The second, third, and fourth trifoliate leaves show similar changes in sterol ratio. The apex also shows the light effect but to a smaller degree. It might also be worth noting the increase in stigmasterol with lower leaf position.

Of special interest is the larger amount of campesterol found in the plant apex as compared to the leaves, and the rapid decrease in campesterol concentration as the leaves develop (table 7). A similar high level of campesterol was also found in germinating seeds (table 1). It appears that campesterol is required during rapid cell division and loses its importance as the cells enlarge and mature. Campesterol has relatively good membrane stabilizing abilities (Grunwald 1968) and it might be this characteristic that is needed during rapid cell division. During phases of cell enlargement a relatively high sitosterol level appears to be required, and it almost appears as if stigmasterol has something to do with tissue ageing; but it is difficult to believe that stigmasterol is the cause and not the result of senescence.

If it is assumed that stigmasterol biosynthesis is through sitosterol, it is possible that under low

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light intensity, or short photoperiod, the conversion of sitosterol to stigmasterol is increased and/or that the catabolism of stigmasterol is decreased. To examine the possible conversion of sitosterol to stigmasterol, tobacco seedlings were incubated in [4-^{14}C]sitosterol and the radioactivity in stigmasterol was determined under light and dark conditions (table 8). As can be seen, essentially the same quantity of sitosterol was converted to stigmasterol whether or not the

TABLE 8. CONVERSION OF SITOSTEROL TO STIGMASTEROL BY TOBACCO SEEDLINGS 6 DAYS OLD

(Sitosterol and stigmasterol contents are shown as disintegrations min^{-1} μg^{-1} sterol.)

incubation time/h	light	sitosterol	stigmasterol	conversion (%)
6	yes	5.3	0.17	2.4
6	no	4.8	0.15	2.3
12	yes	8.1	0.26	2.4
12	no	5.0	0.17	1.5

Incubation medium was 10 ml of 10 mM phosphate buffer, pH 6.6, containing 0.5 μCi [4-^{14}C]sitosterol.

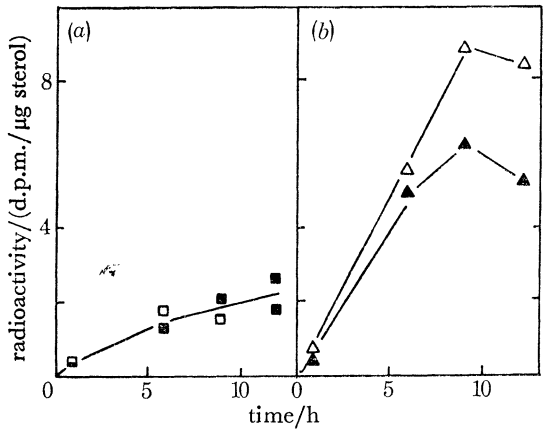

FIGURE 8. Effect of light on [2-^{14}C]mevalonate (0.46 μCi) incorporation into free sterols of tobacco seedlings 6 days old. ●, Dark; ○, light (2.2 J m^{-2} s^{-1}). (*a*) Stigmasterol; (*b*) sitosterol. (Adapted from Bush & Grunwald 1973.)

incubation was in the light or the dark, even after 12 h of incubation. The data suggest that light does not increase the 22,23-dehydrogenation of sitosterol. This observation is not in disagreement with an earlier experiment in which tobacco seedlings were incubated with mevalonate in the light and dark (Bush & Grunwald 1973). As is shown in figure 8, mevalonic acid incorporation into stigmasterol is not influenced by light but light did stimulate mevalonic acid incorporation into sitosterol. These data would suggest that the increase in the stigmasterol:sitosterol ratio is a function of decreased sitosterol biosynthesis and not due to a stimulated conversion of sitosterol to stigmasterol.

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Discussion

P. F. Saunders (*Department of Botany and Microbiology, University College of Wales, Aberystwyth, Dyfed, U.K.*). Dr Grunwald has argued convincingly against a hormonal function for plant sterols. In dismissing the possibility of such a rôle I would not wish to imply that the *biochemical* function of the compounds is necessarily different in kind from that of compounds whose physiological function can be described by the term 'hormone'.

Compounds such as the gibberellins clearly have a very important, though as yet unidentified, function in the biochemical events leading to a variety of developmental changes. We recognize them as hormones, and the plant is able to utilize them as hormones, simply because their concentrations at certain points in space or time become rate-limiting for specific processes. The sterols might well perform equally important and perhaps similar biochemical functions without their concentrations ever becoming rate-limiting.

Professor Wareing has already emphasized the possible importance of a 'balance' between various hormones in the control of plant development. In drawing up a balance sheet and in understanding how the various items interact, it may well be necessary to consider the possibility of an interaction with endogenous sterols even though they do not have a recognizable 'hormonal' function.

C. Grunwald. I agree to some extent with Dr Saunders's comment, and a good example to emphasize his point is ethylene. Not too many years ago the term phytohormone was only rarely employed when ethylene was discussed; however, today much less reservation is expressed in this regard. Maybe some day we will change our mind about the function of sterols in plants. In this regard, I should like to point out that steroids other than the sterols might be more likely candidates. After all, hormones are required in only small amounts to alter the morphology and physiology of plants, and some of the steroids, if they indeed occur in plants, occur in minute quantity. I think the oestrogens and androgens are good examples, but I believe that more research is needed.

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